Effects of water stress, only or in interaction with a pathogen, on plant functioning and fruit quality, depending on genetic variation

Julie Ripoll

ACADEMIE D'AIX-MARSEILLE
UNIVERSITE D'AVIGNON ET DES PAYS DU VAUCLUSE

THESE

Présentée pour obtenir le grade de **Docteur en Sciences**
De l'Université d'Avignon et des Pays du Vaucluse

Spécialité : Sciences Agronomiques

Effets de la contrainte hydrique, seule ou en interaction avec un pathogène, sur le fonctionnement de la plante et la qualité du fruit de *Solanum lycopersicum* L., en fonction du génotype

Par : Julie Ripoll

Soutenue le 21 avril 2015 devant un jury composé de:

Pierre MARTRE	Directeur de recherches INRA, Montpellier	Rapporteur
Jérémie LECOEUR	« Head of Environmental and Crop Modeling », Syngenta	Rapporteur
Philippe VIVIN	Chargé de recherches INRA, Bordeaux	Examinateur
Benoit JEANNEQUIN	Ingénieur de recherches INRA, Alenya	Examinateur
Jawad AARROUF	Maître de Conférences (HDR) - Université d'Avignon	Examinateur
Nadia BERTIN	Directrice de recherches INRA, Avignon	Directrice de thèse
Laurent URBAN	Professeur à l'Université d'Avignon	Directeur de thèse

Ecole doctorale : Sciences et Agrosciences, Avignon

Laboratoires d'accueil :

INRA –UR1115 « Plantes et Systèmes de culture horticoles », Avignon
UAPV-Laboratoire EA4279 « Physiologie des Fruits et Légumes », Avignon

Remerciements

Mes premiers remerciements vont à mes directeurs de thèse Nadia Bertin et Laurent Urban qui m'ont offert la chance de réaliser cette thèse. Ces quelques années à vos côtés sont passées très vite et ont été très riches en enseignements. Merci Nadia pour ta bonne humeur, ta disponibilité continue, tes encouragements et ton investissement personnel tout au long de cette aventure. Merci Laurent pour nos enrichissantes discussions, ta disponibilité et ton esprit de synthèse hors pair.

Je suis aussi reconnaissante envers la SFR Tersys, l'unité Plantes et Systèmes de culture Horticoles (INRA, Avignon) et le laboratoire EA4279 de Physiologie des Fruits et Légumes (UAPV, Avignon) qui m'ont permis de réaliser ma thèse.

Je remercie également les membres du jury: Pierre Martre, Jérémie Lecœur, Philippe Vivin, Benoit Jeannequin et Jawad Aarrouf qui ont accepté de juger mon travail.

Mes remerciements vont aussi aux membres des comités de pilotage de la thèse: Félicie Lopez-Lauri, Gilles Vercambre, Michel Havaux, Michael Staudt et Thierry Simonneau pour leurs conseils et leurs remarques. J'adresse un merci tout particulier à Félicie et Michael pour vos précieux conseils et vos aides dans les projets réalisés en commun (analyses génétiques ou de volatils) et pour ta bonne humeur Félicie lors des TP de physiologie végétale.

Je remercie également François Lecompte et Philippe Nicot pour la réalisation du projet Surbot qui m'a permis de me familiariser avec le milieu de la pathologie végétale. Merci à Mohamed El Maataoui et à Astrid-Kim Rimbault pour leur aide précieuse dans ce projet notamment pour les analyses d'histologie et des gènes.

Je tiens à remercier toutes les personnes qui ont contribué à l'élaboration des expérimentations, leurs suivis, aux récoltes et à l'analyse d'échantillons, notamment Béatrice Brunel qui m'a accompagné tout au long de ces années, merci pour ton énergie et ton efficacité mais aussi pour toutes les idées de randonnées ! Merci à Jean-Claude L'Hôtel pour ta disponibilité pour les mesures de potentiels hydriques et les petits déjeuners qui suivaient et pour m'avoir initiée au tir à l'arc ! Merci à Guillaume Garcia et José Fabre pour vos

enthousiasmes, vos disponibilités et vos aides précieuses pour toutes ces expérimentations ! Merci à Alain Goujon pour ta bonne humeur et tes blagues ! Merci à Yolande Carretero, Christophe Giraud et toute l'équipe tomate du GAFL pour m'avoir permis de réaliser cette expérimentation et pour son suivi ! Merci à Michel Pascal, Joël Béraud et François Debruyne pour vos aides pour les expérimentations en pathologie végétale, on en aura déplacé des plantes ! Un grand merci à l'équipe du laboratoire d'analyses de PSH notamment Emilie Rubio, Sylvie Sérino, Patricia Laugier et Doriane Bancel pour m'avoir formée aux analyses et pour tous ces échantillons analysés ! Merci également à Thibault Soubrat et Florent Avril qui ont contribué à une partie des analyses ! Merci à Bruno Buatois, Rachid Al Halabi et Michael Besostri pour leurs aides précieuses ! Je remercie également Claude Bruchou pour ses conseils en analyses statistiques.

Je remercie également Florence Charles, Christophe Mazzia et David Roux pour les bons moments passés lors des TP en licence.

Je tiens à remercier tous ceux qui ont contribué à la bonne ambiance de travail et aux à-côtés: Rémy, Refka, Philippe, Vincent, Sébastien, Maryline, Mitra, Juliette, Sandrine, Sanders, Christine (alias Kiki), Saad…

Un grand merci à mes parents qui m'ont épaulée dans les moments difficiles et pour toutes ces cartes postales. J'adresse aussi une pensée à mon grand-père qui m'a donné le goût des sciences, j'aurai aimé que tu puisses lire ce manuscrit. Un immense merci à Nicolas pour ta patience, ton aide et ton amour qui m'ont permis d'avancer. Merci aussi à tous mes ami(e)s qui m'ont soutenue (Pauline, Ilvira, Cécile, Julie, Lili…).

Sommaire

Liste des abréviations

$\Psi_{predawn}$: predawn water potential

Ψ_{midday}: midday water potential

$\Psi\pi$: leaf osmotic potential

Ψ_{soil}: soil water potential

Ψ_F: fruit water potential

$\Psi\pi_F$: fruit osmotic potential

ABA: abscisic acid

AGP-ase: ADP-glucose pyrophosphorylase

ANOVA: analysis of variance

AO: ascorbate oxidase

APX: ascorbate peroxidase

AsA: ascorbic acid

ATP: adenosine triphosphate

AUC: area under the curve

BT: before treatment

CA: carbonic anhydrase

CD: cell division treatment

CE: cell expansion treatment

DAA: Day After Anthesis

DHAR: dehydroascorbate reductase

DI: Deficit Irrigation

DM: dry matter content

ET: éthylène

ETc: daily evapotranspiration

ETP: potential evapotranspiration

FW: fresh weight

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

GDBH: growth differentiation balance hypothesis

g_s: stomatal conductance

HPLC: high-performance liquid chromatography

JA: jasmonic acid

LET: linear electron flox

LOX: lipoxygenase

MAGIC TOM: Multi-Parent Advanced Generation Inter-Cross population of Tomato

MDHA: monodehydroascorbate

MDHAR: monodehydroascorbate reductase

MT: maturation treatment

NIRS: near infrared spectroscopy

NMR: nuclear magnetic resonance

NO: nitric oxide

NS: not significant

PAR: photosynthetically active radiation

PEG: polyethylene glycol

PRD: partial root zone drying

PRI: photochemical reflectance index

PS: photosystems

QTL: quantitative trait locus

RDI: regulated deficit irrigation

ROS: reactive oxygen species

RP: recovery period

RWC: relative water content

SA: salicylic acid

SLA: specific leaf area

SSC: soluble solids concentrations

SUSY: sucrose synthase

TA: titratable acidity

TFs: transcription factors

TSS: total soluble solids

VOC: volatile organic compound

WD: water deficit (/ déficit hydrique)

WUE: water-use efficiency

Introduction

I- Introduction

1- Contexte général

Face aux changements climatiques, à la raréfaction des ressources naturelles (en particulier l'eau, Shao *et al*., 2008), aux nouvelles législations mises en place (ex. Plan EcoPhyto 2018) et à l'urgence de diminuer les effets néfastes de l'activité humaine sur l'environnement, les systèmes horticoles doivent évoluer vers des systèmes moins intensifs et plus durables (réduction des apports d'eau et de minéraux, réduction des rejets vers les nappes, diminution de l'utilisation des pesticides…) tout en maintenant de forts rendements. De plus, les consommateurs recherchent de plus en plus des produits cultivés dans une optique de développement durable, et ayant une valeur nutritive et gustative améliorées. L'amélioration de la qualité nutritive et gustative des fruits et légumes peut s'appuyer sur différents leviers, en particuliers la sélection génétique ou la modification des modes de production.

2- La qualité des fruits

La qualité des fruits s'élabore tout au long du développement et implique de nombreux processus. Les composés primaires tels les sucres solubles et les acides organiques déterminent la qualité gustative des fruits (Nora *et al*., 2012). Certains composés secondaires synthétisés au cours de l'adaptation et de la résistance de la plante au stress ont aussi un intérêt micronutritionnel comme les vitamines ou les composés antioxydants (constituant la "valeur santé" du fruit) ou un intérêt aromatique (Atkinson *et al*., 2011). Dans la thèse, les sucres, les acides, les caroténoïdes et l'acide ascorbique ont été analysés pour caractériser la qualité des fruits. Les voies de synthèse de ces composés primaires et secondaires sélectionnés sont reliées via les voies métaboliques de la plante schématisées dans la figure 1. Cependant leurs teneurs ne sont pas directement dépendantes les unes des autres mais sont plutôt influencées par les modifications des chaines métaboliques qui les relient (Ripoll *et al*., 2014). Ainsi, de nombreux facteurs peuvent influencer la qualité finale des fruits notamment des facteurs génétiques ou des stress biotique ou abiotique. Par exemple, des stress légers comme un déficit hydrique (water deficit: WD) peuvent avoir des effets positifs sur les composés d'intérêt que ce soit par un effet de concentration (diminution des teneurs en eau) ou bien par une stimulation de leur synthèse en réponse au stress (Ripoll *et al*., 2014).

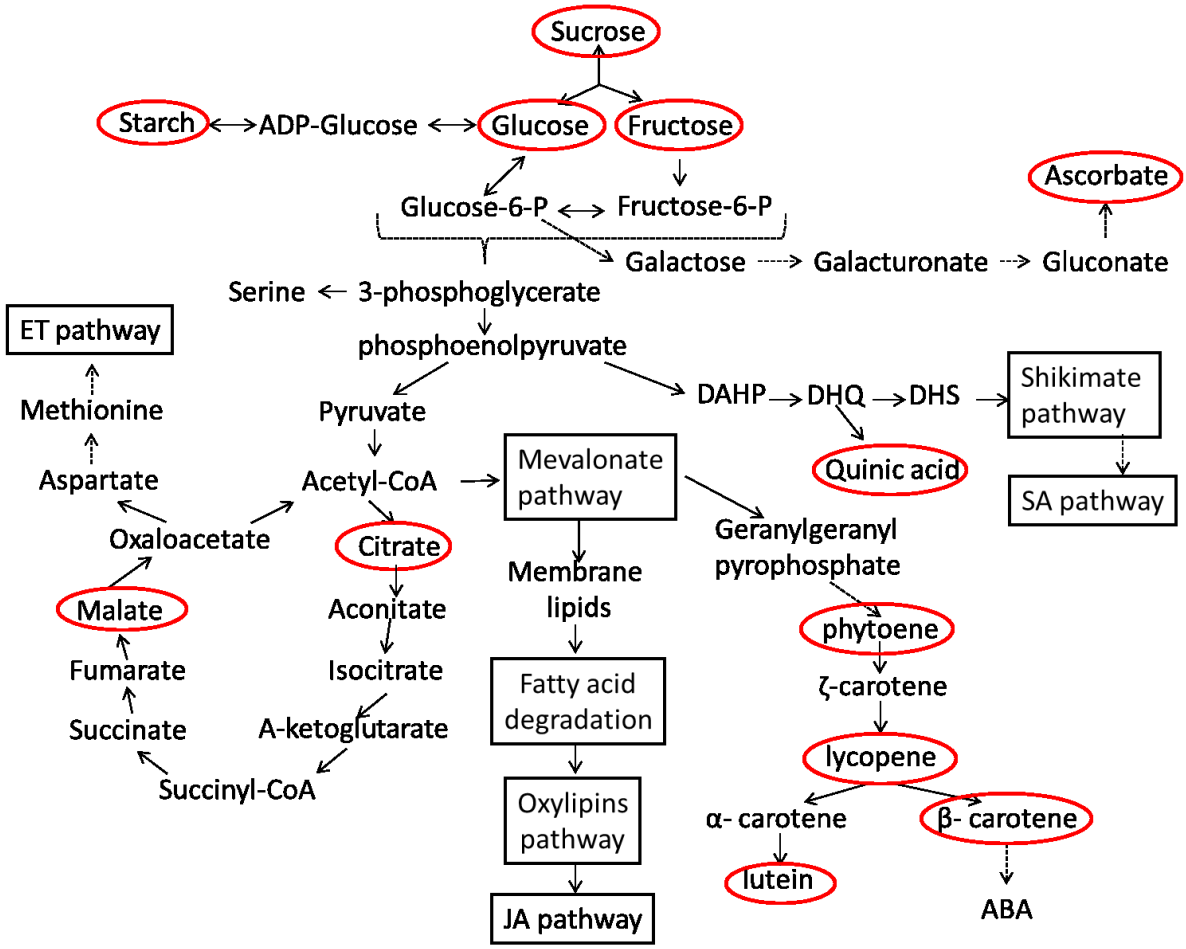

Figure 1: Voie de biosynthèse générale et simplifiée des acides organiques, des caroténoïdes et de l'acide ascorbique (ascorbate) à partir des sucres solubles issues de la photosynthèse chez les plantes, inspiré de Baba and Shiraiwa (2013), Bräutigam *et al*. (2009), Diretto *et al*. (2006), Dixon *et al.* (2002), Howles *et al.* (1996), Ran *et al.* (2001), Whitt *et al.* (2002). Les flèches pleines symbolisent une relation directe tandis que les flèches en pointillées symbolisent un raccourci dans les voies de biosynthèse. Les encadrés noirs représentent des voies spécifiques amenant aux composés d'intérêt et non détaillées. Les cercles rouges représentent les composés d'intérêt ciblés pour évaluer la qualité dans cette étude.
Abréviations utilisées : ABA: acide abscissique, ADP-glucose: Glucose-1-phosphate adenylyltransferase, DAHP: 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate, DHS: 3-dehydroshikimic acid, DHQ: 3-dehydroquinic acid, ET: éthylène, Fructose-6-P: fructose-6-phosphate, Glucose-6-P: glucose-6-phosphate, , JA: acide jasmonique, SA: acide salicylique

3- Le déficit hydrique

Le principal facteur étudié dans la thèse est l'eau qui est une ressource naturelle dont la gestion exerce des contraintes fortes en agriculture (Gao and Giorgi 2008). Le stress engendré par le manque d'eau peut être un bon levier pour améliorer la qualité gustative (modification de l'équilibre sucres-acides; Etienne *et al*., 2013; Beckles *et al*., 2012) et nutritive des fruits (synthèse d'antioxydants, de composés phénoliques et de vitamines; Nora *et al*., 2012) ainsi que pour stimuler les défenses de la plante face à des pathogènes ou des ravageurs (AbuQamar *et al*., 2008; Quiros-Gonzalez, 2000). Le WD est parfois utilisé en conditions de production afin d'accélérer la maturation des fruits de tomate et d'accroitre leurs teneurs en matière sèche (Mercier *et al*., 2009). Cependant les effets du WD sont plutôt mal maitrisés entrainant des pertes de rendement. Afin de trouver le bon équilibre entre la réduction des apports d'eau, le maintien du rendement et l'amélioration de la qualité des fruits, il est important de mieux quantifier les effets du WD sur la plante en production.

La contraite hydrique s'exerce de par la perception du manque d'eau au niveau des racines (sécheresse, salinité...) et par la demande évaporative (impact du climat) de la plante. Les réponses de la plante au WD vont variées en fonction de cette contrainte. Quatre étapes de réponses peuvent être décrites (cf. review de Tardieu *et al*., 2006). La première étape consiste en des ajustements osmotiques des cellules afin de maintenir la turgescence des tissus. En suivant, des mécanismes de réduction des pertes en eau sont mis en place avec une réduction des flux de transpiration, notamment en limitant l'ouverture des stomates (Bradford et Hsiao, 1982) ou en réduisant la surface d'échange via une limitation de la croissance (taille et/ou nombre de feuilles) ou un enroulement des feuilles (flétrissement). Egalement, la synthèse de molécules de signalisation et l'activation de gènes de réponses au WD notamment pour la production d'enzymes détoxifiantes, de transporteurs ou de protéines régulatrices est une étape importante dans la réponse au WD. Enfin, l'accroissement de l'exploration racinaire du sol afin d'obtenir des ressources en eau supplémentaires consistitue la dernière étape.

L'ajustement osmotique mis en place afin de réguler les potentiels hydrique et de turgescence des tissus en déshydratation, se fait par une accumulation de solutés organiques comme les sucres, les ammoniums quaternaires, des protéines solubles ou des acides (Mitchell *et al*., 1991). Si le stress persiste, la baisse du potentiel hydrique des tissus va engendrer une limitation de l'expansion cellulaire ainsi que des divisions cellulaires affectant la production de biomasse et le rendement. Ces ajustements osmotiques sont aussi importants

pour l'équilibre rédox des tissus et la protection des membranes cellulaires. En effet, la turgescence cellulaire intervient de manière plus ou moins directe au niveau du chloroplaste: par le maintien de son volume et indirectement, par son effet sur l'ouverture stomatique, qui en contrôlant la conductance au CO_2, conditionne l'utilisation de l'énergie photochimique (ATP, NADPH) dans le chloroplaste. Par exemple, la limitation de l'ouverture des stomates conduit à une diminution des échanges gazeux provoquant un ralentissement de la photosynthèse ayant pour conséquence une limitation biochimique du chloroplaste pour fixer le CO_2, probablement associée à la régénération limitante du ribulose biphosphate, substrat du cycle de Calvin (Damour *et al*., 2009). La non-utilisation de cette énergie peut induire ou exacerber des phénomènes de photo-inhibition qui se traduisent par la diminution du potentiel photochimique, liée à l'activation des mécanismes de dissipation de l'énergie et/ou à l'altération des photosystèmes (PS) II. Un stress carboné et un stress oxydatif se superposent alors au WD. L'ensemble stress hydrique, stress carboné et stress oxydatif va impacter les échanges entre organes sources et puits et modifier la qualité des fruits. Une diminution de l'offre en eau et en ressources carbonées va conduire à une diminution de la croissance des fruits donc du calibre final et augmenter sa teneur en matière sèche liée à des effets de dilution ou de modification du métabolisme primaire (Baldazzi *et al*., 2013). L'importance relative des effets influence la qualité gustative du fruit. Le stress oxydatif peut qu'à lui impacter le statut redox des fruits et donc conduire à une modification de la qualité nutritive en affectant les teneurs en antioxydants (Ripoll *et al*., 2014).

4- Le modèle d'étude : la Tomate

La plante modèle étudiée est la tomate qui occupe une place importante au niveau mondial (légume le plus cultivé et le deuxième pour la consommation, FAO, 2010) avec un large choix de variétés permettant son utilisation dans de nombreux programmes de sélection, et qui est également un modèle au niveau de la caractérisation physiologique des fruits charnus. De plus, la tomate présente une forte valeur santé de par sa richesse en minéraux, vitamines, acides organiques, acides aminés et antioxydants (comme les caroténoïdes). Cette richesse perdue au bénéfice d'un productivisme accrue, revient à l'ordre du jour de par la demande des consommateurs.

La tomate est une plante herbacée pérenne (dans des conditions climatiques adaptées) mais elle est cultivée comme une plante annuelle. Elle est originaire d'Amérique du Sud

(Andes) et appartient à la famille des Solanacées. La croissance de la tomate peut être indéterminée ou dite "déterminée" (Atherton et Harris, 1986). Chez les variétés dites à "croissance déterminée", la fonction végétative sur chaque tige s'arrête précocement (entre le premier et le cinquième bouquet), chaque tige se terminant par un bouquet floral, la croissance continue sur les drageons auxiliaires. Pour les variétés à croissance indéterminée, chaque bouquet floral est séparé par une à quatre feuilles constituant un étage/sympode et la plante peut croître indéfiniment. Ainsi, sur une même plante, des fruits d'âge différents coexistent en fonction des sympodes et sur un même bouquet. En effet, les fleurs de tomate (hermaphrodites et autofécondes) sont regroupées en inflorescence formant des grappes. De manière générale, les plantes à croissance déterminée sont utilisés dans les systèmes de culture au champ pour la production de tomates d'industrie et celles à croissance indéterminée sous serre pour la production de fruits frais. Dans le cadre de cette étude, seules des tomates à croissance indéterminée ont été étudiées (Fig. 2).

La tomate est une baie composée du péricarpe et de la pulpe (Fig. 3). Son développement peut être subdivisé en trois phases (Gillaspy *et al*., 1993). La première correspond à la phase de division cellulaire, elle peut être plus ou moins longue (de 10 à 15 jours pour les variétés de type cerise, et de 20 à 25 jours pour les variétés à gros calibre). Cette phase se caractérise par une importante activité mitotique notamment dans le péricarpe. La division cellulaire est suivie de l'expansion cellulaire et du développement de l'embryon contenu dans les graines (de 25 à 30 jours selon les fruits). Pendant l'expansion cellulaire, l'importation des ressources carbonées est maximale et induit une entrée massive d'eau dans les cellules stimulant l'expansion de celles-ci. A la fin de l'expansion cellulaire, les graines sont arrivées à maturité et le fruit a quasiment atteint sa taille maximale, il est dit « vert mature ». La dernière phase de développement consiste en la maturation des fruits, déclenchée par une augmentation de la respiration du fruit et une augmentation de la synthèse d'éthylène. Cette phase est courte (10 jours environ), elle débute avec le changement de couleur du fruit passant par le stade breaker (moitié vert et moitié orange) lié à la transformation des chloroplastes en chromoplastes jusqu'à atteindre une couleur rouge uniforme (en raison de la forte présence de lycopène). Pendant la maturation les teneurs en sucres, acides et composés volatils du péricarpe varient généralement au profit d'un enrichissement en sucres, une diminution des acides et une augmentation de la synthèse des composés volatils aromatiques.

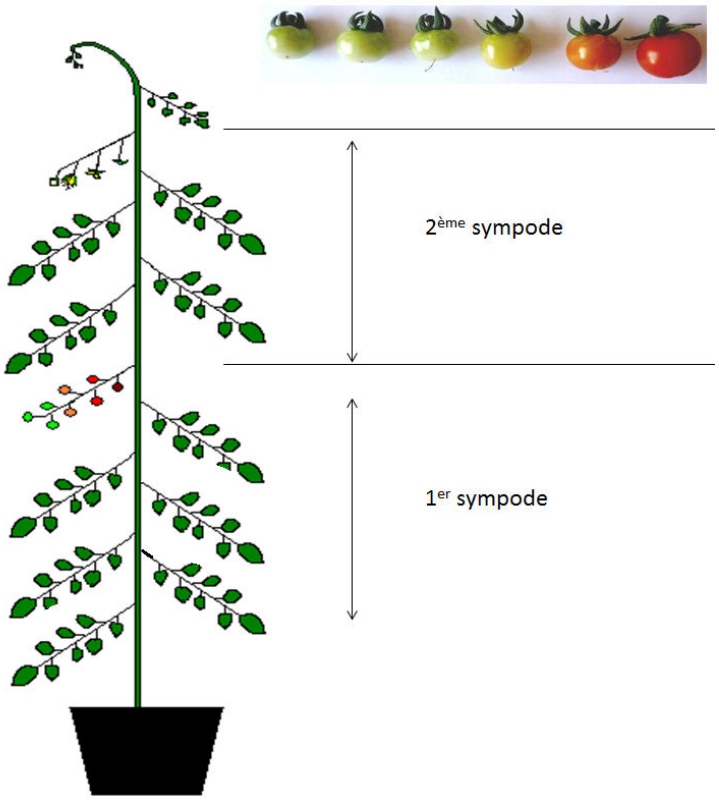

Figure 2: Schéma d'un plant de tomate à croissance indéterminée avec représentation de deux étages foliaires et photographie de fruits provenant d'un même bouquet, le fruit le plus âgé étant le plus proche de la tige centrale.

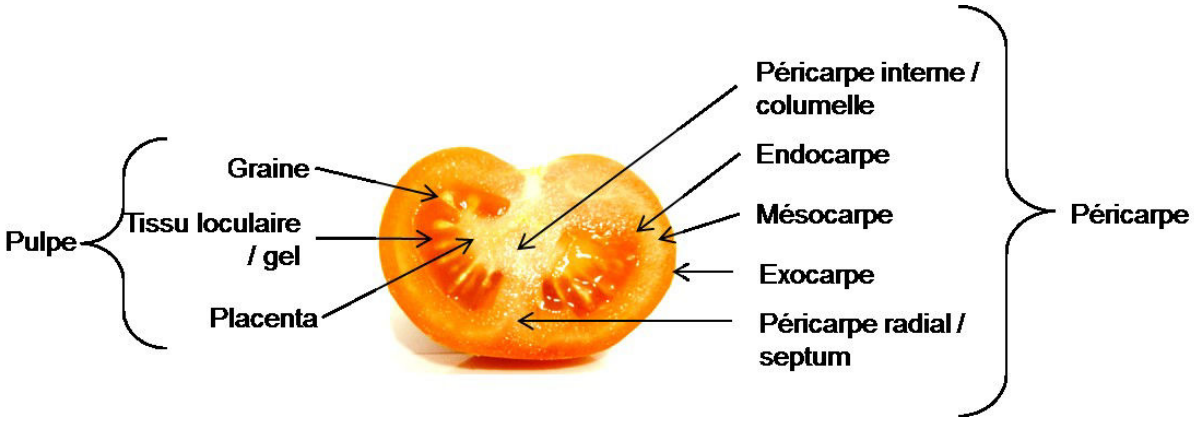

Figure 3: Structure du fruit de tomate en coupe longitudinale

5- La problématique

De manière générale, les contraintes abiotiques ou biotiques peuvent modifier le développement et la croissance de la plante et du fruit, donc affecter le rendement. Toutefois, ces effets dépendent du stade de développement affecté par le stress. En effet, un stress apparaissant pendant la phase végétative peut avoir des effets moindres sur le rendement, qu'un stress apparaissant pendant la phase reproductive (Craufurd & Wheeler, 2009). De même, la réponse de la plante peut varier fortement en fonction de l'intensité du stress (Niinemets, 2010) et des stress modérés peuvent stimuler la résistance de la plante à des stress plus sévères (Bruce *et al*., 2007).

En conditions naturelles, les plantes sont souvent exposées à des stress multiples, combinant des stress abiotiques et biotiques, ou bien des alternances de périodes de stress avec des périodes de récupération. Des études récentes ont montré un recouvrement des mécanismes de réponse et d'adaptation de la plante aux différents types de stress abiotiques et/ou biotiques (Fujita *et al*., 2006). Certaines interactions entre stress ont des effets additifs qui peuvent être délétères pour le développement de la plante comme dans le cas d'un WD et d'un stress de chaleur (Mittler, 2006). Cependant, des interactions entre stress abiotique et biotique ont aussi des effets positifs par exemple entre des insectes et un WD (Quiros-Gonzalez, 2000) ou bien lors d'interactions entre un pathogène et un stress osmotique ou oxydatif (Wiese *et al*., 2004). Ces interactions vont également impacter la qualité des fruits comme résumé dans le chapitre I.

Il parait donc possible d'exploiter ces mécanismes pour « endurcir » la plante et diminuer sa sensibilité à des stress successifs, tout en favorisant la qualité des fruits. Pour cela il est nécessaire de mieux comprendre et quantifier les processus impliqués dans la réponse au WD en lien avec la qualité et l'adaptation de la plante à différents stress.

6- Les objectifs

L'objectif de la thèse est d'évaluer les effets bénéfiques et délétères du déficit hydrique sur des critères de rendement et de qualité des fruits et de défenses de la plante vis à vis d'autres stress. Pour cela, nous avons quantifié les effets d'un WD sur la qualité et la production de la tomate en fonction du stade affecté par le stress et de l'intensité du stress. De même, l'impact du stress hydrique sur la sensibilité de la plante à un autre stress de même nature (succession de stress hydriques) ou de nature différente (biotique) a été évalué. Les différentes hypothèses de travail sont résumées dans la figure 4.

Ainsi, nous avons cherché à:

- Identifier de(s) seuil(s) d'humidité du sol et des critères pertinents pour caractériser la réponse de la plante au cours du dessèchement du sol.
- Déterminer si les impacts du WD sur la qualité organoleptique, la valeur santé et le rendement sont dépendants des stades de développement de la plante ou du fruit affectés par le WD.
- Caractériser l'impact de stress répétés sur l'adaptation des plantes et sur la qualité du fruit
- Caractériser l'implication du métabolisme primaire sur les modifications de la sensibilité des plantes à un pathogène majeur de la tomate (*Botrytis cinerea*) lors d'interactions avec un WD
- Caractériser les changements métaboliques en réponse au dessèchement à l'aide de leur signature volatile (ces travaux nécessitent des études complémentaires et ne sont pas présentés dans le manuscrit)
- Mesurer la variabilité génétique des réponses

Nous nous sommes donc placés volontairement dans des conditions de culture proches de celles des serres de production (Fig. 5) mais aussi en condition climatique contrôlée (phytotrons). Des déficits hydriques d'intensités variables ont été appliqués selon la saison de culture et la problématique visée. Le matériel génétique utilisé pour chaque expérimentation comprenait toujours au minimum deux génotypes ayant une réponse contrastée aux types de stress visés, sélectionnés selon la littérature (Tab. 1) et par des sélectionneurs. Nous avons choisi d'explorer une grande variabilité génétique en étudiant les 8 parents de la population Multi-Parent Advanced Generation Inter-Cross de la tomate (MAGIC TOM) pour établir un

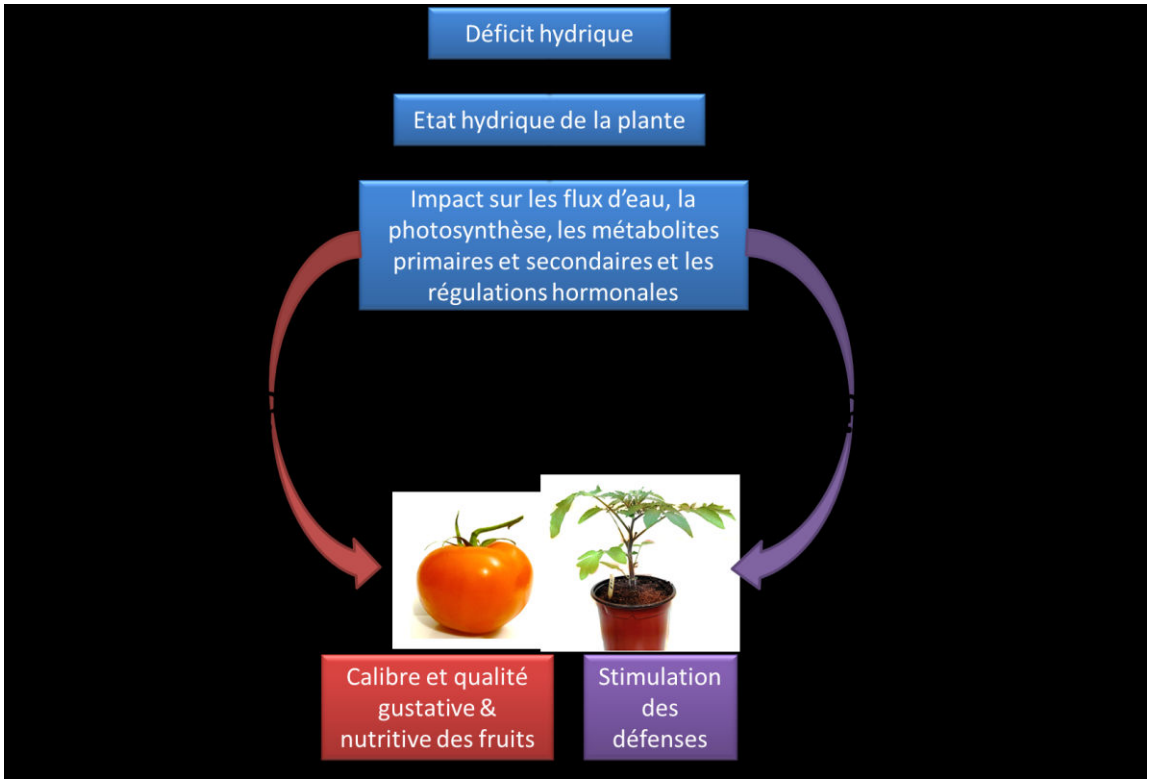

Figure 4: Hypothèses de travail : on peut stimuler les défenses de la tomate et améliorer la qualité organoleptique et nutritive des fruits via un déficit hydrique modéré (cf. chapitre 1). Ces hypothèses ont été testées expérimentalement par l'étude d'alternances de périodes de déficit hydrique et de récupération (chapitre 2), lors d'interactions entre un WD et un pathogène (*Botrytis cinerea*) (chapitre 5) et en fonction du stade de développement de la plante (chapitre 4) et du fruit (chapitre 3). Les effets du WD ont été évalués sur les principaux processus de croissance (flux d'eau et de carbone, division et expansion cellulaires dans les fruits) qui déterminent le rendement et la qualité gustative (sucres, acides) et nutritive (acide ascorbique, caroténoïdes) des fruits. La variabilité due au génotype a également été évaluée dans la plupart des expérimentations.

Figure 5: Photographies des différents modes de culture utilisés lors de cette étude à savoir un compartiment de serre en verre de l'unité GAFL (INRA-PACA) et une chambre climatique de l'unité PSH (INRA-PACA), avec deux stades de développement visibles : stade jeune / plantule (A) et stade reproducteur (B).

Génotypes	Espèce	Taille du fruit	Spécificité	Références
Cervil	*Solanum lycopersicum cerasiforme*	< 5 g	Parent MAGIC TOM	Ranc, 2010
Criollo	*Solanum lycopersicum cerasiforme*	< 15 g	Parent MAGIC TOM	Ranc, 2010
Ferum	*Solanum lycopersicum esculentum*	< 130 g	Parent MAGIC TOM	Ranc, 2010
LA0147	*Solanum lycopersicum esculentum*	< 130 g	Parent MAGIC TOM	Ranc, 2010
LA1420	*Solanum lycopersicum cerasiforme*	< 50 g	Parent MAGIC TOM	Ranc, 2010
Levovil	*Solanum lycopersicum esculentum*	< 130 g	Parent MAGIC TOM	Ranc, 2010
Momor	*Solanum lycopersicum esculentum*	< 130 g	Sensible au DH Résistant Fusarium	Peter and Rai, 1976 Fazio et al., 1999
Monalbo	*Solanum lycopersicum esculentum*	< 130 g	Sensible à Botrytis cinerea	Données pathologie
Plovdiv XXIVa	*Solanum lycopersicum cerasiforme*	< 50 g	Parent MAGIC TOM	Ranc, 2010
Stupicke Polni Rane	*Solanum lycopersicum esculentum*	< 70 g	Parent MAGIC TOM	Ranc, 2010

Tableau 1: Récapitulatif des génotypes utilisés lors de cette étude, de la taille moyenne des fruits et de spécificités connues.

screening des réponses au WD. Ces parents vont servir à la génération de lignées introgressives commercialisables. La caractérisation de leurs réponses en conditions de déficit hydrique représente un intérêt pour les sélectionneurs. Pour d'autres expérimentations, nous avons travaillé sur du matériel végétal spécifique par rapport aux thématiques étudiées comme dans le cas de l'étude des réponses au pathogène nécrotrophe *Botrytis cinerea*. Au total, quatre expérimentations de longue durée (plus de 6 mois) et quatre expériences de durées moyennes (entre 3 et 4 mois) ont été réalisées.

7- Cadre de la thèse et collaborations

Ma thèse s'inscrit dans ce contexte et fait partie intégrante des programmes de recherche de la Structure Fédérative de recherche TERSYS regroupant différents acteurs de la recherche agronomique tel l'INRA et l'Université d'Avignon et des Pays du Vaucluse.

Mes travaux de recherche se sont déroulés sous la cotutelle de l'Unité Plantes et Systèmes de culture Horticoles (PSH) de l'INRA PACA et le laboratoire EA 4279 de Physiologie des Fruits et Légumes de l'Université d'Avignon et des Pays du Vaucluse, avec le support de la SFR Tersys et du CASDAR CTPS TOMSEC (projet de recherche sur la conception d'idéotypes culturaux adaptés à la contrainte hydrique et améliorés sur des critères de qualité gustative et nutritionnelle).

Ces travaux ont également fait l'objet de plusieurs collaborations notamment avec l'Unité de Pathologie Végétale de l'INRA PACA, l'Unité de Génétique et Amélioration des Fruits et Légumes (GAFL) de l'INRA PACA et le Centre d'Ecologie Fonctionnelle et Evolutive (CEFE) du CNRS de Montpellier.

Les expérimentations ont été réalisées au sein des différentes structures ayant participé à ces travaux. Fort du soutien de tous ces acteurs, nous avons tenté d'apporter des éléments de réponses aux problématiques qui nous lient dans un objectif d'adaptation des cultures à la sécheresse et la production de produits de qualité.

II- Plan du travail

La thèse est organisée en 7 chapitres.

Le chapitre I est une synthèse bibliographique portant sur les effets de la sécheresse sur les critères de qualité des fruits charnus notamment la tomate en relation avec les métabolismes primaire et secondaire. Un examen des méthodes innovantes qui pourraient être utilisées à l'avenir pour évaluer l'état physiologique des plantes en condition de stress a également été réalisé.

Le chapitre II porte sur l'étude des effets stimulants, pour les défenses de la plante et la qualité du fruit, de périodes de WD alternée avec des périodes de récupération (RP). Cette étude a été réalisée sur les 8 génotypes parents de la population MAGIC, possédant la plus grande variabilité génétique connue sur une sélection de 360 accessions de tomate. Ce traitement alternatif a permis de limiter les pertes de rendement tout en améliorant la qualité des fruits en fonction de l'intensité du WD, de la phase de développement du fruit ciblé, du génotype et de l'accumulation de cycles de WD et de RP par les plantes.

Le chapitre III porte sur l'étude des impacts d'un WD modéré sur la qualité des fruits en fonction de la phase de développement du fruit affectée par le WD. Deux génotypes contrastés ont été utilisés dans cette étude afin de comparer les réponses à l'échelle de la plante et du fruit. Cette étude a permis de mettre en évidence qu'un WD modéré n'engendre pas seulement une réponse modérée par rapport à un stress plus intense, mais bel et bien une réponse spécifique fortement dépendante du génotype. La phase de division cellulaire serait la plus sensible au WD modéré en termes d'amélioration de la qualité des fruits, y compris de leur croissance.

Le chapitre IV présente les résultats des effets de cinétiques de dessèchement du sol sur l'état physiologique de la plante et du fruit, à deux stades de développement (végétatif et reproductif), sur une sélection de quatre génotypes parents de la MAGIC TOM. Cette étude a révélé des stratégies différentes entre plantes végétatives et reproductives concernant la gestion des impacts du WD au niveau des photosystèmes. Une sélection de paramètres issus des mesures de la fluorescence de la chlorophylle *a,* a pu être déterminée en fonction des stades de développement de la plante et du génotype.

Le chapitre V porte sur l'hypothèse de la stimulation des défenses de la plante par un WD vis à vis d'un stress biotique. Pour cela un WD modéré et une infection par un pathogène nécrotrophe, *Botrytis cinerea* ont été combinés. Les régulations hormonales mises en jeu lors de cette interaction favorisent le développement du pathogène sur tige, et ce, en lien avec les teneurs en métabolites primaires. Cependant, un effet systémique entre les infections sur tige et les infections foliaires pour les plantes en WD a été observé et soulève de nouvelles questions de recherche. Une augmentation de la teneur relative en fructose par rapport aux autres sucres dans les tissus infectés semble limiter le développement de l'infection. Des hypothèses concernant le rôle que pourrait avoir l'enzyme sucrose synthase dans cette interaction méritent d'être vérifiées.

Les derniers chapitres VI et VII sont consacrés à la synthèse de l'ensemble des résultats obtenus et à leur mise en perspectives.

III- Publications et communications scientifiques réalisées au cours de la thèse

1- Articles publiés

Ripoll J., Urban L., Staudt M., Lopez-Lauri F., Bidel L.P.R., Bertin N. (2014). Water shortage and quality of fleshy fruits - making the most of the unavoidable. Journal of Experimental Botany, 65(15), p. 4097-4117.

Urban L., Staudt M., Ripoll J., Lauri F., Bertin N. (2015). Less can make more - revisiting fleshy fruit quality and irrigation in horticulture. Chronica Horticulturae, 54 (4), p. 24-31.

2- Articles en préparation ou soumis

Ripoll J., Urban L., Brunel B., L'Hôtel J.-C., Garcia G., Bertin N. (2014). Impact of water deficit on tomato fruit growth and quality depends on the fruit developmental stage. Acta Horticulturae. Accepted.

Ripoll J., Urban L., Brunel B., Bertin N. Beneficial effects of water deficit on tomato quality depend on fruit developmental stage and genotype. Soumission de la version finale prévue à « Journal of Plant Research ».

Ripoll J., Urban L., Bertin N. The MAGIC TOM Parents: an important source of genetic variability for adaptation to repeated cycles of deficit irrigation at the plant and fruit levels. Soumission de la version finale prévue à « Environmental and Experimental Botany ».

Ripoll J., Bertin N., Brunel B., Urban L. Adaptive strategies of vegetative and reproductive plants to soil drying revealed by parameters derived from fluorescence induction curves. Soumission de la version finale prévue à « Plant Physiology ».

Ripoll J., Lecompte F., Nicot P., Rimbault A.K., Urban L., El Maataoui M., Lauri F., Bertin N. Impact du déficit hydrique sur la sensibilité de la tomate au pathogène *Botrytis cinerea* - Implications du métabolisme primaire et des voies de régulations hormonales. Soumission de la version finale prévue à « Journal of Experimental Botany »

3- Communications orales et posters

Ripoll J., Urban L., Brunel B., L'Hôtel J-C., Bertin N. (2014). Impact of water deficit on tomato fruit growth and quality depends on the fruit developmental stage. 29e International Horticultural Congress, Brisbane (17-22 aout). Poster digital.

Ripoll J., Bertin N., Al Halabi R., Buatois B., Staudt M. (2014). Monitoring plant odors in tomato culture for in-situ stress detection. Plant Biology Europe FESPB/EPSO Congress, Dublin (22-26 juin). Poster.

Ripoll J., Urban L., Brunel B., Goujon A., L'Hôtel J-C., Causse M., Bertin N. (2014). Variability of the genetic response of tomato during soil drying and repeated cycles of water deficit and recovery. Plant Biology Europe FESPB/EPSO Congress, Dublin (22-26 juin). Poster.

Ripoll J., Urban L., Brunel B., Goujon A., L'Hôtel J-C., Causse M., Bertin N. (2014). Combining deficit irrigation and recovery periods can save water and maintain yield and fruit quality in tomato. XVIIIth EUCARPIA Meeting of the Tomato Working Group, Avignon (22-25 avril). Poster. Prix du meilleur poster étudiant.

Ripoll J. (2014). Les enjeux du déficit hydrique face aux changements climatiques - Améliorer la qualité des fruits et les défenses de la plante. Communication orale, Concours Ma thèse en 180 secondes, Finale locale d'Avignon (4 avril).

Ripoll J. (2013). Effets de la contrainte hydrique, seule et en interaction avec d'autres stress biotique et abiotique, sur les mécanismes d'adaptation de la plante et la qualité du fruit, en fonction de la variabilité génétique. Communication orale, Journée Tersys 2013, Avignon (12 septembre).

Ripoll J., Lecompte F., Nicot P., Lauri F., Urban L. and Bertin N. (2013). How to improve Fruit Quality and Plant Resistance? - Impacts of Deficit Irrigation at the Plant and Fruit Scales. Journée Tersys, Avignon (12 septembre). Poster. Prix du meilleur poster étudiant.

Ripoll J., Brunel B., L'Hôtel J-C., Bertin N., Urban L. (2012). Adaptation des cultures au changement climatique : Réponse adaptative de la Tomate au déficit hydrique – Comparaison et sélection de différentes variétés commercialisables. Journée des Ecoles doctorales, Avignon (29-31 oct 2012), Poster.

Water shortage and quality of fleshy fruits – making the most of the unavoidable

En horticulture, l'augmentation des périodes de sécheresse dans le cadre du changement global pourrait être mise à profit pour compenser les pertes de rendement associées au déficit hydrique (WD). En effet, des effets positifs sur la qualité des fruits et la stimulation des défenses de la plante ont été observés en réponse à des WD. Concilier les avantages du WD et en réduire les inconvénients permettraient aux professionnels de s'adapter aux conditions de culture futures.

Nous défendons ici l'idée que les producteurs de fruits pourraient piloter le déficit hydrique (WD) pour optimiser ces effets sur la plante et le fruit. En effet, le WD stimule les métabolismes primaire et secondaire, ce qui pourrait accroître la qualité, en particulier les concentrations en phytonutriments. Toutefois, des compromis entre qualité et rendement doivent être trouvés.

Ainsi, le succès de la gestion des cultures dans des conditions d'approvisionnement en eau limité requiert une meilleure compréhension des impacts du WD sur les processus physiologiques, le rendement et la qualité, ainsi que sur la façon dont les plantes gèrent les métabolismes primaire et secondaire, et leurs interactions. Nous proposons ici une mise à jour des connaissances sur les effets du WD en fonction des critères de qualité des fruits.

La question de l'effet du WD sur le rendement et la qualité étant complexe, nous fournissons également un examen assez original de ce que nous savons sur les effets des différentes formes de sécheresse sur la qualité des fruits par rapport aux métabolismes primaire et secondaire. Nous discutons également le rôle de la phase de développement de la plante lors de WD et les interactions avec d'autres facteurs de stress et les effets génétiques.

Améliorer notre compréhension est certainement important, mais les producteurs devront s'appuyer sur des indicateurs pertinents de l'état de l'eau et des critères de qualité. Nous avons donc décrit brièvement les indicateurs de l'état de l'eau qui pourraient être utilisées à des fins de surveillance, tels que les composés volatils.

Ce chapitre est présenté sous la forme d'un article publié en 2014 dans « Journal of Experimental Botany ».

Water shortage and quality of fleshy fruits – making the most of the unavoidable

Julie Ripoll[1,2], Laurent Urban[2], Michael Staudt[3], Félicie Lopez-Lauri[2], L.P.R. Bidel[4], Nadia Bertin[1]

1. INRA - Centre d'Avignon, UR 1115 Plantes et Systèmes de culture Horticoles, Domaine Saint Paul - Site Agroparc, 228 route de l'Aérodrome, CS 40509, 84914 Avignon Cedex 9, France
2. Laboratoire de Physiologie des Fruits et Légumes, Université d'Avignon et des Pays du Vaucluse, Bât. Agrosciences, 301 rue Baruch de Spinoza, B.P. 21239, F-84916 Avignon Cedex 9, France
3. Centre d'Ecologie Fonctionnelle et Evolutive Montpellier, CNRS, 1919 Route de Mende, 34293 Montpellier Cedex 5, France
4. INRA, UMR AGAP, Place P. Viala, F-34060 Montpellier, France

Résumé

Les événements climatiques extrêmes, tel la sécheresse, devraient augmenter en fréquence et intensité ainsi qu'au niveau géographique en conséquence des changements climatiques. De manière générale, pour que les cultures aient une bonne croissance dans le futur, les agriculteurs auront besoin de s'adapter à la faible quantité d'eau disponible et apprendre à prendre avantage sur les effets de la sécheresse. Heureusement, la sécheresse est aussi associée à des effets positifs. La sécheresse est connue pour stimuler le métabolisme secondaire et ainsi d'augmenter les défenses de la plante et les concentrations en métabolites secondaires impactant la qualité des produits récoltés, notamment au niveau du goût et des bénéfices pour la santé. Cet effet de la sécheresse sur la production de métabolites secondaires est d'une importance cruciale pour la culture des fruits. Toutefois, afin de gérer efficacement les cultures dans des conditions d'approvisionnement en eau limitée, par exemple en appliquant un déficit hydrique, les producteurs doivent tenir compte non seulement de l'impact de la sécheresse sur la productivité mais aussi de la façon dont les plantes gèrent les métabolismes primaires et secondaires. Cette question est évidemment complexe, car lors de déficit hydrique, les compromis entre productivité, défense et qualité dépendent de l'intensité, la durée et la répétition des événements de déficit hydrique. Le stade de développement de la plante au cours de la période de déficit hydrique est également crucial, tout comme les effets d'autres facteurs de stress. En outre, les producteurs doivent s'appuyer sur des indicateurs pertinents de l'état de l'eau, c'est-à-dire, les paramètres impliqués dans les processus métaboliques pertinents, y compris ceux affectant la qualité. Bien que de nombreux rapports sur les effets de la sécheresse sur le fonctionnement des plantes et la productivité des cultures aient été publiés, ces questions n'ont pas été examinées à ce jour. Dans cette revue, nous fournissons une mise à jour des connaissances actuelles sur les effets des différentes formes de la sécheresse sur la qualité des fruits par rapport aux métabolismes primaires et secondaires et de leurs interactions. Nous examinons aussi les indicateurs classiques et moins classiques de l'état de l'eau qui pourrait être utilisés à des fins de surveillance, tels que les composés volatils. Nous nous concentrons sur les cultures de fruits en raison de l'importance du métabolisme secondaire dans la qualité des fruits et de l'importance des fruits dans l'alimentation humaine. La question de la défense est également brièvement discutée.

Mot clés: Déficit hydrique, qualité des fruits, gestion de l'irrigation, mécanismes d'adaptation et leurs interactions, métabolisme volatile

Abstract

Extreme climatic events, including drought, are predicted to increase in intensity, frequency and geographic extent as a consequence of global climate change. In general, to grow crops successfully in the future, growers will need to adapt to less available water and to take better advantage of the positive effects of drought. Fortunately, there are positive effects associated with drought. Drought stimulates the secondary metabolism, thereby potentially increasing plant defences and the concentrations of compounds involved in plant quality, particularly taste and health benefits. The role of drought on the production of secondary metabolites is of paramount importance for fruit crops. However, to manage crops effectively under conditions of limited water supply, for example by applying deficit irrigation, growers must consider not only the impact of drought on productivity but also on how plants manage the primary and secondary metabolisms. This question is obviously complex because during water deficit, trade-offs among productivity, defence and quality depend upon the intensity, duration and repetition of events of water deficit. The stage of plant development during the period of water deficit is also crucial, as are the effects of other stressors. In addition, growers must rely on relevant indicators of water status, i.e., parameters involved in the relevant metabolic processes, including those affecting quality. Although many reports on the effects of drought on plant function and crop productivity have been published, these issues have not been reviewed thus far. Here, we provide an up-to-date review of current knowledge of the effects of different forms of drought on fruit quality relative to the primary and secondary metabolisms and their interactions. We also review conventional and less conventional indicators of water status that could be used for monitoring purposes, such as volatile compounds. We focus on fruit crops due to the importance of secondary metabolism in fruit quality and the importance of fruits in the human diet. The issue of defence is also briefly discussed.

Key-words: Water Shortage, Fruit Quality, Irrigation Management, Adaptation & Interaction Mechanisms, Volatile Metabolome.

I- Introduction

Agriculture is facing increasingly frequent periods of drought, and in the future, water reduction is expected to exert the most adverse impact upon growth and productivity among abiotic stress factors (Shao *et al.*, 2008). This trend is of particular concern in the Mediterranean region, which will experience more frequent periods of intensive drought, leading to the extension of arid areas (Gao and Giorgi, 2008). In many countries where the lack of water is not yet critical, crops are currently irrigated in excess to avoid water shortage and promote plant growth; however, this irrigation may be detrimental to crop quality and water resources around the world. In the Mediterranean area, agriculture consumes approximately 177.7 10^9 m^3 $year^{-1}$ (an average figure obtained from data collected between 2003 and 2007 from Mediterranean countries with the exception of countries of the African continent), and a marked reduction in irrigation would save a considerable amount of water [1, FAO website]. It is generally accepted that this objective can be achieved by increasing water use efficiency (WUE). For instance, regulated deficit irrigation or partial root drying have been proposed to reduce water consumption in fruit orchards and to stimulate plant adaptation to stress-prone environments (Stikic *et al.*, 2003). These techniques enable the plants to more effectively explore the soil with their roots and stimulate the production of compounds such as abscisic acid (ABA), a major phytohormone involved in the responses of plants to abiotic stress (Shen *et al.*, 2014). These strategies are promising but they have been only partially "explored" in order to preserve yield and overlooked other traits such as crop quality. Based on our knowledge of the mechanisms involved in plant and fruit responses to WD, we believe that other irrigation strategies involving a greater reduction in water supply could be developed. Moreover, in this review, we propose a re-examination of commonly held ideas on water requirements for crop performance in light of current knowledge of the effect of drought on quality criteria, such as the content of phytonutrients, and on plant defences. We focus on fruit crops due to their agronomic and dietary importance. Fleshy fruits are the most important crops in the world after grains, and they represent a key ingredient of a healthy diet [2, FAO website] due to their high contents of phytonutrients, vitamins and antioxidants.

Fruit quality is measured according to several criteria, the relative importance of which differs among producers, consumers and distributors (Shewfelt, 1999). Fruit size and external attractiveness (colour, form, size…) determine commercial quality, whereas adequate flesh

firmness ensures a reasonable shelf-life. The consumer purchases fruit based on appearance and aroma, which are associated with taste and shelf-life. However, flavour promotes fruit consumption, and the characteristics of high-quality fruit include high sugar levels and an appropriate sugar-to-acid ratio. Consumers are also increasingly concerned with the nutritive value of fresh fruits, which is typically related to the antioxidant content (primarily polyphenols and vitamin C). The global demand for high-quality fruits that taste good, are rich in vitamins and antioxidants, are environmentally friendly and can endure the demands of worldwide supply chains is growing rapidly and requires continuous effort to improve varieties and respond to environmental constraints.

A wealth of studies have compared the behaviour of different plant species under well-irrigated and deficit irrigation conditions. The molecular response to drought has been intensively investigated in many species and numerous responsive genes have been identified which are involved in many signaling and metabolic pathways (Atkinson and Urwin, 2012). To date, only few candidate genes for stress resistance have been characterized and the links between gene or protein expression and plant or organ phenotype under field conditions are still poorly understood. At the plant or organ level, many studies have discussed the overall negative impact of water deficit (WD) on yield, which leads to growth limitation or even plant death or organ abortion under extreme conditions. One of the major and most well-documented effects of drought is stomatal closure (Damour *et al.*, 2010). The associated decrease in transpirational water loss is beneficial for plant survival because it helps the plant to maintain water balance; however, this positive effect comes at the price of a reduction in net CO_2 uptake. A decrease in net photosynthesis not only translates into a decrease in carbohydrate supply to the fruits, it also creates conditions that are conducive to photo-oxidative stress, i.e., the photosynthesis-associated production of reactive oxygen species (ROS), including $O_2^{\cdot-}$, $^{\bullet}OH$ and possibly H_2O_2 (Grassmann *et al.*, 2002). Because oxidative stress stimulates the accumulation of antioxidant compounds, a beneficial effect of WD on the health value of fruits may be expected and has been reported in many descriptive studies (Nora *et al.*, 2012). More generally, a recent review (Wang and Frei, 2011) outlined some typical patterns in crop-quality response to several abiotic stressors and indicated a tendency towards a loss in taste but an increase in nutritional value. Thus, trade-offs between crop yield and quality might be achieved under controlled WD conditions provided that growers have access to quantitative information not only related to productivity but also to crop quality. In this perspective, experimental conditions should better cover the range of conditions

experienced by plants under natural conditions (West *et al.*, 2004). Indeed, compared with experimental conditions that focus on one or two stress factors applied at a given period, in the field, plants are prone to stresses of varying intensity (from mild to severe stress), the progressive development of stress and soil dehydration and repeated alternating cycles of stress and recovery that occur during different periods of plant development. Moreover, in the field, one single stress factor rarely occurs in isolation; thus, several complex interactions and feedback cycles involved in plant responses must be considered. Finally, cumulative or transient effects are integrated by the plant throughout the crop cycle, which may lead to markedly contrasting responses with respect to plant health, productivity and quality.

We review in this paper our current state of knowledge about the effects of WD on quality criteria of fleshy fruits (soluble sugars, organic acids, volatile aromas and texture), with special emphasis on the underlying physiological mechanisms. Because of the growing importance of the concentration in phytochemicals as a quality criteria of fruits, we examine in detail the roles played by the reduction in supply of precursors and the oxidative stress, which are both associated with WD. During WD, trade-offs among productivity, defence and quality depend upon the intensity, duration and repetition of the phases of water deficit. The stage of plant development during the period of water deficit is also crucial, as are the effects of genetic factors and other co-occurring stresses. We therefore examine the range of plant/fruit responses to WD depending on genotype, stress intensity, timing and interactions with other abiotic and biotic stresses. Finally, we review briefly novel non-destructive methods and tools that can be used to assess plant physiological status and fruit quality criteria in response to WD.

To avoid redundancy with several recent reviews on WD or stress (not specifically devoted to fruit), we do not detail the impact of drought on photosynthetic machinery, ROS synthesis and the damaging effects of ROS on metabolic pathways (Silva *et al.*, 2013), genes responding to water stress (Tardieu *et al.*, 2011) or the regulation of these genes (Atkinson and Urwin, 2012; Claeys and Inze, 2013; Sujata and Kshitija, 2013) or hormonal pathways (Albacete *et al.*, 2014; Perez-Alfocea *et al.*, 2011; Wilkinson and Davies, 2002). Similarly, we do not discuss the impact of WD on post-harvest quality (Nora *et al.*, 2012). Because the notion of physiological stress is a subject of debate, the term water deficit (WD) was used throughout this review.

II- The impact of water deficit on processes underlying fruit organoleptic quality

Important characteristics of fruit organoleptic quality include a fruit's external appearance, size, texture and taste. Fruit's taste and texture are primarily determined by the amount of dry matter and its sugar, acid, cellulose and protein composition in addition to the ratio between sugars and acids. Fruit size results from cell division and cell expansion, which are relative to carbohydrate and water fluxes and carbon metabolism within the fruit. All of these processes are regulated based on the fruit ontogenetic program and in response to environmental conditions (Génard *et al.*, 2007). Many studies have reported that in several species, WD typically results in depressed plant growth, enhanced fruit quality (e.g., increased sugar and acid levels) and an acceleration in fruit maturation but low marketable fruit yield (Guichard *et al.*, 2005; Ho, 1996b; Mirás-Avalos *et al.*, 2013). However, the reported effects of WD on fruit quality are highly variable and occasionally conflicting due to the large number of underlying processes that interact during fruit development and the timing and intensity of WD and because different species show different sensitivities. Therefore, an analysis of the effects of WD at the level of the processes involved should contribute to an understanding of global effects observed at the fruit level.

2.1 The effect of water deficit on fruit development and growth processes

Independent of the species, fruit growth may be divided into distinct developmental phases, including a period of intense cell division followed by a period of cell expansion and ending with the ripening period.

Cell division, which is typically restricted to a short period of fruit development and does not lead to a large increase in tissue volume, strongly influences the final fruit size in many species (e.g. in tomato (Bertin *et al.*, 2003; Bohner and Bangerth, 1988; Prudent *et al.*, 2010); pear *Pyrus* L. (Zhang *et al.*, 2006) and melon *Cucumis melo* L. (Higashi *et al.*, 1999)). Only a few studies have addressed the effects of WD on cell division in fruit tissues. In grape berries *Vitis vinifera* L., negative or no effects were reported depending on the timing of treatment (McCarthy *et al.*, 2002; Ojeda *et al.*, 2001). An absence of effects was also observed in olives *Olea europaea* L. (Gucci *et al.*, 2009) and pear fruit (Marsal *et al.*, 2000), with the exception of severe stress (Gucci *et al.*, 2009). Under intensive WD, the induced carbon starvation may negatively regulate cell division, as has been observed in tomato fruit at the tissue (Bertin, 2005; Prudent *et al.*, 2010) and at the gene (Baldet *et al.*, 2006) level.

When cell division ceases, an increase in tissue volume is induced due to cell growth via an increase in cytoplasmic volume and an expansion of the vacuoles. Cells are smaller in fruits grown under WD, as has been observed in olives (Gucci *et al*., 2009), pears (Marsal *et al.*, 2000), grapes (Ojeda *et al.*, 2001) and tomatoes (our experimental observations). Cell expansion is supported by the pressure of cell contents and constrained by cell wall properties (Cosgrove, 1997). The decrease in cell turgor and water potential resulting from cell-wall relaxation and loosening enables water to enter the cell and stimulate expansion (Lockhart, 1965). Water enters the fruit via xylem and phloem tissues and follows the stem-to-fruit gradient of water potential, which is generated by a gradient of osmotic potential between sources and sink tissues and that links cell expansion to sugar metabolism and subcellular compartmentalisation. Thus, WD may affect tissue expansion via its effects on the biophysical, metabolic and hormonal factors involved in the regulation of cell turgor and osmotic pressures and cell-wall extension. Although the fruit-water balance and osmotic regulation under WD are likely to have major impacts on tissue expansion, other hypotheses have been proposed. Mingo *et al.* (2003) demonstrated that fruit growth is affected by WD without changing fruit cellular turgor and proposed that cell expansion is regulated by sub-epidermal pH, which is consistent with previous results (Thompson, 2001). Modifications of the biochemical and physical properties of the cell wall under WD have also been suggested (Boyer, 1988). Several cell wall-loosening factors have been identified in plant cells, including numerous acid-induced or hormone-induce proteins (for example expansins hydrolases) and hydroxyl radicals (OH), the production of which can be catalysed by peroxidase (Schopfer, 2001). Other studies have supported the hypothesis that ROS participate in cell-wall softening, for example, during pear fruit maturation (Fry *et al.*, 2001). Thus, the potential effect of WD on cell-wall loosening and water balance is complex and involves many factors that may have opposing effects on the final cell size.

The onset of fruit ripening coincides with a rapid slowdown in cell expansion. Under WD, the onset of fruit ripening is hastened in peach *Prunus persica* L. (Mercier *et al.*, 2009), apple *Malus domestica* B. (El-Soda *et al.*, 2014) and detached avocado *Persea americana* M. (Adato and Gazit, 1974). These effects have been attributed to a stress-induced increase in endogenous ethylene, which plays an important role in the coordination of fruit-ripening processes in many fruit crop species, including climacteric (e.g., tomato, apple or banana *Musa x paradisiaca* L.) and non-climacteric (e.g., strawberry *Fragaria x ananassa* D., citrus

Citrus spp. L. or grape berries) fruits (Barry and Giovannoni, 2007). The biochemical features of the ethylene biosynthesis pathway under both normal and water-stress conditions and associated genes are well defined and have been reviewed previously (Apelbaum and Yang, 1981; Barry and Giovannoni, 2007; Fray *et al.*, 1994).

2.2 The effect of water deficit on fruit texture

The overall structure and spatial organisation, the cellular morphology of primary tissues (Aurand *et al.*, 2012), the cell turgor and fruit-water status (Jackman *et al.*, 1992; Shackel *et al.*, 1991), the accumulation and distribution of osmotically active solutes (Saladie *et al.*, 2007), the chemical and mechanical properties of cell walls (Rosales *et al.*, 2009; Toivonen and Brummell, 2008) and the cuticle properties and loss of water by transpiration (Saladie *et al.*, 2007) predominate in the determination of texture. Although many of these processes and traits are regulated by various environmental factors (Sams, 1999), of which water is of primary importance, very few studies have investigated the mechanisms involved in the environmental control of texture/firmness, and little information is available on the mechanisms affected (Harker *et al.*, 1997). Fruit texture is a primary determinant of consumer acceptance, and it greatly impacts organoleptic quality, flavour and aroma perception, shelf-life and transportability (Causse *et al.*, 2003; Seymour *et al.*, 2002). Several descriptive studies have reported significant but contrasting variations in fruit firmness (physical component of texture) under WD. For example, WD increases the firmness of pears (Lopez *et al.*, 2011); however, an absence of effect was reported for apricots *Prunus armeniaca* L. (compression test; Perez-Pastor *et al.*, 2007), kiwi fruit *Actinidia deliciosa* (puncture test; Miller *et al.*, 1998) and apples (puncture test, Hooijdonk *et al.*, 2007). A recent study on tomatoes revealed that a moderate WD protocol decreased firmness when measured using the compression test but increased firmness when measured using the puncture test, with strong interactions between WD and genotypes (Aurand pers. comm.). These conflicting results may arise from different methods of texture evaluation, different stress intensities, strong genotype interactions with the environment and complex interactions among the numerous mechanisms involved in final fruit texture.

The most likely hypotheses are that WD impacts texture via its effect on cell size, cell turgor, solute transport and the accumulation of osmotically active solutes at the cell level. Indeed, a positive link between dry matter or total soluble solids (TSS) and firmness was reported for the tomato (Aurand *et al.*, 2012; Saha *et al.*, 2009) and kiwi fruit (Nardozza *et al.*,

2011). In addition, drought stress has been shown to cause alterations in the chemical composition and physical properties of the cell wall (Peleman *et al.*, 1989). Similarly, in cherry tomato fruit, environmental stressors promote the solubilisation of cell wall pectin and reduce the concentration of calcium (Rosales *et al*., 2009), which is involved in the maintenance of cell-wall structure and fruit firmness (Poovaiah *et al.*, 1988). In addition, the pectin matrix has many critical functions in the development of plant organs, such as the determination of apoplast porosity (Baron-Epel *et al.*, 1988), ionic-exchange capacity (Gillet *et al.*, 1998) and cell adherence (Knee, 1978). The oxidative stress-induced accumulation of antioxidant compounds, which prevent oxidative damage, may also impact texture. For example, ascorbate has been shown in vitro to solubilise tomato pectins (Dumville and Fry, 2003), which may explain the positive correlation between fruit firmness and reduced ascorbate content observed in tomato in response to post-harvest chilling injury (Stevens *et al.*, 2008).

The effect of water supply during fruit growth on post-harvest texture is an additional important issue. Kiwi (Reid *et al.*, 1996), apple (Hooijdonk *et al.*, 2007) and pear (Lopez *et al.*, 2011) fruits grown under WD have been shown to have a better shelf life after harvest. In contrast, no effect of growth conditions on post-harvest firmness has been reported for apricot (Perez-Pastor *et al.*, 2007) or kiwi (Miller *et al.*, 1998) fruits. This phenomenon requires further investigation.

2.3 The effect of water deficit on fruit taste relative to sugar and acid content

Soluble sugars and organic acids (primarily malic and citric acids) are major osmotic compounds that accumulate in fleshy fruits. These compounds determine taste and represent more than half of the total dry matter in tomatoes. The metabolic pathways underlying acid and sugar syntheses and the links between enzymatic activities and product accumulation in fruits have been well documented (reviewed by Etienne *et al.* (2013) for acids and by Beckles *et al.* (2012) for sugars). Under WD, fruit sugar content increases in tomato fruit depending upon the cultivar and timing of stress (Bertin *et al.*, 2000; Veit-Köhler *et al.*, 1999). WD applied near ripening shows the greatest positive impact on soluble sugar accumulation in various fleshy fruits (i.e., sucrose, glucose and fructose in Satsuma Mandarin fruit (Yakushiji *et al.*, 1996); glucose and fructose in grape berries (Castellarin *et al.*, 2007); and glucose and fructose in tomato fruit (Ho, 1996a)). In contrast, the effects of WD on fruit acidity are more conflicting. In many species (peach, clementine, mandarin, pear, tomato), water supply has been shown to correlate negatively with organic acid content in ripe fruits, but in grapes,

nectarines (Etienne *et al.*, 2013) and tomatoes (Bertin *et al.*, 2000; Mitchell *et al.*, 1991; Veit-Köhler *et al.*, 1999), this correlation has been shown to be positive. The variations in soluble sugar and acid accumulation in response to WD, often reported on the basis of fresh weight, may result either from dilution/dehydration effects (Etienne *et al.*, 2013; Guichard *et al.*, 2001), from active solute accumulation (Lo Bianco *et al.*, 2000; Hummel *et al.*, 2010), or from starch breakdown, as observed on tomatoes under salinity-induced WD (Balibrea *et al.*, 2003). For example, in strawberries, water stress increases sugar content and does not affect acid content relative to fresh weight; however, it does not affect sugar content and reduces acid content relative to dry weight (Terry *et al.*, 2007). In addition, water stress-induced carbon starvation is thought to decrease fruit sugar content, as has been observed in grape berries, peaches, tomatoes, mangoes *Mangifera* L. and clementines (Poiroux-Gonord *et al.*, 2012; Poiroux-Gonord *et al.*, 2013a), whereas organic acids typically show the opposite trend (Poiroux-Gonord *et al.*, 2012).

In the tomato, sucrose-metabolising enzymes (acid invertases and sucrose synthase, SUSY) and the starch-synthesising enzyme (ADP-glucose pyrophosphorylase, AGPase) play important roles in sugar import and metabolism (Beckles *et al.*, 2012). Invertases, whether alone or combined with plant hormones, have been recognised as key metabolic enzymes involved in plant responses to environmental stimuli due to their role in sugar signalling and sensing (Roitsch and Gonzalez, 2004; Ruan *et al.*, 2010). For instance, the reduction in apoplastic invertase activity has been suggested as an early step in the signal transduction cascade induced by water stress that leads to irreversible fruit abortion (Zanor et al., 2009). However, the effects of WD on sugar-metabolising enzymes in sink organs remain poorly documented. An increase in SUSY activity has been observed in water-stressed fruits in orange *Citrus sinensis* L. (Hockema and Etxeberria, 2001). In peach fruit, water stress-induced ABA stimulates sugar accumulation by increasing the activity of sorbitol oxidase; however, this effect was observed under moderate water stress but not severe water stress (Kobashi *et al.*, 2001). A recent study of transformed tomato lines (Centeno *et al.*, 2011) revealed a negative link between malate accumulation and levels of transitory starch and final soluble sugar content and suggested the regulation of AGPase activity by the cellular redox status in developing fruit. Although links between enzyme activities and sugar accumulation have been studied extensively (Steinhauser *et al.*, 2011), the relationships involving hormones, sugar metabolising enzymes and sugar accumulation in fleshy fruits under water-stress conditions merits much more consideration in future research.

For many fleshy fruits, consumer acceptance not only correlates with individual concentrations of sugars and acids but also with the sugar/acid ratio. Based on the above-described effects of water stress on sugar and acid content, it is difficult to anticipate the effect of water stress on this ratio. In strawberries, the ratio increases under WD due to an increase in sugar content (Terry *et al.*, 2007). In tomato fruit, the sugar/acid ratio was shown to increase under high air-vapour pressure deficit, but the effect was dependent on the plant fruit load and harvest period (Bertin *et al.*, 2000). The sugar/acid ratio increases from spring to summer; it correlates better with acid content in spring but correlates equally with both components (soluble sugars and organic acids) in summer. These variations are likely influenced by water deficit-induced carbon starvation. In clementine fruit, the ratio is lowered by carbon starvation due to the decrease in sugar accumulation and increase in acid accumulation (Poiroux-Gonord *et al.*, 2013b).

2.4 The effect of water deficit on fruit aromas

All fleshy fruits contain and release a great variety of volatiles that confer their typical aroma. For example, in tomatoes, over 400 volatile compounds have been identified (Buttery *et al.*, 1987), many of which affect consumer taste perception together with sugars and acids (Baldwin *et al.*, 1998). Although fruit aroma represents a fundamental criterion of the organoleptic quality, few studies on its dependence on irrigation strategies were available, except in grapes. Several independent studies on grapevines have shown that water limitation increases aromatic compound content, in particular carotenoid breakdown volatiles (so-called norisoprenoids), which confer berries and wine a more fruity character (Bindon *et al.*, 2007; Chapman *et al.*, 2005; Koundouras *et al.*, 2009; Song *et al.*, 2012). Consistent with these findings, Deluc *et al.* (2009) observed an increased abundance of a carotenoid cleavage enzyme transcript in grapes grown under WD. The increase in norisoprenoids in fruits may be associated with metabolic responses to excess light energy and the build-up of oxidative stress under drought (Deluc *et al.*, 2009; see also Section 2). Other irrigation experiments in vineyards reported that aroma and flavour improved with irrigation (Reynolds *et al.*, 2007) or were not affected (Bravdo, 2001). In addition to grapes, some studies on apples reported significant positive effects of WD on the concentration of aroma compounds (Behboudian *et al.*, 1998; Hooijdonk *et al.*, 2007; Mpelasoka and Behboudian, 2002), although one study reported no effect (Mpelasoka *et al.*, 2000). Two studies on tomatoes and strawberries

reported significant aroma enrichment in response to WD (Modise *et al.*, 2006; Veit-Köhler *et al.*, 1999).

In summary, across all fruit types, the majority of studies have reported beneficial effects of reduced water availability on fruit aroma, as has been generally acknowledged with respect to the aroma content of spices and medicinal plants (Nowak *et al.*, 2010). However, in many studies, the gain in aroma content was accompanied by a loss in fruit size (e.g., Song *et al.*, 2012). Therefore, it remains unclear whether the reported enhancement in aroma was due to the true stimulation of aroma biosynthesis or, rather, due to a dilution/concentration effect based on changes in fruit size and water content, as has been discussed with respect to soluble sugars and organic acid contents (Koricheva, 1999). Further, the effects of water limitation on fruit aroma may be indirect, in part due to changes in the fruit microclimate (reduction in canopy density) or the nutrient status of the entire plant. Indeed, fertilisation, shading and pruning practices have been shown to affect fruit aromas (Pelayo-Zaldívar, 2010). Many more studies are required to unravel the multiple mechanisms underlying the effects of WD on fruit aromas. Particular attention should be paid to the timing and intensity of the applied water stress because the majority of aroma compounds and/or their precursors accumulate primarily during fruit growth and subsequently change during ripening and/or senescence. Future studies should also include quantitative measurements of aroma compounds in the headspace of intact fruits and not only their concentrations in fruit tissues. Indeed, the profiles of aromas released by intact fruits may differ greatly from their aroma content because not all volatiles produced and released by fruits accumulate in fruit tissues at detectable amounts, a phenomenon that is well known for many volatiles produced by vegetative plant organs (Staudt and Bertin, 1998).

III- The effect of water deficit on health-promoting phytochemicals

Fruits supply a large range of health-promoting phytochemicals, of which secondary metabolites, primarily terpenoids (carotenoids, ABA and others) and phenolic compounds, are the largest group along with ascorbate. Very little is known, for example, about the effect of ABA, the single most important plant hormone associated with drought, on fruit development and physiology. An increasing body of evidence suggests that ABA produces powerful biological effects. ABA is effective against a large range of diseases, including type II diabetes, obesity and hypertension associated with atherosclerosis (Guri *et al.*, 2007; Guri *et al.*, 2010). Although the nutritional benefits of fruits and vegetables have been long established, consumption remains insufficient. Fortunately, there is great potential to increase the concentrations of phytochemicals in plant products using genetic approaches either by conventional breeding or breeding assisted by markers or metabolic engineering in addition to using agronomic approaches (Poiroux-Gonord *et al.*, 2010). Of all of the environmental factors that play a stimulating role in the synthesis and accumulation of useful phytochemicals in fruits, moderate stress, and more specifically, controlled drought, appear promising (Poiroux-Gonord *et al.*, 2013b). We now review briefly current knowledge on the effects of WD on the concentrations of the primary health-promoting phytochemicals in fruits and related physiological mechanisms involved in their synthesis and accumulation.

3.1 Drought can increase phenolic compounds, carotenoids and vitamin C content in fruits

Many observations of the effects of WD on the accumulation of phytochemicals in berry fruits have been made since the 1990s (Lovisolo *et al.*, 2010). With respect to phenolic compound content, the response to drought generally appears positive (Anttonen *et al.*, 2006; Deluc *et al.*, 2009; Esteban *et al.*, 2001; Keutgen and Pawelzik, 2007; Krauss *et al.*, 2006; Navarro *et al.*, 2006) and peaks at +40%. The picture is slightly different for carotenoids, for which the effects range from negative (De Pascale *et al.*, 2007; Riggi *et al.*, 2008) to non-significant (Krumbein *et al.*, 2006) to positive (De Pascale *et al.*, 2001; Favati *et al.*, 2009; Krauss *et al.*, 2006; Marin *et al.*, 2009; Navarro *et al.*, 2006; Wu *et al.*, 2004; Zushi and Matsuzoe, 1998) and in the last case can reach greater than +150%. With respect to vitamin C, the findings are quite similar, with many reports showing positive effects of WD (Favati *et al.*, 2009; Murshed *et al.*, 2013; Veit-Köhler *et al.*, 1999; Zushi and Matsuzoe, 1998). It is important to stress that these studies show variable effects depending on genetic and seasonal

factors or the intensity and duration of treatment. Drought may influence the metabolism of these phytochemicals via at least two major mechanisms that are not mutually exclusive and that may even interact (Fanciullino *et al.*, 2013). First, drought typically induces a decrease in leaf stomatal conductance, resulting in a decrease in net photosynthesis. The decrease in net photosynthesis may result in a reduced transport of primary metabolites to the fruits that are the major source of precursors for the biosynthesis of phenolic compounds, carotenoids and ascorbate. Second, drought may exacerbate oxidative stress/oxidative signalling. Oxidative stress is known to directly and indirectly influence the biosynthetic pathways of these compounds. Both mechanisms appear closely linked because the accumulation of carbohydrates may exacerbate photo-oxidative stress in photosynthetic organs, such as leaves (Urban and Alphonsout, 2007), whereas the latter mechanism may influence primary metabolism in nearby fruits (Poiroux-Gonord *et al.*, 2013b). Moreover, WD may influence the metabolism of health-promoting phytochemicals by hastening fruit development.

3.2 Are drought-induced variations in phytochemicals due to a reduction in the availability of primary metabolites?

It has often been reported that the availability and long-distance transport of primary metabolites determines the biogenesis of phytochemicals in fruits, such as ascorbate (Wheeler *et al.*, 1998) or carotenoids (Cunningham, 2002). Consistent with this idea, sucrose limitation was observed to delay and reduce lycopene and phytoene accumulation in green tomato fruit pericarp disks (Telef *et al.*, 2006), whereas sucrose feeding was shown to promote colour break in citrus fruit (Telef *et al.*, 2006). However, in both cases, the reported effects are ethylene dependent, which suggests that the positive effects of sucrose on carotenoid synthesis before maturity are likely indirect, i.e., mediated by the maturation process itself. Other observations on clementines suggest that during the cell division phase, low carbohydrate supply to fruits does not inhibit but, rather, stimulates the accumulation of carotenoids, possibly due to an indirect effect on plastid formation (Poiroux-Gonord *et al.*, 2012; Poiroux-Gonord *et al.*, 2013a). In tomato fruit, the absence of a correlation between sugars and reduced ascorbic acid (AsA) content also suggests that fruit AsA content is not limited by leaf photosynthesis or sugar availability (Gautier *et al.*, 2009). All of these observations appear to refute the idea that carbohydrate availability determines the synthesis and accumulation of secondary metabolites or vitamin C, at least in fruits. Historically, the issue of the effect of carbon supply on secondary metabolites was addressed by ecologists who predicted how plants allocated resources between differentiation-related processes

(including the production of secondary metabolites involved in defence) and growth-related processes, giving rise to the Growth Differentiation Balance Hypothesis (GDBH) (Herms and Mattson, 1992; Loomis, 1932; Wilkens *et al.*, 1996). However, the GDBH is unlikely to apply to storage organs such as fruits. For example, in tomatoes grown under nitrogen deficit conditions, phenolic compounds accumulate in leaves, as predicted by the GDBH (Le Bot *et al.*, 2009), but not in fruits (Benard *et al.*, 2009). Carotenoids accumulate in fruits to advertise the nutritional status of fruits to potential seed disseminators rather than to fulfil defence functions. More recently, sugar signalling (Gibson, 2005; Rolland *et al.*, 2002) has modified our simplistic view of the effect of carbohydrate availability on secondary metabolism. The emerging mechanistic view is instead one of a modulating role of carbohydrates with regard to the biogenesis of secondary metabolites (Cazzonelli and Pogson, 2010; Fraser *et al.*, 2007; Lillo *et al.*, 2008; Telef *et al.*, 2006).

3.3 Stimulation of the synthesis of health-promoting phytochemicals by drought-induced oxidative stress

There are ample grounds to consider that the cellular redox state, resulting from the stress-induced production of ROS, ROS regulatory processes, and the accumulation of reducing power, controls tightly the synthesis of carotenoids in leaves and fruits (Fanciullino *et al.*, 2013). Similarly, the entire biosynthetic pathway of phenolic compounds is under ROS/redox control (Lillo *et al.*, 2008; Wingate *et al.*, 1988). The idea that the biosynthetic pathways of carotenoids and phenolic compounds are under ROS/redox control is consistent with knowledge of the gene-controlling role of redox-sensitive systems (Buchanan *et al.*, 2012; Potters *et al.*, 2010). However, the specific molecular mechanisms involved are not well understood, and the genes expressed in various fleshy fruits during drought, for example, in grapes (Castellarin *et al.*, 2007), require further investigation. In addition, recent results suggest that ROS formed during WD, osmotic stress and salt stress may indirectly orchestrate phenylpropanoid and flavonoid biosynthetic pathways via the initiation of phosphorylation cascades by H_2O_2 and MAPK that are highly activated during WD (Grassmann *et al.*, 2002; Mateos *et al.*, 2003).

AsA also plays a major role in the antioxidant scavenging of H_2O_2 via ascorbate peroxidase (APX) or AsA itself. In fruits, AsA content depends upon its biosynthesis, its recycling after APX- or ascorbate oxidase (AO)-mediated oxidation into monodehydroascorbate (MDHA), its eventual travels from the leaf to the fruit and its

catabolism. There is evidence that drought tolerance correlates with plant AsA content and/or its regeneration by monodehydroascorbate reductase (MDHAR), which plays a fundamental role in ROS detoxification (Wang *et al.*, 2012). This role of ROS detoxification is supported by the behaviour of AsA mutants under drought stress. For example, ascorbate-deficient mutants of *Arabidopsis* are more sensitive to WD (Niu *et al.*, 2013). Moreover, tomato plants with reduced expression of AO show 30% greater AsA content than control plants and show improved tolerance to WD resulting in higher stomatal conductance and photosynthesis rate (Garchery *et al.*, 2013; Zhang *et al.*, 2011).

3.4 Photo-oxidative stress in leaves impacts secondary metabolism and antioxidant metabolism in fruits

The role of ROS and redox status in the synthesis of secondary metabolites in the pulp of fruits raises an intriguing question. At the time of maturation, when chloroplasts have been transformed in chromoplasts, ROS cannot originate from photosynthesis because the pulp has lost its photosynthetic machinery. In the pulp of stressed fruits, ROS may originate from NADPH oxidase located in the membranes, from xanthine oxidase in peroxisomes (Luis *et al.*, 2006; Mateos *et al.*, 2003) or from the respiratory electron transport chain of mitochondria. Recently, Poiroux-Gonord *et al.* (2013b) provided evidence that leaves of orange trees undergoing photo-oxidative stress can influence metabolism in the pulp of nearby fruits. Among other findings, they observed modifications of antioxidant metabolism and a 15% increase in the concentration of total carotenoids 99 hours after exposure of the leaves to stressful conditions. The idea that stressed leaves may be the source of oxidative stress or redox signalling in fruit or that ROS may even be exported from stressed leaves to nearby fruits is appealing and deserves attention. This idea is consistent with the concept of systemic acquired resistance (SAR) in cases of biotic stress (Ryals *et al.*, 1996) and systemic acquired acclimation (SAA) in cases of abiotic stress (Karpinski *et al.*, 1999). In *Arabidopsis thaliana* L., it has been observed that in response to wounding, an ROS auto-propagating signal may be carried over a long distance at a rate of up to 8.4 cm min^{-1} (Miller *et al.*, 2009; Mittler *et al.*, 2011). Eventually, even secondary metabolites, such as phenolic compounds accumulated in leaves in response to stress, may move directly to fruit. Indeed, highly soluble phenolic acids and hydroxycinnamic acids have been found in phloem and xylem saps (Bidel *et al.*, 2010). When applied on *Arabidopsis* roots, flavanones (i.e., naringenin, hydroxykaempferol and hydroxyquercetin) move from root to shoot using the symplastic pathway, whereas shoot-to-root transfer appears to be limited to the vascular tissues and

depends upon ABC-C-type carriers (Buer *et al.*, 2007). The importation of flavanol derivatives to fruits may occur when they are highly abundant, as in the tomato (e.g., naringenin-chalcone) or in *Citrus* species (e.g., naringenin-glycosides). No data exist that assesses the participation of these derivatives in flavonoid accumulation in fruits or the role of water stress in long-distance transport. Here, we detail the role of leaf-to-fruit signalling; however, it is quite clear that skin-to-pulp signalling may also exist at least as long as the photosynthetic machinery is effective.

Fig. 1 resumes the effects of water deficit on quality criteria of fleshy fruits associated with photooxidative stress and reduced carbon gain.

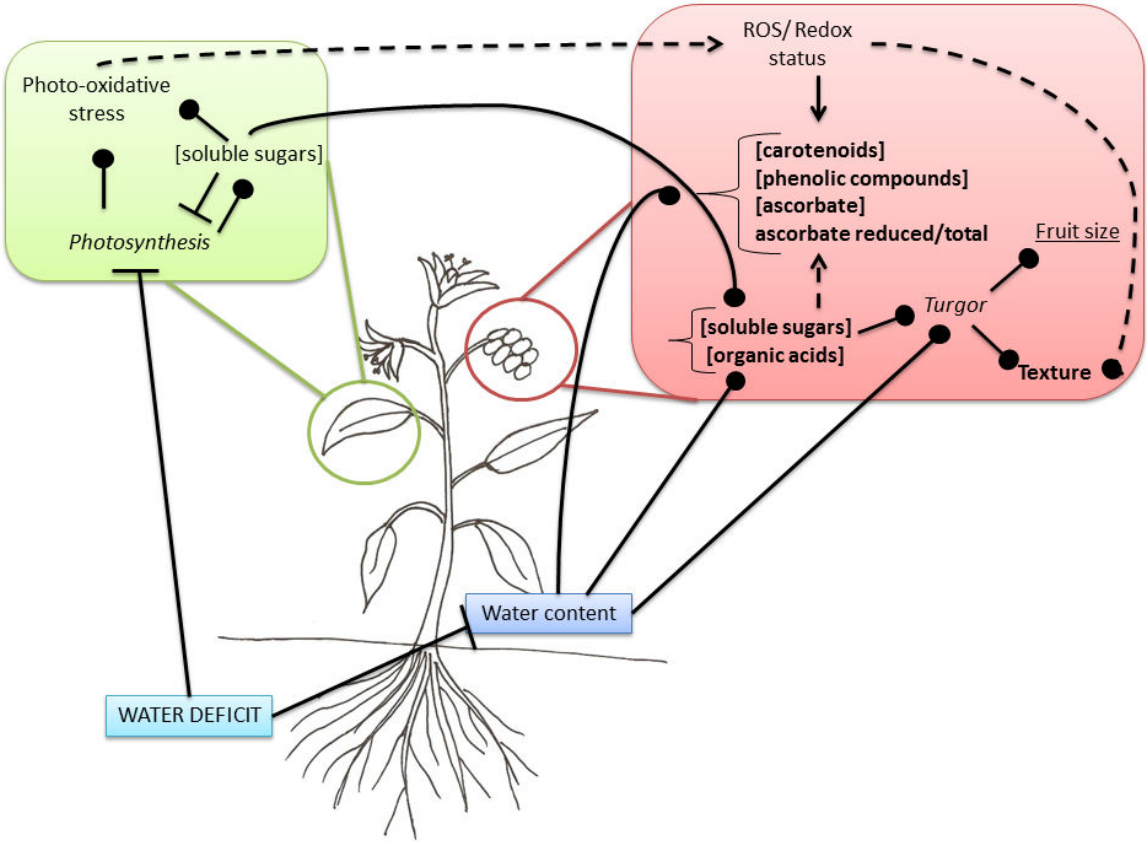

Figure 1: A simplified model of the prevailing effects of water deficit (WD) on quality criteria of fleshy fruits, derived from the available literature on peach, tomato and orange fruits.

This model brings forward the way WD influences quality criteria in fruits either directly or indirectly through its effects on leaves. Up- and down-regulations are indicated by arrow endings (circles and bars, respectively), except when no general trend predominates (arrow endings), depending on species, genotypes, plant and fruit stage, and intensity of WD. WD induces a decrease in leaf water status, and also in leaf stomatal conductance and net photosynthesis. The water deficit-associated decrease in photosynthesis increases the risk of photooxidative stress in leaves while decreasing the amount of carbohydrates for export to fruits. The decrease in water supply to fruits is at the origin of a decrease in fruit size but may also increase levels in carbohydrates, secondary metabolites and ascorbate, by exerting a concentration effect or by stimulating the synthesis of these compounds. But then the water deficit-associated decrease in carbohydrate supply to fruits may have the opposite effect by decreasing the amount of carbohydrates. At the fruit level, the effect of carbohydrate availability on the concentrations of secondary metabolites and ascorbate, being a matter of debate, is represented using a broken line. We also used a broken line to represent the potential effect of the water deficit-associated increase in photooxidative stress in leaves on the redox status and the concentrations in secondary metabolites of fruits (Poiroux-Gonord *et al.*, 2013). Eventually we represented the effects of the redox status and of turgor (as influenced by water supply and the concentrations in carbohydrates) on texture (Pilati *et al.*, 2007).

IV- How do different types of water deficit affect fruit quality?

The great number of studies on WD highlight highly variable responses of plants and fruits depending on the length and intensity of the deficit, the plant/fruit development stage affected by the WD protocol, the genotype and the presence or absence of other stress factors (high temperature and light, salinity, pathogens) (Bray, 1997; Tardieu *et al.*, 2006). In this section, we review the variable effects of a few of these primary factors on fruit quality. When available in the original studies, indicators of stress intensity are mentioned.

4.1 The effect of water deficit applied at different developmental stages and intensities

Contrasting effects of WD have been observed depending on the plant developmental stage. In germinated tomato seeds, WD applied by the addition of polyethylene glycol was mostly lethal, whereas the rare surviving seeds produced either resistant or very sensitive plants (Kulkarni and Deshpande, 2007). These responses to PEG are dependent on the concentration and duration of the treatment and might be mediated by epigenetic changes (Parra *et al.*, 2007). The vegetative growth stage is highly sensitive to WD, and a major consequence of WD during this stage is the reduction of plant growth and the subsequent fruit yield due to numerous fruit abortions (Gladden *et al.*, 2012). In grapes, WD applied during the vegetative stage (no irrigation until veraison) reduces the maximum rate of shoot elongation and node production, accelerates periderm development, decreases fruit growth (Matthews *et al.*, 1987) and increases total phenolic content in contrast to malate (Matthews and Anderson, 1988). Thus, early WD applied during the vegetative phase may affect the entire reproductive period and negatively impact yield and improve fruit quality. In tomatoes, WD applied from flowering to fruit set primarily impacts the number of reproductive organs and potentially leads to an increase in fruit size and quality by increasing the availability of assimilates for the remaining fruits (-50% of daily evapotranspiration ETc, Patanè and Cosentino, 2010; -66% of control, Wang *et al.*, 2011). However, intensive stress (-80% of control irrigation) may lead to abnormal ovule development (Rapoport *et al.*, 2012).

At the level of the fruit, the effects of WD also depend upon the stage of fruit development during treatment. In peaches, moderate WD applied during cell division (stage I) promotes fruit size but does not impact fruit water content (Li *et al.*, 1989). When applied

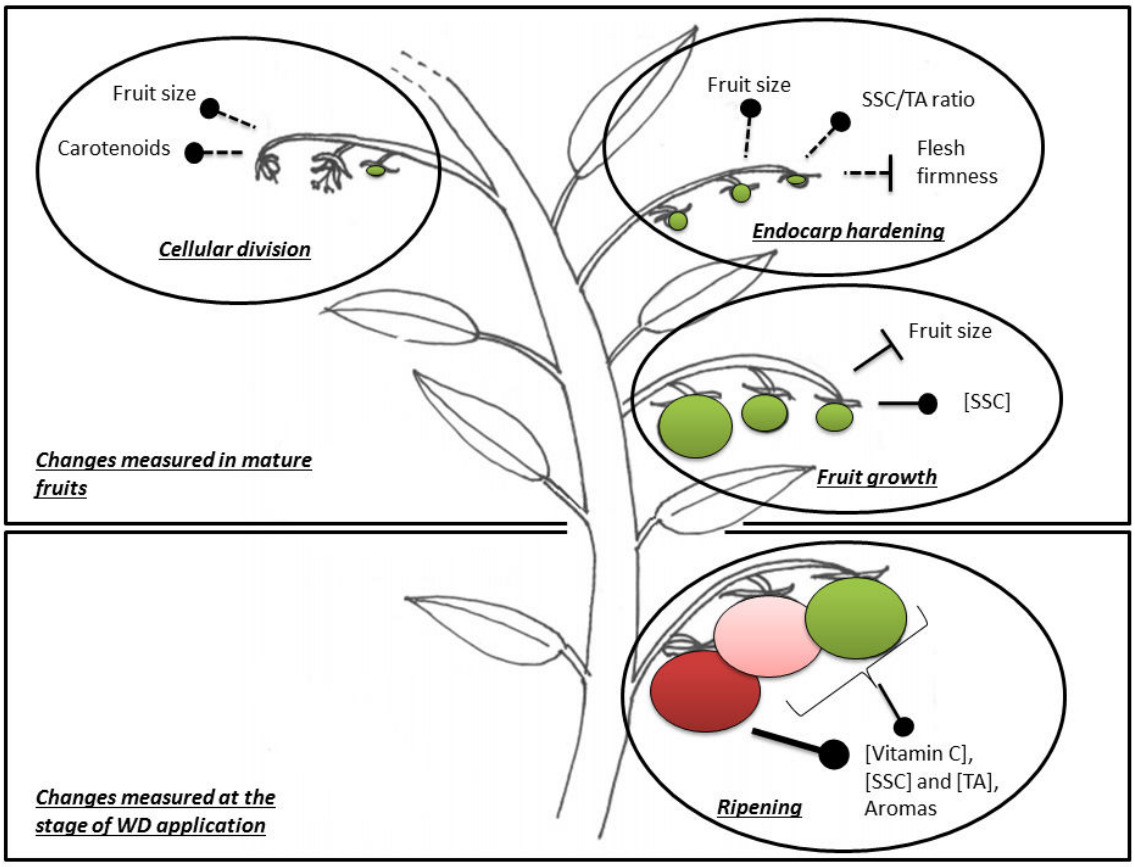

Figure 2: A simplified model of the effects of water deficit (WD) applied during specific fruit developmental stage on fruit size and criteria of quality according to the literature on peach, tomato and Citrus.

This model summarizes the effects of WD applied during specific fruit developmental stages on fruit size, soluble solids concentration (SSC), titratable acidity (TA), firmness, aromas and the concentration in phytonutrients. A stimulating effect is expressed by lines ending with circles; lines ending with bars express an inhibitory effect, whereas dotted arrows express hypothetical or year-dependent effects of WD. At the stage of cellular division, the effect of WD is believed to be an increase in fruit size as observed in peach (Li *et al.*, 1989) and tomato (Nuruddin *et al.*, 2003) although the behaviour of tomatoes seems to depend on year-to-year variations. Observations on Citrus fruits by Fanciullino *et al.* (2014) led us to represent the hypothetical positive effect of WD during cellular division on carotenoids. WD applied during rapid endocarp hardening can increase the SSC/TA ratio but the effects appear contrasted on flesh firmness and fruit size as a consequence of year-to-year variations (Vallverdu *et al.*, 2012). During fruit growth, WD increases soluble sugars content and reduces fruit size (Girona *et al.*, 2004; Li *et al.*, 1989). At the ripening stage, the increase in vitamin C, soluble sugars and organic acids is more marked when fruits are red than when they are at the turning point. Finally, aromas are improved by WD during ripening as indicated by increased emissions of hexanal, (Z)-3-hexenal, (E)-2-hexenal and benzaldehyde (Veit-Köhler *et al.*, 1999).

during the phase of rapid endocarp hardening (stage II), WD (-1.8 MPa stem water potential compared with control with -1.0 MPa at maximum) improves sweetness and flavour intensity and increases consumer acceptance, with contrasting effects on yield depending on the year (Vallverdu *et al.*, 2012). WD applied during the main fruit-growth stage (Stage III) results in a negative impact on yield due to carbon limitation (Girona *et al.*, 2004) but is associated with a marked decrease in fruit water content (Li *et al.*, 1989). Finally, in peaches, the ripening stage is the most sensitive stage to WD, and the stress-induced reduction in yield is proportional to the reduction in water supply up to 25% of ETc (Wang and Gartung, 2010). Similarly, in tomatoes, WD applied during ripening (50% of water capacity) shows the greatest increase on fruit quality (soluble sugars, organics acids, aromas and vitamin C) when applied during the red stage compared with the mature green or orange stages (Veit-Köhler *et al.*, 1999).

WD has also been applied during the entire growing season. This stress leads to a significant decrease in fruit fresh weight and yields in tomatoes (under 65% and 80% reductions of water supply) (Nuruddin *et al.*, 2003) and mangoes (33% to 75% ETc) and promotes TSS accumulation in mangoes. The response intensities correlate with the intensity of WD (Durán Zuazo *et al.*, 2011).

In addition to the stage of application, the intensity of WD is an important determinant of the plant and fruit responses. To our knowledge, the effects of different stress intensities during targeted periods of fruit development have rarely been investigated. In oranges, different combinations of two stress intensities (55% and 70% of control) were applied during two phases (flowering, fruit growth or ripening), and the results indicated that the improvement in fruit quality is counterbalanced by the decrease in yield when at least one development phase is exposed to intensive stress (García-Tejero *et al.*, 2010).

On their whole, these results suggest that compromise between yield and quality could be achieved if WD occurs at the right intensity and at the right period of plant and fruit development. However, the intensity that optimises both traits and enables the developmental stages to be preserved from stress remains unclear and is likely species and season dependent. Finally, the impact of WD on fruit yield and quality is not as simple as it first appears. Figure 2 summarises the potential impact of WD on fruit yield and quality depending on fruit development phase, excluding severe stress, which is clearly deleterious.

4.2 Water deficit and plant priming

The capacity of plants to enhance their tolerance to future biotic or abiotic stresses upon appropriate stimulation by a prior exposure to stress is called "primed acclimation" or "priming" (see the reviews by: Bruce *et al.*, 2007; Conrath, 2011; Filippou *et al.*, 2013). Therefore, plant acclimation to water stress may lower the sensitivity to more severe drought phases and to other biotic or abiotic stressors often sharing common response pathways. Thus primed plants are expected to minimize yield losses through adaptation, but also to increase fruit quality through the up-regulation of the synthesis of some health compounds. Alternating cycles of stress have been applied to the entire root system or to part of the root system, i.e., partial root drying, to stimulate plant adaptation to WD and improve fruit quality (Stikic *et al.*, 2003). For example, two short periods (10 days) of WD (40% to 50% soil humidity) applied to tomato plants were observed to increase the carotenoid content in fruits harvested after the recovery period due to an increase in antioxidant enzyme activity during the stress period (Stoeva *et al.*, 2010 ; 2012). Similar results were observed in cucumber (Akinci and Losel, 2009).

In addition to the cascade of responses to priming that cause morphological, physiological and biochemical changes that make the plant more tolerant to subsequent stress, it has been shown that plants have a “memory” of encountered stress conditions that enables them to enhance their adaptation to changing environments. Indeed, primed plants demonstrate a faster activation of defence responses following stress perception. In recent years, the molecular mechanisms involved in priming have been investigated in the field of plant pathology, and the existence of a stress imprint in the primed plant that conserves information from a previous stress to be applied when the next stress occurs has been suggested by Bruce *et al.* (2007). Many mechanisms of response to stress are shared among several abiotic or biotic stresses (so-called cross-tolerance) and could have an analogous effect on the induction of priming. ABA, which is central in regulating the plant response to abiotic stress, appears to be involved in plant priming in addition to jasmonic and salicylic acids (Li and Zhang, 2012). Priming induces the accumulation of signalling proteins and transcription factors (TFs) in their inactive forms and enables their rapid upregulation after a subsequent exposure to secondary stress. Thereby priming is expected to mitigate the damages and yield reduction associated to stress. The accumulation of dormant MAP kinases (MPK3 and MPK6), which are implicated in signal transduction, also appears to be necessary for the stress imprint (Beckers *et al.*, 2009); moreover, both MPKs are linked to the activation of phenylalanine

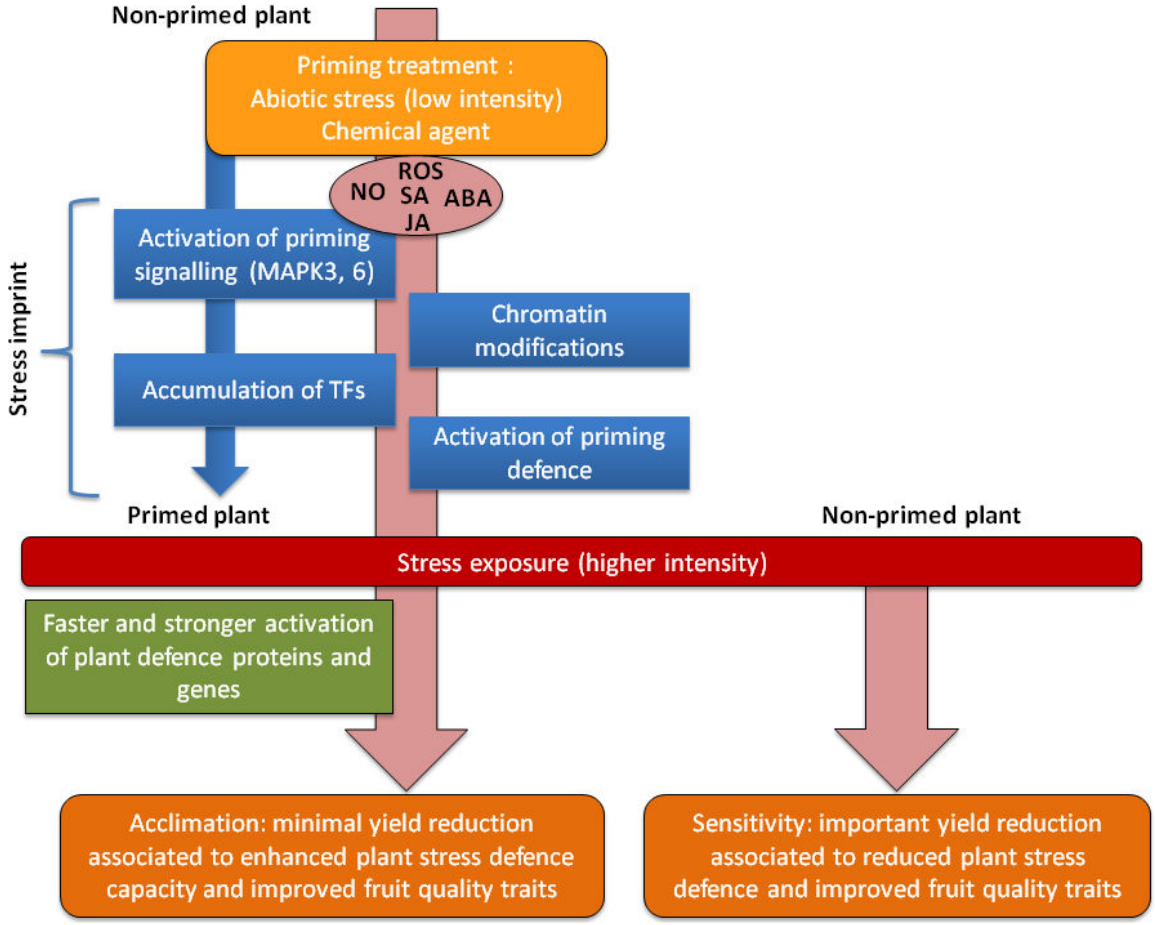

Figure 3: Plant priming mechanisms for acclimation to abiotic stress and improvement of fruit quality.

Abiotic stress or chemical agents applied to plants can induce ‘‘priming’’ to prepare plants to better cope with subsequent and more severe stressing conditions. The priming treatment induces early accumulation of ROS and nitric oxide (NO) that interact with hormonal responses and leads to the “primed state” of plant cells. The resultant “stress imprint” is characterized by epigenetic alterations such as chromatin modifications (histone modifications and DNA methylation), as well as accumulation of transcription factors (TFs) and inactive forms of mitogen-activated protein kinases (MAPKs). Therefore, primed plants are able to respond with increased or faster induction of defence responses upon exposure to subsequent stress. Finally, priming treatment causes a reduction of stress symptoms through enhanced tolerance while non-primed plants exhibit severe damages. Thereby, primed plants are expected to minimize the impact on yield, and at the same time to trigger antioxidant mechanisms that positively affect fruit quality.

lyase, which is involved in polyphenol synthesis, thus involving plant defence and fruit quality. It has been also suggested that nitric oxide (NO) induced in response to priming stress could involve S-nitrosylation and denitrosylation of proteins (Floryszak-Wieczorek *et al.*, 2012). By using a proteomic approach, Arasimowicz-Jelonek *et al.* (2013) demonstrated that the priming induced by chemical agents (β-amino-butyric acid, GABA, INA and Lamarin) involved NO and redox-regulated enzymes implicated in primary metabolism and oxidative responses, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), carbonic anhydrase (CA) and ascorbate recycling (with the dehydroascorbate reductase DHAR). GAPDH is a key enzyme involved in oxidative and NO signalling pathways that acts as an NO sensor (Kornberg *et al.*, 2010; Morigasaki *et al.*, 2008; Muñoz-Bertomeu *et al.*, 2010), whereas CA exhibits antioxidant activity and SA response (Slaymaker *et al.*, 2002). Positive side-effects on the accumulation of health-promoting compounds are expected, as observed by Stoeva *et al.*, (2010; 2012).

Often, the tolerance due to priming can persist for several days, but the stress imprint is in some cases transmitted to the next generation, suggesting that plants may inherit acclimation capacity (Molinier *et al.*, 2006; Slaughter *et al.*, 2012). Thus, plant priming represents an adaptive and cost-efficient defence strategy that increases the plant's ability to survive in hostile environments, resumed in Fig. 3. Priming may also be applied to improve fruit quality and fruit tolerance to stresses encountered during harvest and post-harvest treatments (Capanoglu, 2010). However, as outlined in Section 3.1, it will be crucial to determine when and how much stress should be applied to maintain and even improve quality.

V- Factors affecting the fruit response to water deficit

In the field, WD may induce very different effects depending on the genotype and the interactions with other biotic or abiotic stresses. Commercial genotypes have primarily been selected based on plant productivity, fruit size, shelf life and resistance to specific pathogens. Thus, cultivated genotypes became less tolerant to multiple stress environments, in particular to abiotic stress factors. Indeed, breeding has favoured yield and economic criteria, and natural allelic variations of genes favouring adaptation to environmental stresses have been lost (Gorovits and Czosnek, 2007; Gur and Zamir, 2004; Tanksley and McCouch, 1997).

5.1 Interactions between water deficit and genetic factors

Understanding the genetic variability and the genotype x environment interactions involved in fruit quality and plant tolerance to WD is crucial (Panthee *et al.*, 2012). A great deal of research has been conducted to identify genes that are sensitive to WD. The majority of the molecular mechanisms (involved in plant growth and ABA signalling) have been elucidated, in particular in *Arabidopsis* (Blum, 2011). However, their use and transfer to cultivated genotypes is far from simple. In tomato, only a small proportion of genes involved in adaptation to water stress is known (Fischer *et al.*, 2011; Labate *et al.*, 2007). Genetic sources of variability to abiotic stress adaptation in tomato primarily include a small number of wild species, which made their use for breeding long and intricate (Foolad and Lin, 1999; Labate *et al.*, 2007). Moreover, water stress-responsive genes are involved in a myriad of physiological processes (osmotic regulation, photosynthesis, hormone synthesis, antioxidant activities, etc.), but they do not necessarily confer plant resistance (Gong *et al.*, 2010) or yield stability. Genes and quantitative trait loci (QTLs) for plant stress adaptation should be identified by comparing genotypes with contrasting behaviour under stress conditions (Labate *et al.*, 2007). For example, a transcript study of metabolic pathways affected by WD carried out on two cultivars of grape berries revealed 6,000 unigenes which vary with the cultivar and WD treatment and play a role in the phynelpropanoid, the ABA, the isoprenoids, the carotenoids, the amino acids and the fatty acids pathways (Deluc *et al.*, 2009). Furthermore, Genome-Wide Association Mapping could permit to explore the genome variability and to discover new QTLs and candidate genes (Ranc *et al.*, 2012). Moreover, using QTLs for stress adaptation based on physiological underlying processes instead of complex traits, such as yield or quality, would be more efficient for understanding the genetic control and interactions with the environment (Bertin *et al.*, 2010; Martre *et al.*, 2011). Because WD is

expected to enhance fruit quality (see Sections 1 and 2), interesting QTLs that enable adaption to WD should improve plant development, carbon acquisition and allocation to fruits, thereby maintaining yield under WD. In practice, several genotypes have been identified as tolerant to drought, either in terms of metabolic content and plant survival (for example in cucumber *Cucumis sativus* L. (Akinci and Losel, 2009) and in tomato (Sanchez-Rodriguez *et al.,* 2010; 2011), or in terms of yield stability (Foolad, 2007). Such cultivars represent important genetic resources for breeding.

5.2 Interactions between water deficit and other stress factors

Because plants are exposed simultaneously to multiple abiotic and biotic stresses under natural crop conditions, interactions among stress factors under realistic ranges of variability are important. Endogenous phytohormones act as signals to combat many stress factors. Some phytohormones, such as ABA, are specific to abiotic factors, in particular WD, whereas others, such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), are more specific to biotic stresses (Fujita *et al.*, 2006). These phytohormones influence plant growth processes (ET and JA) and plant survival mechanisms (ABA and SA) (Albacete *et al.*, 2014). However, hormonal signalling pathways are interconnected (Atkinson and Urwin, 2012; Fujita *et al.*, 2006); thus, multiple individual stress factors do not show simple additive effects when combined (Mittler, 2006). Interactions between WD and other abiotic stress factors in relation to fruit quality have rarely been studied. Generally, interactions among abiotic stress factors, such as a combination of very high temperatures and WD, induce more deleterious effects for plant health than individual factors (Mittler and Blumwald, 2010). Thus, deficit irrigation should be sufficient to improve fruit quality, but it must be well balanced to avoid weakening the plant's ability to respond to other abiotic stress factors.

In contrast, interactions between biotic and abiotic stresses often show beneficial effects of one or both stressors. For example, molecular studies have shown that *Botrytis cinerea* infection triggers the expression of genes involved in pathogen resistance, like *BOS1* in *A. thaliana* (Mengiste *et al.*, 2003) and *SlAIM1* in tomato involved also in adaptation to abiotic stress by modulating responses to ABA (AbuQamar *et al.*, 2008). In these studies, the co-occurrence of *B. cinerea* and abiotic stresses (water stress, osmotic stress and oxidative stress) reduces the susceptibility to the pathogen and induces tolerance to the abiotic stress. Similar positive interactions have been reported between powdery mildew and water stress, saline stress or proton stress (low-pH nutrient solution) in barley (Wiese *et al.*, 2004).

Beneficial interactions between pests and drought have also been reported in *Citrus latifolia* (Quiros-Gonzalez, 2000) and in apple (Gutbrodt *et al.*, 2012), independent of stress intensity. Fruit quality may be impacted by interactions between stressors. For example, in tomato, nematode attacks combined with 3-week WD promoted sugar and flavonoid levels compared to control and stressors alone (Atkinson *et al.*, 2011). However, this stress combination often reduced the above- and below-ground biomass (Grinnan *et al.*, 2013) and thus may induce negative effects on plant yield. In contrast, symbiosis with mycorrhizal fungus was shown to increase fruit production under WD and to improve fruit quality by increasing ascorbic acid concentration and reducing tomato acidity (Subramanian *et al.*, 2006). Indeed biotisation, which comprises the inoculation of young plants with beneficial micro-organisms such as bacteria or mycorrhizal fungi, may increase antioxidant contents in fleshy fruit and improve tolerance to abiotic stress (Gollotte *et al.*, 2009).

These results indicate that interactions between drought and plant pathogens may advantageously stimulate the accumulation of sugars, secondary metabolites and vitamin C in fruits. In addition, plant acclimation based on crosstalk responses to combined stress factors may be used to boost plant performances under non-optimal conditions including defence and quality.

VI- New perspectives for monitoring and sensing water deficit and its impact on fruit quality

Setting up innovative irrigation-sparing strategies requires efficient and non-destructive methods enabling the real-time monitoring of relevant indicators of plant physiological status and fruit quality. Although methods exist for assessing soil humidity, leaf photosynthetic activity, water fluxes or plant growth, the majority of current measurements of fruit quality are destructive, particularly with respect to biochemical components. Complementary non-destructive methods that could be used to better assess plant and fruit physiological responses to WD or to understand the complex interactions among the numerous factors involved are reviewed here. Moreover fruit quality under different scenario of WD could be predicted.

6.1 The volatile metabolome as a potential indicator of plant health status

Plants lose a considerable portion of carbon and biochemical energy gained in photosynthetic processes via the biosynthesis of volatile organic compounds (VOCs). Indeed, more than one thousand plant metabolites are volatile under ambient conditions, including saturated and non-saturated hydrocarbons, oxygen, nitrogen and sulphur-containing molecules, with carbon skeletons ranging from C1 (e.g., methane, methanol, formaldehyde) to C20 (e.g., diterpenes, Schwartzenberg *et al.*, 2004). The release of VOCs by plants is not restricted to flavour-producing flowers, fruits or vegetative secretory organs such as resin ducts and trichomes. All plant organs produce at least traces of volatiles under certain conditions (Loreto and Schnitzler, 2010). The metabolic origins and biosynthetic pathways of the majority of plant VOCs are well described. The majority of plant VOCs can be assigned to one of the three following biochemical classes: terpenoids, also called isoprenoids, that are synthesised in two distinct pathways in plants; lipoxygenase (LOX) products, also called oxylipins, the majority of which are oxygenated C6 compounds that are derived from the peroxidation of unsaturated fatty acids; and volatiles product from the shikimate pathway (phenylpropanoids, e.g., methyl salicylate). Low-molecular-weight VOCs, such as ethylene, methanol, ethanol, formaldehyde, acetaldehyde and acetone, are formed via other biosynthetic routes.

The number of known volatiles has increased steadily in recent decades, not least due to the emergence of breakthrough technologies and improvements to already existing analytical techniques used for VOC measurement. For example, the design of portable gas

chromatographs and electronic noses enables the *in situ* monitoring of VOCs released by organisms (Kunert *et al.*, 2002; Laothawornkitkul *et al.*, 2008), and the development of proton-transfer mass-spectrometry enables real-time measurements of various classes of trace gases at the sub-ppb level (Harren and Cristescu, 2013). Plant release of VOCs can be measured non-destructively when using appropriate enclosure techniques (Niinemets *et al.*, 2011), under close-to natural conditions or in fully controlled environments. This fact has prompted great interest in using VOC measurements for the in vivo monitoring of metabolic processes and the determination of the developmental, phenological and health status of plants or plant organs. For example, methanol emissions are associated with cell growth, likely due to the methanol produced by the demethylation of pectin during cell-wall formation (e.g., Oikawa *et al.* (2011) and references therein). Thus far, the majority of efforts have been towards the use of VOC signatures as indicators of stress responses in plants and, more particularly, of responses to biotic stress. A large number of plant VOCs are produced specifically in response to biotic stresses, most notably the LOX volatiles, methyl-salicylate, monoterpenes such as β-ocimene and linalool, various sesquiterpenes and the two homoterpenes DMNT ((E)-4,8-dimethyl-1,3,7-nonatriene) and TMTT ((E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene) that originate from the sesquiterpene (E)-nerolidol and the diterpene (E,E)-geranyl-linalool, respectively (McCormick et al., 2012). For example, in tomato plants, increased emissions of methyl-salicylate (among other VOCs) was associated with caterpillar (Vercammen *et al.*, 2001) and spider mite parasitism (Dicke *et al.*, 1998; Kant *et al.*, 2004) and infestations with tobacco mosaic virus (Deng *et al.*, 2004) and *Botrytis cinerea* (Jansen *et al.*, 2010). Although certain VOCs are typical in many stress responses, numerous studies have shown that the quantity and composition of VOCs released differs with the type and intensity of stress (McCormick *et al.*, 2012; Niinemets *et al.*, 2013). For example, using an electronic nose, Laothawornkitkul *et al.* (2008) discriminated VOC bouquets among cucumber, pepper and tomato leaves subjected to mechanical damage and diverse pests and diseases.

Changes in VOC signatures may also indicate abiotic stress. Above all, wounding and mechanical stress can produce large amounts of volatile LOX products that could even be detected under field or greenhouse conditions without using plant enclosure systems (Jansen *et al.*, 2010; Ruuskanen *et al.*, 2011). In addition to LOX products, aromatic crop plants, such as tomatoes, release bursts of terpenes from their trichomes upon mechanical damage. Emissions of LOX volatiles and terpenes were also found to increase after exposure to ozone (Penuelas *et al.*, 1999) and to cold and heat treatments (Copolovici *et al.*, 2012). In the latter

study, the emissions increased gradually with the severity of stress. Water logging, i.e., an excess of water, and the resulting hypoxia in the root zone were shown to trigger foliar ethanol and acetaldehyde emissions that originated from fermentation in the root cells (e.g., Copolovici and Niinemets, 2010). Regarding water shortage, numerous studies have described the effects of drought on constitutive VOC emissions ranging from decreased emissions to no effect or increased emissions (for an overview, see Penuelas and Staudt, 2010). However, apart from ethylene (Shakeel *et al.*, 2013), emissions of specific VOCs induced by WD have rarely been reported. Ebel *et al.* (1995) reported increased LOX emissions from apple trees during severe drought. Nevertheless, several independent studies have reported that WD stimulated biotic stress-induced VOC emissions (Gouinguene and Turlings, 2002; Niinemets *et al.*, 2013; Takabayashi *et al.*, 1994), perhaps by accentuating ROS formation and the associated stress signalling (see above).

The VOCs produced by the plant in response to biotic stress are not only symptoms but may form part of the defence reaction to cope with the aggressor. Stress-elicited VOCs may directly deter the attacker or act as olfactory cues to orientate predators and parasitoids of the pest to the plant under attack (McCormick *et al.*, 2012 and references therein). Genetic manipulation of these defensive traits in plants may serve as a form of pest control in agriculture. For example, the introduction of a sesquiterpene biosynthetic pathway into cultivated tomatoes resulted in improved herbivore resistance (Bleeker *et al.*, 2012). In addition to their potential role as defence compounds to deter pests, stress-induced VOCs may be involved in within-plant and between-plant signalling, thereby regulating the protective responses of plants against both biotic and abiotic stresses. As discussed above, several well-known phytohormones (or their derivatives) implemented in stress responses and priming are volatiles, including ethylene, NO, methyl-salicylate and methyl-jasmonate. Other VOCs with strong potential to act in the plant stress signalling network are volatiles containing α,β-unsaturated carbonyl groups (molecules collectively referred to as reactive electrophile species), such as the LOX volatile (*E*)-2-hexenal (Farag and Pare, 2002) and β-cyclocitral, a terpenoid formed from the non-enzymatic breakdown of β-carotene by singlet oxygen (Ramel *et al.*, 2012).

Thus, there is increasing evidence that the quality and quantity of VOCs released by plants indicate not only the presence of stress but also its intensity and the plants' capacity to cope with stress. Therefore, in vivo monitoring of VOC signatures from plants offers a novel avenue for the control of abiotic and biotic stresses in crop management and in phenotyping platforms for breeding or engineering stress-resistant genotypes. The potential use of stress

volatiles as defence elicitors and as olfactory cues for recruiting natural enemies in agroecosystems has been tested (Holopainen *et al.*, 2009; James, 2005) and shown to be promising.

6.2 Alternative non-destructive methods to determine fruit quality and plant health status

There are several non-destructive methods available to determine fruit quality and plant physiological status. Here, we describe briefly nuclear magnetic resonance (NMR), near infrared spectroscopy (NIRS), parameters based on measurements of UV/visible wavelengths such as the photochemical reflectance index (PRI) or Dualex/Multiplex (Force A, ©), and analysis of fluorescence transient of chlorophyll a, which are often used for monitoring plant physiological status but rarely fruit quality. These methods are based on the measurement of physical properties which correlate with the physiological status of plants but also with quality criteria, like electromagnetic (or optical) properties which could related to fruit appearance, mechanical properties to texture and chemical properties to flavour (Abbott, 1999).

NMR has primarily been used to study plant anatomy, changes in water content and transport in stems or in root systems and has rarely been used to assess internal fruit quality. The quantification of extracts of fruit offers the possibility of quantitative measurements of each compound contributing to fruit quality but requires extraction of the compounds (Deborde *et al.*, 2009). However, even without extraction, NMR permits an evaluation of maturity, worm damage or bruises (Chen *et al.*, 1989).

Visible/near-infrared spectroscopy has been recently preferred over infrared thermography for the screening of genotypes in controlled environments by measuring different plant traits, such as leaf water content (Zhang *et al.*, 2012), leaf nitrogen content (Ulissi *et al.*, 2011), seed viability (Kranner *et al.*, 2010) and metabolic content in fruits (Clement *et al.*, 2008). NIRS measurements correlate with sensory analyses of apple (Mehinagic *et al.*, 2003) and mandarin *Citrus reticulata* B. (Gomez *et al.*, 2006) and have been applied to measure colour, total soluble solids and vitamin C in orange (Magwaza *et al.*, 2013). NIRS spectra reflect the unique chemical fingerprint of organic material and thus are potentially important for non-destructive assessments of fruit quality.

Spectral analysis in the UV/visible wavelength range has been used to characterise other factors involved in fruit quality or leaf chlorophyll content. For example, this method was

used in grape to detect anthocyanins, which absorb in the visible light, and flavonoids, which absorb in light in the UV range (Rustioni *et al.*, 2013; Tuccio *et al.*, 2011).

The PRI has been also used to evaluate the epoxidation state of xanthophylls and the light use efficiency of photosynthesis. These spectral analyses use visible wavelengths (from 531 nm to 570 nm) and have enabled the detection of short-term stress responses to nitrogen stress and WD and evaluations of specific carotenoid content in fruits (Araus and Cairns, 2014; Murshed *et al.*, 2013; Suarez *et al.*, 2012).

However, all of these spectral analyses are based on calibrations using predictive statistical models, which depend on extensive databases. Moreover, these techniques may not be appropriate for fruit species with heterogeneous internal structure, such as the tomato (de Oliveira *et al.*, 2014; Jouquet *et al.*, 2014). Others instrument based on optical properties like Dualex or Multiplex permits to assess the phenolic content in grape berries (Cerovic *et al.*, 2008) and to evaluate its change in relation to WD and fruit maturity (Esteban *et al.*, 2001).

Parameters of chlorophyll fluorescence derived from measurements performed using a pulse amplitude-modulated fluorimeter, have been used to follow the maturation of mangoes fruits (Lechaudel *et al.*, 2010). Chlorophyll fluorescence measurements could also be used for post-harvest detection of damaged or infested fruits containing chlorophyll in peel like lemon (Nedbal *et al.*, 2000). Besides minimal fluorescence, maximal fluorescence and variable fluorescence, innovative parameters such as the Performance Index of Strasser (Strasser *et al.*, 2004) could be used in the future on fruits to assess their physiological status in response to WD.

Thus, fruit quality traits, including colour, soluble sugar and organic acid contents or nutritional value, can be quantified using these different methods. All of these methods can be used *in situ* with the exception of NMR.

6.3 Process-based models: promising tools for the analysis and prediction of fruit quality during water deficit

Because the variations in fruit quality under WD involve many mechanisms and feedback loops at the plant and fruit levels, a modelling approach may help define relevant strategies for irrigation and designing ideotypes of plants adapted to drought, i.e., genotypes capable of maintaining yield and producing high-quality fruits, may be useful. Indeed, process-based models are appropriate tools for integrating knowledge from the gene to the fruit (Struik *et al.*, 2005), predicting the behaviour of complex systems such as fruits in fluctuating environments (Génard *et al.*, 2007) and analysing gene-environment interactions

(Bertin *et al.*, 2010). Thus, the virtual fruit model (Génard *et al.*, 2007) may be a basis for understanding fruit quality in response to environmental fluctuations and analysing interactions between WD and other environmental or genetic factors or cultural practices. This model describes water and carbohydrate transport combined with stimulations of cell-wall extension driven by the influx of water and turgor pressure. The original virtual fruit model was developed based on the peach; however, the model has proven to be quite generic and has been adapted for different species, including tomato (Liu *et al.*, 2007), mango (Lechaudel *et al.*, 2005) and grape (Dai *et al.*, 2009). Interestingly, the virtual tomato model has been recently combined with a plant model that describes water and carbon fluxes within the plant architecture and the induced gradients of water potential and phloem sap concentration in carbon within the plant (Baldazzi *et al.*, 2013). This integrated model would be a powerful tool for understanding the complex interactions between water and carbon balance in response to WD at the plant and fruit levels. This model focuses on fruit fresh and dry mass and soluble compound content but it could be improved in the future for the prediction of other quality traits, including the accumulation of healthy compounds such as vitamins and carotenoids. Future developments of this model could encompass genetic factors (Bertin *et al.*, 2010), which would make it suited not only for generating novel ideas for future research, but also as a tool for breeding programs.

VII- Conclusions

Global climate change entails many threats and challenges for the majority of crops. Above all, a reduction in yield is expected in many parts of the world, and drought is generally believed to represent one of the most important negative results of climate change. Fruit crops will certainly also suffer from the increased extension of drought conditions; however, yield is arguably not as important for fruit as for grain crops or oil crops. Yield does matter for fruit crops, but quality criteria are as important if not more important. Fruits are expected to supply health benefits and to bring hedonistic pleasures associated with specific aromatic compounds. We may thus distance ourselves from the dominant deleterious effect of drought on crop performance and consider the potential benefits. In this review, we discussed fundamental and agronomic research and demonstrated that fruits from drought-stressed plants may be superior, in particular with respect to the content of health-promoting phytochemicals. The stimulation of secondary metabolism may also be beneficial to plant natural defences. A reduction in pesticide use may represent an additional benefit for consumers and, thus, the fruit industry. However, existing data also strongly suggest that taking advantage of stress will require a better understanding of the underlying mechanisms of drought adaptation, and much work remains along these lines. Laboratory studies of the effect of a single severe stress applied during a very short period of time should be de-emphasised in the future. Instead, as shown in this review, we must increase our understanding of the effects of variable and repeated periods of drought applied at different periods of the crop cycle, possibly combined with other forms of stress, because these conditions more accurately reflect actual crop conditions. Integrated models must be developed to address the complexity involved and to generate novel research ideas and avenues for plant physiologists. We also require novel monitoring tools that are based on innovative ideas, such as VOC signatures and parameters derived from measurements of chlorophyll fluorescence. Fortunately, the information analysed as part of this review is sufficiently mature and promising to encourage researchers who are considering a shift in their approach to drought research.

The MAGIC TOM Parents: an important source of genetic variability for adaptation to repeated cycles of deficit irrigation at the plant and fruit levels

Lors du précédent chapitre, nous avons synthétisé les effets positifs du WD sur la qualité des fruits mais aussi sur l'utilisation de ceux-ci comme évènement stimulant pour les défenses de la plante au travers des mécanismes associés au priming.

Les plantes en conditions naturelles sont très souvent confrontées à des variations climatiques brusques et répétées, en particuliers des alternances de périodes de stress et de récupération. Nos objectifs dans ce chapitre étaient d'étudier les effets de cette alternance de périodes de WD et de récupération sur le développement de la plante et du fruit, en fonction de la variabilité génétique. Pour ce faire, les huit parents composant la population Multi-Parent Advanced Generation InterCross (MAGIC) de la tomate ont été sélectionnés. Ces parents possèdent une riche variabilité allélique.

La mise en place de mécanismes d'adaptation des plantes a été évaluée au niveau des potentiels hydriques de base et au midi solaire, de la conductance stomatique, de la photosynthèse, de la fluorescence de la chlorophylle *a*, de la composition en chlorophylles, sucres solubles et acides organiques des feuilles et de la croissance de la plante. La qualité des fruits récoltés a été évaluée en termes de croissance et de teneurs en sucres solubles, acides organiques, caroténoïdes et acide ascorbique.

Ce chapitre est présenté sous la forme d'un article en cours de préparation.

Résumé

En conditions naturelles, les plantes subissent des cycles répétés de périodes de déficit hydrique et de récupération, qui ont un impact négatif sur la croissance des plantes et le rendement en fruits, mais qui peuvent améliorer la qualité des fruits. En outre, un déficit hydrique modéré (WD) peut induire un "effet priming" pour la plante, qui est connu pour stimuler l'adaptation et la défense des plantes au cours des prochaines périodes de stress. Cette étude vise à analyser les impacts positifs et négatifs de WD répétés, à l'échelle de la plante et du fruit. Trois périodes de WD (-38 %, -45 % et -55 % d'approvisionnement en eau par rapport au témoin), suivies par trois périodes de récupération (alternance de traitements), ont été appliquées sur les huit parents de la population Multi-Parent Advanced Generation InterCross (MAGIC) qui offre la plus grande variabilité allélique observée chez la tomate. La réponse physiologique de la plante (potentiel hydrique base et au midi solaire, conductance stomatique et croissance) et les traits de qualité des fruits (sucres, acides, caroténoïdes et acide ascorbique (AsA)) ont été mesurés. D'importantes variations génotypiques ont été observées tant à l'échelle de la plante que du fruit, et les réponses mesurée à l'échelle du fruit et de la feuille ne sont pas corrélées. Sur la base de la conductance foliaire et de la photosynthèse, un effet mémoire du stress a pu être observé entre les deux premiers cycles successifs de WD. Globalement, l'alternance de traitements a induit d'importantes régulations hydrique et osmotique, la réduction de la croissance des feuilles à long terme, l'adaptation de la capacité photosynthétique en lien avec l'augmentation de la teneur en chlorophylles et du flux cyclique d'électrons entre les photosystèmes PSII et PSI. Les effets sur les teneurs du fruit en sucres, acides, caroténoïdes et AsA, exprimées en matière sèche, étaient variables, de positif, à nul, à négatif selon le génotype et l'intensité du stress. Exprimées en matière sèche, seuls les génotypes à petits fruits présentaient une amélioration de la qualité nutritive avec ue augmentation des teneurs en AsA. La qualité des fruits pourrait donc être essentiellement améliorée par des effets de dilution. De plus, les résultats suggèrent que l'accumulation d'amidon pourrait être une stratégie d'adaptation pour maintenir la croissance des fruits lors d'un WD. Ces résultats soulignent la complexité des réponses de la plante en termes de critères de performances ou de variabilité des effets génotypiques et représentent un véritable défi pour les études à venir.

Mot clés: Déficit hydrique, Qualité des fruits, Population MAGIC, Périodes de récupération, *S. lycopersicum* L.

Abstract

Under natural conditions, plants experience repeated cycles of water deficit and recovery periods, which negatively impact on plant growth and fruit yield, but may improve fruit quality. Moreover, a moderate water deficit (WD) may induce plant priming which is known to stimulate plant adaptation and defence during the next stress periods. This study intended to analyse the positive and negative impacts of repeated events of WD at the plant and fruit levels. Three periods of WD (-38 %, -45 % and -55 % of water supply compared to control) followed by three periods of recovery (alternation treatment), were applied on the 8 parents of the Multi-Parent Advanced Generation Inter-Cross population which affords the largest allelic variability observed in tomato. Plant physiological responses (predawn and midday water potentials, stomatal conductance and growth) and fruit quality traits (sugars, acids, carotenoids and ascorbic acid (AsA)) were measured. Important genotypic variations were observed both at the plant and fruit levels and responses of fruit and leaf traits did not correlate. Based on leaf conductance and photosynthesis, a plant memory effect could be observed between two successive cycles of WD. Overall the alternation treatment induced important water and osmotic regulations, reduction of leaf growth, adaptation of the photosynthetic capacity likely through an increase in the chlorophyll content and the cyclic electron flow. The effects on fruit sugar, acid, carotenoid and AsA contents on a dry matter basis ranged from negative to positive to nil depending on genotype and stress intensity. On a fresh matter basis, only genotypes with small fruits had a better nutritional quality with increasing AsA. So, fruit quality could be improved mainly by dilution effects. At the fruit level, starch accumulation might be an adaptive strategy to maintain fruit growth during WD. Our observations underline that the complexity involved in plant responses when considering a broad range of crop performance criteria and the variability of genotypic effects, represent a true challenge for upcoming studies aiming at taking advantage of, not just dealing with water deficit.

Key words: Deficit irrigation, Fruit quality, MAGIC population, Recovery period, *S. lycopersicum* L.

I- Introduction

During the last decade and in the context of climate change, many studies have investigated the effect of water deficit (WD) on plant physiology and its impact on crop yield. These studies usually focus on short periods of stress or on long stress period applied throughout the crop. However, under natural conditions plants often experience repeated cycles of WD and recovery periods (RP), which may negatively impact on plant growth and yield as well. Nevertheless, some beneficial effects of WD and RP periods may be expected. First, during recovery periods plant defence or adaptation mechanisms are expected to be exacerbated by WD thanks to priming mechanisms (Stoeva *et al*., 2010; 2012). Second, fruit quality may be improved by moderate WD periods (Ripoll *et al.*, 2014). Thus, a better knowledge and quantification of the beneficial and detrimental effects of cycles of WD and recovery on yield, plant adaptation and fruit quality is an important issue.

WD affects the leaf physiological activity, usually resulting in a reduction of stomatal aperture, followed by a reduction of photosynthetic activity and an increased risk of photo-oxidative stress, which ultimately leads to growth limitation (Tardieu *et al*., 2006). Reduction of growth is one of the most current adaptive responses of tomato to WD (Chaves *et al.*, 2003). However, the succession of two stress periods induces a positive effect on leaf water supply during the second stress period which attenuates the negative impact of WD on plant growth (Al Gehani I., 2005). During RP, growth may not completely recover according to the duration and intensity of WD. In tomato, after irrigation cut-off during the reproductive period (from nine to 13 days according to the cultivar), leaf water potential, stomatal conductance and net photosynthesis rate recovered their initial values (Rahman *et al.*, 1999). Cell wall extensibility is the mechanism less amenable to recover after drought stress hypothetically due to the rapid accumulation of abscisic acid (Mahdid *et al.*, 2011). Under extreme WD, recovery is partial due to some damages on photosystems (PS) II. Indeed the synthesis of reactive oxygen species (ROS) increases under WD, and recovery depends on the quantity of ROS produced (Xu *et al.*, 2010), although this result was not confirmed on tomato.

Rare studies investigated the impacts of WD and RP cycles on tomato although it is known that WD may have positive effects on fruit quality as well on plant defence/adaptation status (Ripoll *et al*., 2014). Indeed, a first moderate WD may promote plant adaptation to a

second more intensive stress. It has been demonstrated that "plant memory" of stress induces a faster activation of response mechanisms to others stressors (abiotic or biotic stress) through the common hormonal response pathways which convey tolerance to these other stressors (Li and Zhang, 2012). The faster activation of defence response is associated to higher level of gene expression and accumulation of inactive signalling proteins and transcriptions factors in primed plant unlike non primed plant (Bruce *et al*., 2007). In primed tomato plants, the fruit carotenoid content is improved and the response is amplified after a second period of stress due to an increase in antioxidant enzyme activity during the first stress period as well as during RP (Stoeva *et al*., 2010; 2012). Finally it appears that one could take better advantage of priming to promote fruit quality and maintain yield. Although this has to be demonstrated, especially after several cycles of WD and RP of varying intensity as it may occur under natural conditions due to the seasonal fluctuations.

Moreover the impact of WD and RP periods on fruit quality may differ according to the periods of fruit development affected by WD. Indeed, the effects of WD on fruit quality traits (e.g. soluble sugars, organics acids, carotenoids or C vitamin contents) are known to vary with the developmental stage affected by stress (reviewed in Ripoll *et al*., 2014). When WD occurs during the cell division stage, it may induce carbon starvation that negatively regulates cell division. However, a positive effect on carbon supply to the remaining fruits has been suggested through a negative regulation of fruit setting and fruit load. Similarly, WD has been shown to improve peach sweetness, flavour and fruit size when applied during cell division and rapid endocarp hardening stages (Li *et al*., 1989; Vallverdu *et al*., 2012). During the cell expansion stage, triggered by enter of water in the cell, WD mainly impacts on water fluxes among source and sink organs (Münch, 1930). In peach, negative effects on yield have been observed associated with a decrease in fruit water content (Li *et al*., 1989; Girona *et al*., 2004). During ripening, which consists in a rapid slow-down of cell expansion and an increase in metabolic changes, WD may interact with ethylene synthesis (Fray *et al*., 1994; Barry and Giovannoni, 2007). Different combinations of WD applied during flowering and fruit growth, or during flowering and ripening, showed that the improvement in fruit quality is counterbalanced by the decrease in yield when at least one development phase is exposed to intensive stress in oranges (García-Tejero *et al*., 2010). So, anticipating the effects of repeated cycle of WD and RP periods on plant growth, plant priming and fruit quality is challenging and merits more investigations.

In the present study, responses of tomato plant and fruit were quantified during three successive periods of DI of increasing intensity followed by RP. WD was applied by reducing the supply of water by about 38 % during the first DI period, 45 % during the second DI period and 55 % during the last DI period compared to control plants. For ease of reading, the term alternation was used throughout this study to design the succession of DI and RP periods. Physiological plant responses (predawn water potential, stem water potential at midday, photosynthesis, chlorophyll content and chlorophyll *a* fluorescence) as well as leaf composition in soluble sugars and organic acids were assessed after each alternation. Fruit quality (soluble sugars, organic acids, carotenoids and ascorbic acid contents) was measured on 2 lots of fruits which developed under different WD and RP periods. The work was performed on the 8 parents of the population Multi-Parent Advanced Generation Inter-Cross of tomato (MAGIC TOM) to assess the genetic variability in plant and fruit responses. These 8 genotypes afford the largest allelic variability observed in tomato populations (for more description on MAGIC populations, see review of Cavanagh, 2008) and are used by breeders to generate introgression lines. Our objectives were to provide an overview of the beneficial and detrimental impacts of repeated cycles of DI and RP at the plant and fruit levels, to assess some genetic variability of these responses, and to point to interesting traits of adaptation to alternation treatment which could be used in future breeding programs.

Genotype	Fruit weight	Species	Known resistance to stressor	References
Cervil	<5g	*S. l. cerasiforme*	Sensitive to saline stress	(Manaa *et al.*, 2011)
Criollo	<15g	*S. l. cerasiforme*	No references	
LA1420	<50g	*S. l. cerasiforme*	No references	
Plovdiv XXIVa	<50g	*S. l. cerasiforme*	No references	
Stupicke Polni Rane	<70g	*S. l. esculentum*	▪ Stomatal closure after 5 days of no water ▪ Resistant to Phytophtora infestans ▪ Increase photosynthesis rate after 4 days at cold temperature ▪ Strong emission of volatile	(Hnilickova and Duffek, 2004) (Petrikova *et al.*, 2003) (Hnilickova *et al.*, 2002) (Subrtova *et al.*, 1985)
Ferum	<130g	*S. l. esculentum*	No references	
LA0147	<130g	*S. l. esculentum*	No references	
Levovil	<130g	*S. l. esculentum*	Tolerant to saline stress	(Manaa *et al.*, 2011)

Table 1: Description of the 8 parents of the MAGIC TOM population selected for their high degree of allelic variability (Ranc, 2010). The average fruit weight and a bibliographic summary of known resistance to various stresses are indicated.

II- Materials and methods

2.1 Plant material & Experimental conditions

The 8 parents of MAGIC TOM (Tab. 1) have been selected for their allelic variability among 360 accessions of tomato (Ranc, 2010). MAGIC TOM consists in four *Solanum lycopersicum* L. lines Levovil, Stupicke Polni Rane (here called Stupicke), LA0147 and Ferum with large fruits and four cherry-type accessions, *S. l. var cerasiforme* lines, Cervil, Criollo, PlovdivXXIVa (here called Plovdiv) and LA1420. LA1420 seeds were provided by the Tomato Genetics Resource Centre, Davis, California. Cervil and Levovil seeds were provided by Vilmorin Seed Company. The other lines were from the Tomato Genetic Resource Centre in INRA, Avignon, France (Causse *et al*., 2013).

The trial was conducted during spring and summer 2012 in a glasshouse located near Avignon, France. Climate conditions (temperature, humidity, and light intensity) in the glasshouse were recorded every minute and averaged hourly throughout the experiment. Day and night temperatures were stable until June-12 that is until RP2 (around 25 °C and 18 °C, respectively), and then they increased during DI3 and RP3 (around 30 °C and 22 °C, respectively) due to seasonal effects. At the same time, the daily light integral increased (from 5.5 to 8.4 mol m^{-2} day^{-1}), whereas day and night humidity decreased (from 57 % to 37 % at daytime and from 80 % to 60 % at night) from DI1 to RP3. Plants were grown in 4 L pots filled with compost (substrate 460, Klasmann, Champety, France) distributed on two rows (control and stressed plants) of 80 plants each (10 plants per genotype) at a density of 1.3 plant m^{-2}.

Irrigation was monitored according to daily ETP (Penman, 1948), reassessed twice a day and corrected with cultural coefficient (40 %, 50 %, 75 % and 100 %). Drainage was measured daily and monitored around 30 % for the control, which corresponds to commercial production practices. The alternation treatment corresponded to three phases of WD (DI periods) of increasing intensity (-38 %, -45 % and -55 % of water supply) followed by three RP (Fig. 1A). Soil water potential (Ψ_{soil}) was measured daily with a probe (Watermark 253, Campbell Scientific, Antony, France) disposed at the opposite of the dripper (Fig. 1B). One probe per treatment per genotype was used and connected to a data acquisition system (Data

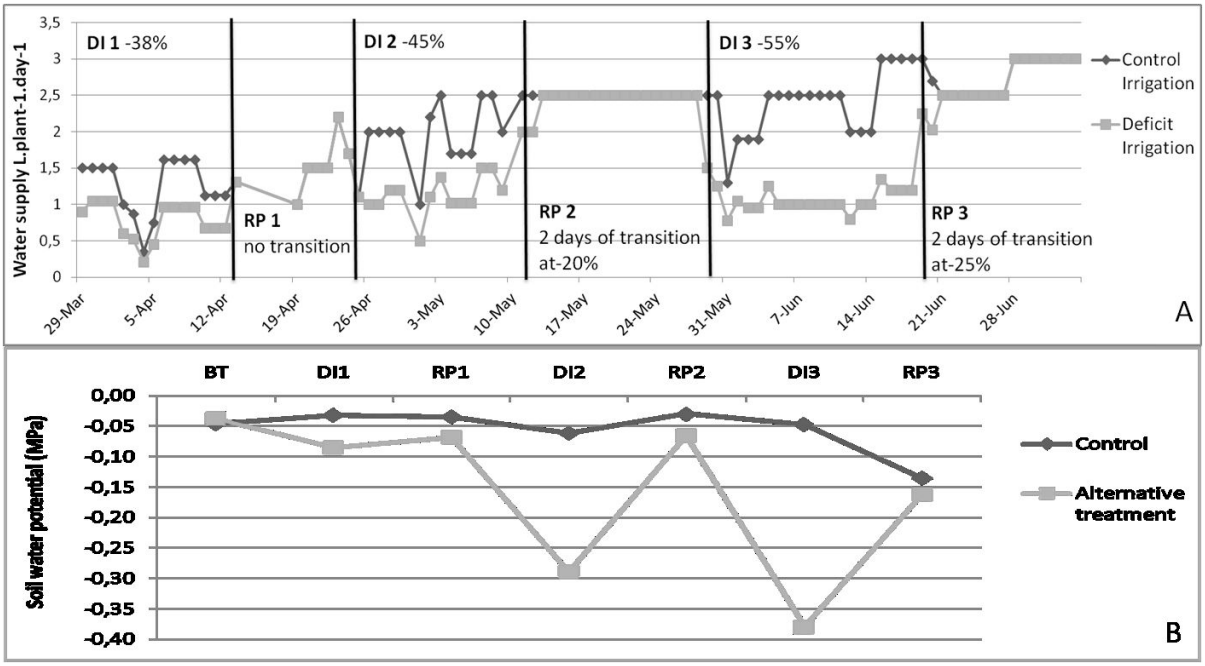

Figure 1: Time table of deficit irrigation (DI) and recovery (RP) periods during the experiment (A) and mean of soil water potentials for each DI and RP (B). Water supply in liter per plant per day in control and stressed irrigations. Irrigation of control plants was monitored according to the potential evapotranspiration measured twice a day. For each DI period the average reduction of water supply is indicated on the first line. Transition period of about two days were applied after each DI period in order to reduce the risk of blossom end rot for fruits. Stressed irrigation corresponded to the alternation treatment which consists in 3 DI of increasing intensity followed by RP periods.

logger, Campbell Scientific, Antony, France). Results were converted into MPa using equation 8 given by Shock (1998). Under control conditions, Ψ_{soil} was rather stable until the DI3 period and decreased during RP3 due to plant development and increasing temperature in the glasshouse. The alternation was well contrasted during the three DI periods compared to control (with sufficient recovery during RP). Averaged Ψ_{soil} throughout the experiment was -0.055 MPa and. -0.174 MPa for control and stressed plants, respectively.

Nutrients were applied daily with a commercial solution (Liquoplant Rose, Plantin, Courthézon, France). Flowers were pollinated three times a week using an electrical bee. Trusses were pruned according to final fruit size (Tab. 1) in order to obtain comparable levels of competition among fruits for all genotypes (Cervil: 12 fruits per truss, Criollo: 10 fruits, LA1420: 8 fruits, Plovdiv: 8 fruits, Stupicke: 6 fruits, Ferum: 5 fruits, LA0147: 5 fruits, Levovil: 4 fruits). No chemical treatment was applied and *Macrolophus caliginosus* were released throughout the culture to protect plants from whiteflies.

WD occurred at different developmental stages according to the different growth rate of the eight genotypes. So, the first WD period (DI1) occurred during anthesis of the fourth truss of Cervil, which corresponded to the end of anthesis of the second truss of Ferum. All DI and RP periods lasted approximately 15 days. During recovery, DI was first reduced by half for 2 days and then brought to the control level. No drainage was observed on stress rows during DI periods.

2.2 Plant measurements

Stem water potential was measured using a pressure chamber (SAM Précis 2000 Gradignan, France) at predawn and at solar noon ($\Psi_{predawn}$ and Ψ_{midday}) at the end of each DI and RP periods ($n \geq 4$ for a given genotype, 64 plants min.). Leaves were bagged the day before, at nightfall.

Photosynthesis, respiration and transpiration rates were measured around noon with an infrared gas analyser (LICOR 6400, Li-Cor, Lincoln, USA) with the rebroadcast of captured light (10 % of blue light). The flow of incoming CO_2 was set at 400 ppm (close to external condition). Respiration was measured in the dark at 400 ppm of CO_2. Measurements were realized on non-senescent mature leaves during the last 3 days of each period ($n \geq 5$ for each genotype, 80 plants min.).

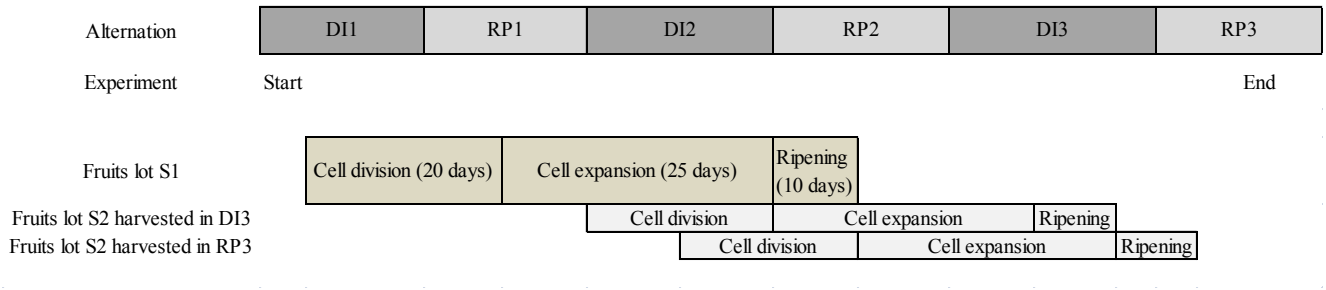

Figure 2: Time table of the fruit development periods and corresponding deficit irrigation (DI) and recovery (RP) periods for the two lots of fruits (S1 and S2). S1 fruits developed from DI1 to RP2 and DI1 occurred during part of the cell division period, whereas DI2 affected the cell expansion period. S2 fruits developed from DI2 to RP3. DI2 occurred during part of their cell division period and DI3 during part of the cell expansion and ripening periods. The two harvest periods corresponded to different developmental rates among genotypes. Durations of fruit developmental phases are means for all the genotypes.

Fluorescence of chlorophyll *a* was measured on 30 min. dark adapted leaves using a fluorimeter (HANDY-PEA, Hansatech, King's Lynn, UK). Dark-adaptation allowed the PSII electron acceptor pool to be gradually re-oxidized to a point where all PSII reaction centres are capable of undertaking photochemistry. Measurements were carried out with an induction period of 1 s and leaves were illuminated to a light level of 3000 μmol m^{-2} s^{-1}. The measurements were carried out on non-senescent mature leaves, at around 11 am during the last 3 days of each period ($n \geq 4$ for a given genotype, 64 plants min.). The performance index of Strasser (PI) was used to assess the plant viability under the alternation (Strasser *et al*., 2004) and J_0^{RE1}/J^{ABS} the quantum yield of the electron transport flux until PSI acceptors (selected according to our previous results) were followed using the equation of Stirbet and Govindjee (2011). Chlorophyll content was evaluated using a chlorophyll meter (SPAD 502, Konica-Minolta, Osaka, Japan).

Plant leaf number and leaf length were measured at the end of each period ($n \geq 5$). The last day of the DI3 period, two non-senescent mature leaves, which have been prone to the alternation, were harvested on each plant and their specific leaf area (SLA) ($n \geq 5$, 80 plants min.) was measured. Leaf area was measured with a Planimeter (Li-3100 C Area Meter, Li-Cor, Lincoln Nebraska, USA) and leaf dry weight was measured after seven days at 70 ° C in a ventilated oven. Furthermore, at the end of each DI and RP periods four leaflets of two leaves from 5 plants per genotype per treatment (80 plants in total) were harvested around 11 am, immediately frozen in liquid nitrogen and stored at -80 °C , for biochemical analysis (soluble sugars, organics acids and starch content).

2.3 Fruit measurements

The dates of anthesis of the successive trusses were recorded on all plants during the whole experiment. Thus, fruits could be pooled according to the developmental stage prone to the successive DI periods: cell division, cell expansion and/or beginning of ripening (Fig. 2). For the first pool of fruits (S1) harvested during RP2, DI1 occurred during the cell division period and DI2 occurred during the cell expansion period. For the second pool of fruits (S2), DI2 occurred during the cell division period and DI3 occurred during the cell expansion period and beginning of ripening. Fruits of lot S2 were harvested at two periods according to the time of ripening: in DI3 for the genotypes Cervil, Criollo, Plovdiv and Stupicke, and at the beginning of RP3 for the genotypes Levovil, LA1420, LA0147 and Ferum.

All measurements were realized on red mature fruits (breaker plus at least five days) harvested on five plants per genotype and per treatment (80 plants in total). Fruits were

harvested at 11 am, avoiding the first proximal and last distal fruits from each truss. Fruit size and fresh weight of all fruits were measured. Then fruits were frozen in liquid nitrogen and kept at -80 °C prior to biochemical analysis of pericarp soluble sugars, organic acids, carotenoids, ascorbic acid, starch content (only for the genotype Cervil) and dry matter content. For the biochemical analyses, fruits were pooled into five lots of three to five fruits for each treatment and genotype, in order to average the variability due to the truss position along the stem.

2.4 Biochemical Analyses

Soluble sugars and organic acids were extracted according to Gomez *et al.* (2002) and measured by HPLC method. Starch was measured on the supernatant after a hydrolysis. The glucose released by starch hydrolysis was measured using the micro-method described in Gomez *et al.* (2007) and starch was calculated. Dry matter content was measured after lyophilisation. Ascorbic acid content (AsA) was evaluated using the method of Stevens *et al*. (2006). Carotenoids were extracted according to the method of Serino *et al.* (2009) and assayed by HPLC.

2.5 Statistical Analyses

All statistical analyses were performed using R3.1.0 (R Core Team, 2014). The evolution of physiological parameters over the experiment was compared between stressed and control plants using the areas under the curves (AUC). AUC were calculated according to the Trapezoidal rule (Atkinson, 2008). Genotype and treatment effects for all parameters were analysed by two-way ANOVA. The residue normality (ANOVA) was tested using the Shapiro-Wilk test (Royston, 1995). Levene's test was allowed to check homoscedasticity of variances of the residues (Car package; Fox and Weisberg, 2011). When authorized, two-way ANOVA was performed and followed by multiple comparisons of means (lsmeans and multcompView packages; Lenth, 2014, Graves *et al*., 2012). Alternatively the non-parametric Kruskal-Wallis test was applied (pgirmess package; Giraudoux, 2014). Clustering analysis was performed on individual leaf data (FactoMineR package; Husson *et al*., 2014) and heat-maps of the fruit were plotted according to control and alternation conditions (gplots package; Warnes *et al*., 2014). Partial correlations network was realised on plant and fruit data based on the residues of the linear regressions (elimination of the genotype effect) performed independently under control or WD treatment (P threshold < 0.001) (GGMselect, GeneNet and igraph packages; Giraud *et al*., 2009; Schaefer *et al*., 2014; Csardi and Nepusz, 2006).

III- Results

Before the alternation treatment started, all the measured parameters had comparable values between control and future stressed plants.

3.1 Mean effects of the alternation treatment at the plant and leaf levels

Effects of the alternation treatment were analysed based on the kinetics of three parameters selected for their know importance in the plant response to WD: minimum stem water potential measured at midday (Ψ_{midday}), leaf net photosynthesis rate and stomatal conductance (Fig. 3). The range of variation of $\Psi_{midday,}$ was rather similar among genotypes indicating similar conditions of WD. Only few significant differences in Ψ_{midday} were observed between control and stressed plants (Fig. 3). A decrease in Ψ_{midday} was observed during DI1 on three genotypes (Criollo, Ferum and LA0147). During DI2, only Ferum and Cervil were affected and the effect persisted during RP2 indicating the absence of recovery for these two genotypes. Interestingly in Levovil, stressed plants could maintain their Ψ_{midday} above that of control plants until a sharp drop during the last DI3 period. The net photosynthesis rate and the stomatal conductance varied with the alternation treatment in similar ranges for all genotypes. In control and stressed plants, the temporal decline in both variables was attributed to an ontogeny effect and/or to summer climatic conditions during DI3 and RP3 periods. In three genotypes (Cervil, Stupicke and Levovil), the net photosynthesis rate significantly dropped in stressed plants during DI1 and only one of these genotypes (Cervil) recovered during RP1. In two genotypes (Ferum and LA0147) a delayed response was observed during RP1. In Criollo, LA1420 and Plovdiv, the net photosynthesis rate of stressed plants decreased during DI2, and Plovdiv did not recover during RP2. Despite the drop of net photosynthesis rate of control plants at the end of the experiment, a significant difference was observed during DI3 between control and stressed plants of two genotypes (Stupicke and LA1420). Negative effects of WD on net photosynthesis rate were observed during one of the two first DI periods (DI1 or DI2), but never during two successive DI periods, indicating an adaptation of the plant. The treatment effects on net photosynthesis rate were not directly related to variations in leaf stomatal conductance. In three genotypes (Criollo, Plovdiv and Stupicke) the stomatal conductance was reduced in stressed plants

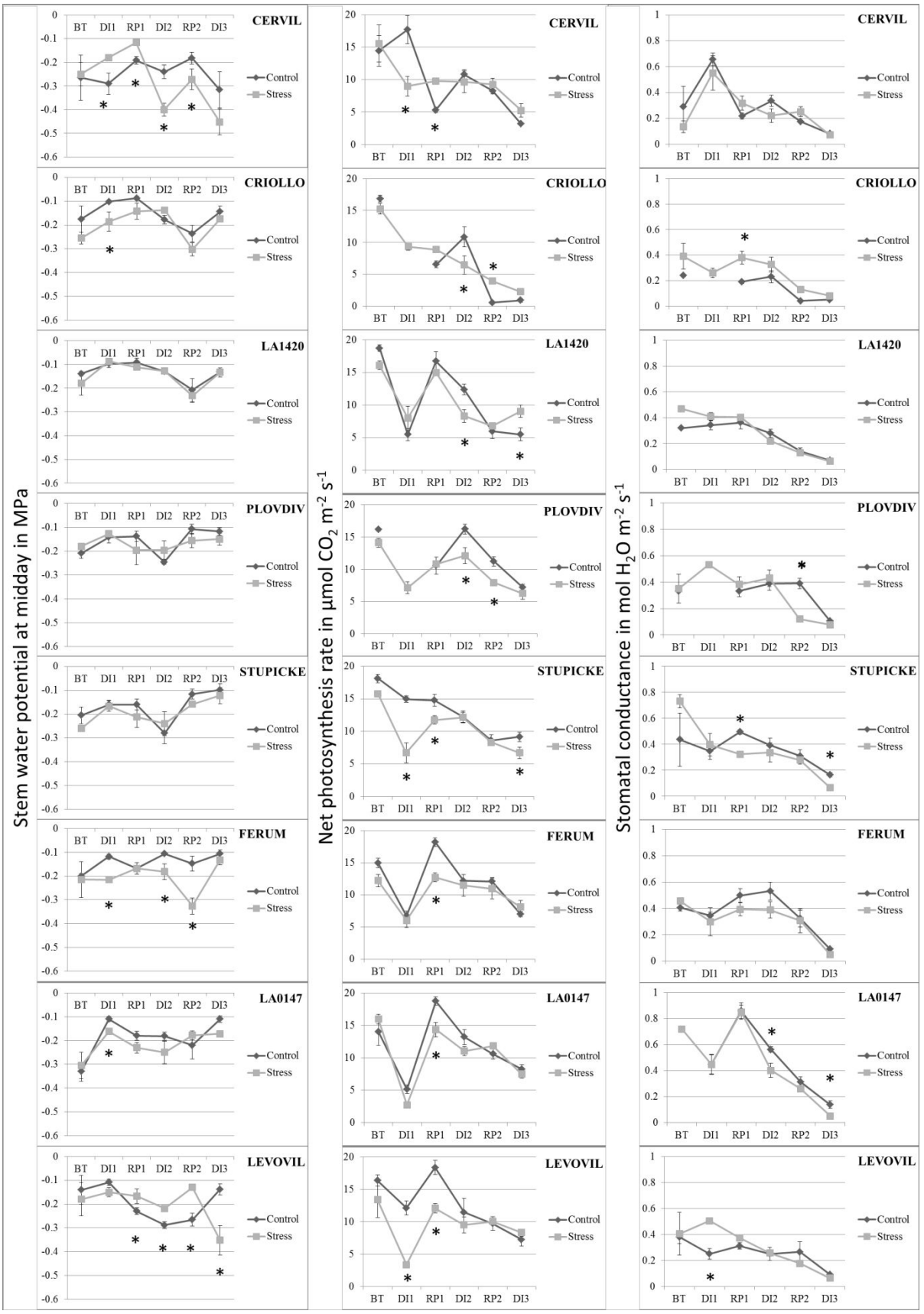

Figure 3: Stem water potential at midday (left), net photosynthesis rate (middle) and stomatal conductance (right) for the 8 parents of the MAGIC TOM (genotypes: Cervil, Criollo, LA1420, Plovdiv, Stupicke, Ferum, LA0147 and Levovil) measured before treatment (BT)

and at the end of each deficit irrigation (DI) and recovery periods (RP). Data are means ± SE ($n \geq 5$). Significant differences between control and alternation treatments are indicated using * symbols for $P < 0.05$ (Student test by genotype). Some data are missing because important daily fluctuations of climate occurred at the time of measurements and data were skipped (i.e. Criollo and Plovdiv at the end of DI1 and LA0147 in BT).

during RP1 or RP2 periods while in Levovil, LA0147 and Stupicke the decreased was measured also during DI periods (DI1 for Levovil, DI2 and DI3 for LA0147, DI3 for Stupicke).

The specific leaf surface area measured at the end of the experiment on non-senescent mature leaves varied by a factor three among genotypes and it diminished in response to the alternation treatment in LA1420 (-26 %), Stupicke (-26 %), LA0147 (-15 %) and Levovil (-36 %) while it increased in Cervil (+21 %) (Fig. 4).

In order to evaluate the global plant response to the alternation treatment, AUC were calculated on a large selection of parameters ($\Psi_{predawn}$ and Ψ_{midday}, dry matter, soluble sugars, organic acids and starch contents, stomatal conductance, net photosynthesis rate, chlorophyll content, respiration and transpiration rates, the maximum efficiency of PSII photochemistry when all the PSII centres were open (F_V'/F_M'), the maximum efficiency at which light absorbed by PSII is used for reduction of Q_A (F_V/F_M), the quantum yield of the electron transport flux until PSI acceptors J_0^{RE1}/J^{ABS} and the PI index of Strasser *et al*. (2004)). AUCs represented the cumulated response from BT to the end of DI3 (Fig. 1), RP3 was excluded because healthy non-senescent mature leaves were rare at this time. AUCs data are available in supplementary data 5 to 8.

Clustering performed on AUCs, under control and alternation conditions highlighted genotype-dependant response in terms of leaf physiological and biochemical measurements (Fig. 5). Under control conditions (Fig. 5A), three genotypes constituted their own cluster Cervil, Criollo and Plovdiv due to specific leaf composition (starch, fructose and sucrose contents) for Cervil, transpiration and photosynthesis rate for Criollo, respiration and citric acid content for Plovdiv. Genotypes LA1420, LA0147, Stupicke, Ferum and Levovil constituted a single cluster due to similar light adaptive fluorescence of chlorophyll *a* value, photosynthesis rate and malic acid content. After the alternation (Fig. 5B), four clusters were observed. The genotypes Cervil and LA1420 had their own cluster, due to high values of $\Psi_{predawn}$ and Ψ_{midday} and high sucrose and fructose contents for Cervil, and to a specific profile of dark-adapted fluorescence of chlorophyll *a* (F_V/F_M, PI and J_0^{RE1}/J^{ABS}) for LA1420. The third cluster included Criollo and Plovdiv which had similar light-adapted fluorescence profile (F_V/F_M and PI), chlorophyll content and transpiration and photosynthesis rates under the alternation treatment. The fourth cluster included Stupicke, Ferum, LA0147 and Levovil, all large fruits genotypes, with similar chlorophyll content and transpiration rate under the alternation treatment.

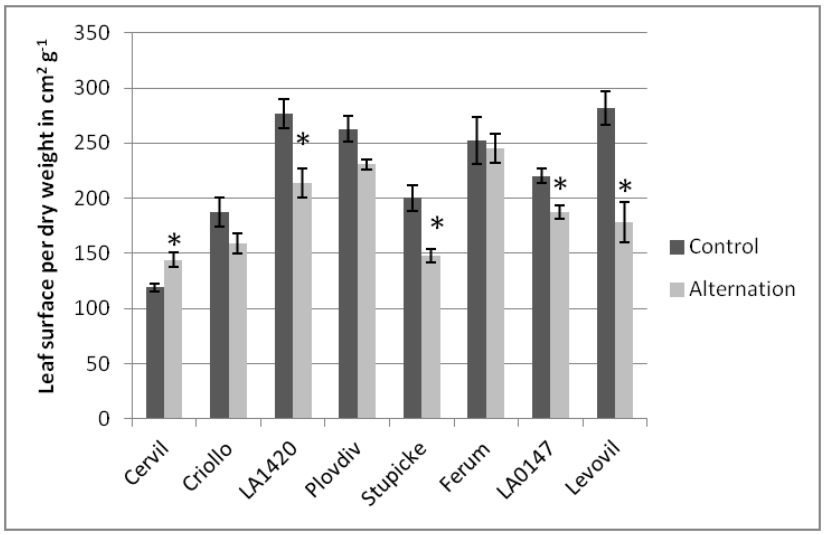

Figure 4: Specific leaf area of the 8 parents of the MAGIC TOM, measured at the end of the experiment on control and alternation treatment (3 deficit irrigation periods of increased intensity followed by recovery periods). Data are means ± SE ($n \geq 5$). Significant differences between control and alternation treatments are indicated by * symbols for $P < 0.05$ (Student test by genotype).

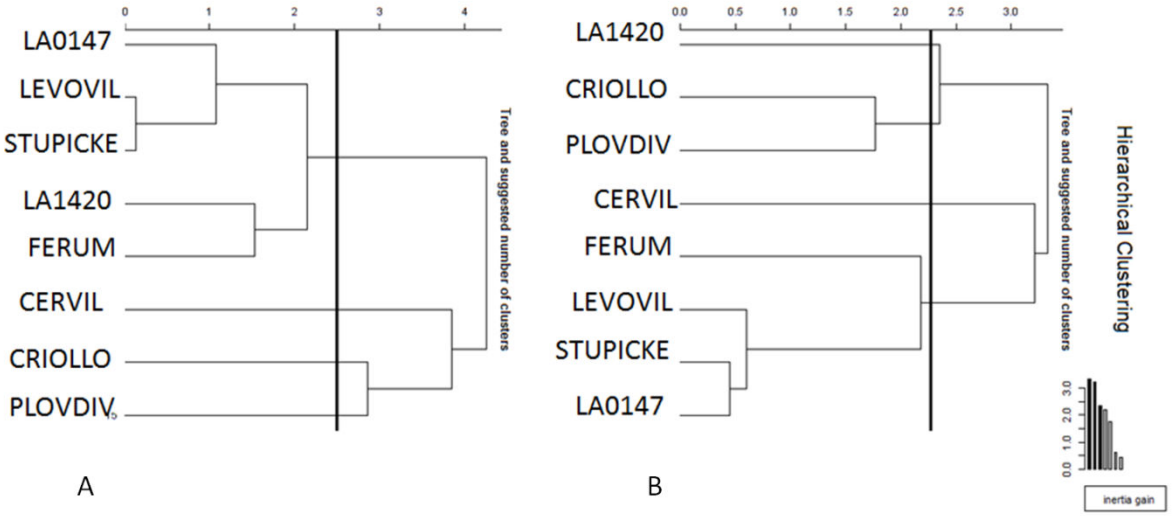

Figure 5: Clustering of genotypes according to the area under the curve (AUC) of leaf physiological and biochemical measurements (i.e. soluble sugars and organics acids) for control (A) and stressed (B) plants. AUC were calculated according to the trapezoidal rule for each variable measured at the end of each DI or RP periods (except RP3 which was not included in the area because of leaf senescence). AUC represent the dynamics of plant behaviour between control and alternation treatment. The alternation treatment consisted in 3 DI of increasing intensity followed by recovery periods. Clusters are defined according to the Euclidean distances (vertical bar in the graph) according to the inertia gain.

	CERVIL	CRIOLLO	LA1420	PLOVDIV	STUPICKE	FERUM	LA0147	LEVOVIL
Predawn water potential	***31,2***	19,0	***28,2***	***33,3***	***32,9***	***30,0***	15,8	5,3
Midday stem water potential	12,7	***28,4***	7,0	14,3	13,6	***54,9***	20,8	-8,7
Length*number of leaves	7,0	***-10,0***	-3,0	***-16,3***	-5,2	***-17,8***	***-8,6***	***-15,3***
Dry matter content	6,5	***18,6***	***12,7***	***13,9***	5,6	***12,5***	***13,5***	***15,8***
Citric acid	***-18,1***	-0,7	-6,9	3,7	-1,0	7,0	-7,4	***-12,1***
Malic acid	-7,3	9,4	***38,1***	***23,9***	***30,6***	***25,5***	5,2	***41,6***
Quinic acid	***21,5***	***41,1***	***24,7***	***34,1***	***25,3***	***27,9***	***66,6***	***64,9***
Total acids	-1,5	9,8	20,8	8,5	***20,3***	***22,0***	***19,3***	***19,6***
Glucose	***14,1***	***16,4***	35,5	***36,6***	***143,1***	9,9	***59,1***	***129,9***
Fructose	11,5	27,1	***11,1***	***30,6***	***71,8***	5,8	***47,6***	***72,1***
Sucrose	-5,0	12,4	4,4	9,6	8,6	5,7	***17,3***	10,5
Total sugars	8,3	***20,8***	***6,6***	***41,9***	***14,6***	65,1	***29,1***	***62,3***
Ratio SS/OA	13,4	5,3	-16,0	29,3	***-7,9***	***30,5***	***6,1***	***34,6***
Starch	-4,9	***79,2***	12,8	***242,0***	101,5	***195,5***	101,3	***212,4***
Stomatal conductance	4,7	83,9	-11,6	***-36,3***	-20,7	-18,2	***-23,6***	-18,8
Net photosynthesis rate	12,8	25,6	2,3	***-23,7***	-9,6	-6,2	-3,9	-4,2
Respiration	***20,1***	11,6	***3,2***	***25,5***	***19,4***	-1,4	21,2	6,3
Chlorophyll content	1,7	***6,8***	***10,9***	***2,7***	***16,1***	4,5	1,8	17,0
Transpiration rate	-13,8	17,5	-22,6	***-48,6***	***-39,1***	***-19,1***	***-31,2***	***-32,3***
Fv'/Fm'	***-15,1***	2,2	-2,1	-6,7	-0,3	-7,2	-1,8	-1,0
Fv/Fm	***-1,7***	-1,0	***-2,3***	***-2,6***	***-3,0***	***-2,8***	***-2,7***	***-2,7***
J_0^{RE1}/J^{ABS}	9,1	***25,1***	***17,6***	7,9	***24,7***	-0,6	***23,1***	11,1
PI_{ABS}	-12,6	-17,0	***-19,8***	***-19,4***	-11,1	***-30,9***	-9,2	***-32,0***

Color scale

-50 -25 -10 0 10 25 50 100 200

Table 2: Relative differences in plant and leaf variables between alternation treatment and control. Stem water potentials (in absolute values), leaf length x leaf number, leaf metabolite contents (soluble sugars, organic acids and starch on a dry matter basis) and leaf photosynthetic activity were measured at the end of the alternation for the 8 parents of the MAGIC TOM (ranged by increasing fruit size). Relative differences were calculated based on total area under the curves (AUC) as:

$$x\ (\%) = \frac{(\text{Mean DI [glucose]} - \text{Mean Control [glucose]})}{\text{Mean control [glucose]}} \text{x}100$$

The AUC of each variable was calculated for each genotype from DI1 until the end of DI3, except photosynthesis for Criollo and Plovdiv genotypes which began at RP1 (because of missing value in control plants). Data are means ± SE ($n \geq 5$). The percentages were scaled by colour (green for high and red for low values). Significant differences are indicated by bold, italic and underlined fonts for $P < 0.05$ (Two-way ANOVA test and Kruskal-Wallis test).

Genotypes gathered within a same cluster did not necessarily respond to the alternation treatment in the same way (Tab. 2, Supplementary data 1 and 2). On the whole, the alternation treatment induced a general decrease in transpiration (except Criollo) in relation with a decrease in stomatal conductance (except Cervil and Criollo). Surprisingly, the net photosynthesis rate was hardly affected by the alternation treatment over the whole experiment (except in Plovdiv), arguably as a consequence of photosynthetic adaptation. The F_V/F_M index decreased in all genotypes except Criollo, but it did not drop below 0.75 U.A (stress threshold see Stewart and Globig, 2011) throughout the experiment (not shown). AUC of the PI index of Strasser, which is more sensitive to WD than F_V/F_M, significantly decreased under the alternation treatment for four genotypes (-19.4 % in Plovdiv, -19.8 % in LA1420, -30.9 % in Ferum and -32 % in Levovil). On the contrary, under the alternation treatment the the quantum yield of the electron transport flux until PSI acceptors (J_0^{RE1}/J^{ABS}) increased in several genotypes (+25.1% in Criollo, +17.6% in LA1420, + 24.7% in Stupicke, +23.1% LA0147) as well as the relative chlorophyll content (+6.8 % in Criollo, +10.9 % in LA1420, +2.7 % in Plovdiv and + 16.1 % in Stupicke) and respiration rate (+3.2 % in LA1420, +19.4 % in Stupicke, +20.1 % in Cervil, +25.5 % in Plovdiv). Then, leaf dry matter content (Tab. 2) increased under the alternation treatment (except in Cervil and Stupicke) as well as the contents in malic (except Cervil, Criollo and LA0147) and quinic acids, in glucose (except LA1420 and Ferum) and fructose (except in Cervil, Criollo and Ferum), and in starch (except in Cervil and LA1420).

3.2 Effects of the alternation treatment on fruit size and composition

Fruit fresh mass, size, dry matter content and concentrations in metabolites (soluble sugars, organic acids, AsA and carotenoids) were measured on mature fruits sampled at different time during the experiment (Fig. 2).

A hierarchical clustering analysis based on fruit composition variations (dry matter basis) among genotypes is presented on figure 6 for the first lot of fruits (S1). Clusters indicate fruit traits that co-varied under a given condition and highlight the differences in metabolites concentrations among genotypes. Under both condition, total soluble sugars, total organic acids, lutein and β-carotene could be pooled together, as could be pooled together AsA and phytoene contents, on the one hand, and lycopene contents and total carotenoids, on the other hand. This figure highlights contrasted composition among genotypes under control conditions. For instance Cervil fruits which contained the most dry matter, had the lowest content in total sugars, acids, carotenoids and lycopene, but the highest content in total AsA

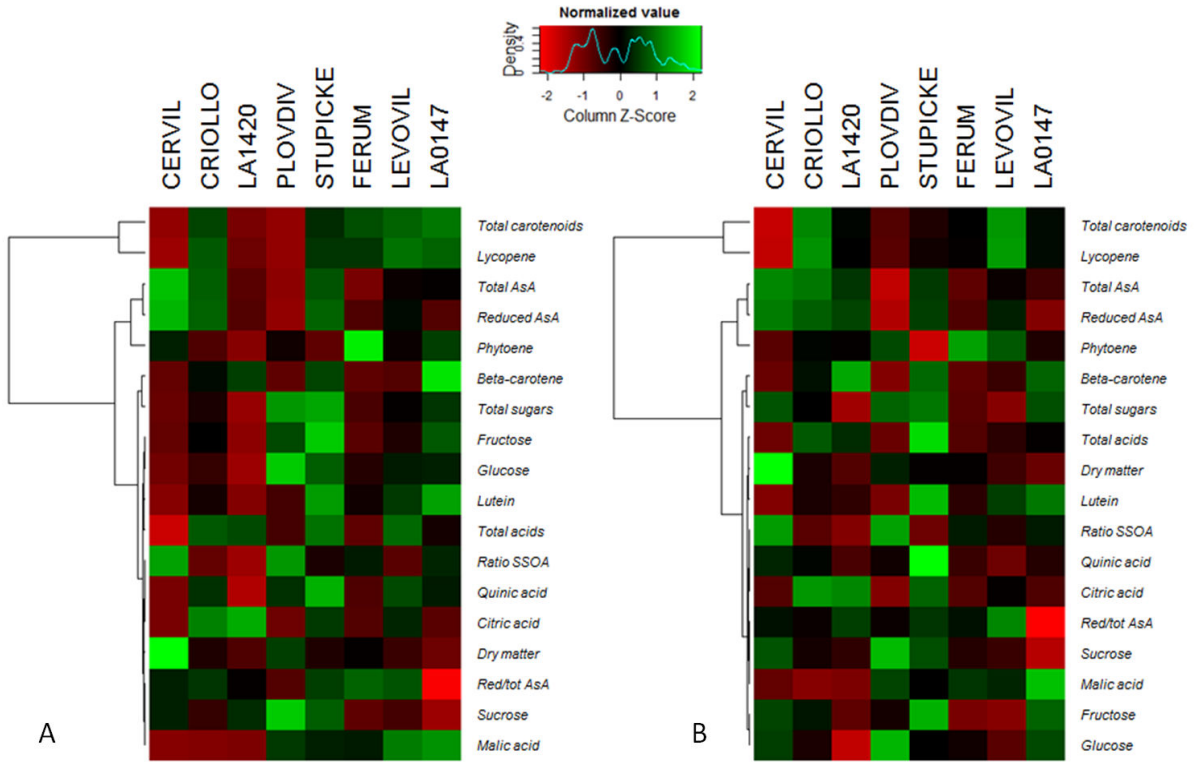

Figure 6: Heat map representation of fruit composition (contents in soluble sugars, organic acids, ascorbic acid (AsA) and carotenoids expressed on a dry matter basis), in MAGIC TOM, grown under control conditions (A) and under the alternation treatment (B) applied during the fruit cell division (DI1) and the cell expansion period (DI2) (lot S1). The alternation treatment consisted in 3 DI of increasing intensity followed by recovery periods. Data are centered and scaled by parameters ($n \geq 5$). Parameters are hierarchically classified according to Euclidean distances and ranged by color (green for high and red for low).

	CERVIL	CRIOLLO	LA1420	PLOVDIV	STUPICKE	FERUM	LA0147	LEVOVIL
Fruit size	2.3	-4.0	-6.8	4.2	-3.5	-3.7	-1.9	-5.6
Fresh weight	7.2	-5.7	-18.9	-3.3	-10.7	-11.0	-5.2	-12.7
Dry matter content	***8.9***	5.4	-0.3	-1.3	*12.7*	5.1	2.2	4.3
Glucose	***19.6***	7.9	7.1	-1.2	-2.9	6.8	-2.9	5.8
Fructose	***20.5***	6.1	12.9	-3.7	-1.8	5.0	-1.6	3.3
Sucrose	**29.7**	21.6	-19.6	11.9	9.3	36.9	8.7	-24.9
Total sugar	***20.3***	7.2	9.4	-2.1	-2.1	6.3	-2.1	4.3
Citric acid	9.4	-10.7	***-19.8***	-10.1	-4.7	-5.1	-20.0	-1.8
Malic acid	26.1	-6.6	-3.9	-5.9	-18.9	1.6	***-30.6***	1.1
Quinic acid	24.7	4.1	16.1	1.8	19.8	8.0	-10.4	1.2
Total acid	18.3	-4.9	-7.6	-3.8	3.5	1.7	-18.1	0.1
Sugar/acid ratio	0.6	12.2	18.0	2.0	-2.6	4.6	16.3	4.1
Lutein	-4.3	-6.6	45.5	-31.8	1.9	-15.2	-3.6	-14.2
Lycopene	-22.8	20.1	34.3	14.6	-11.6	-10.1	16.5	-12.3
Beta-carotene	-9.8	-0.2	24.5	-20.2	8.8	-6.9	1.9	***-20.4***
Phytoene	-19.7	30.6	48.6	27.3	-21.2	-6.2	29.4	-12.3
Total carotenoids	-21.4	19.9	35.3	14.4	-11.2	-9.4	17.2	-12.9
Reduced AsA	-9.0	-3.0	26.0	-16.0	-7.8	-7.0	-0.4	-17.2
Total AsA	-6.2	2.9	22.8	-17.3	-5.1	-1.3	-2.0	-12.1
Reduced/total AsA	-2.7	-6.0	2.3	1.5	-2.6	-5.8	1.7	-4.9
Starch	***56.3***							

Color scale: -40 -30 -20 -10 0 10 20 30 40 50

Table 3: Relative differences in fruit metabolite contents between alternation treatment and control. Soluble sugars, organic acids, ascorbic acid (AsA) and carotenoids were measured on a dry mass basis on the 8 MAGIC TOM parents. Relative differences were calculated as described in legend of Table 2. Data are means of pools of 5 fruits ± SE ($n \geq 5$). Fruits S1 were harvested in RP2 after the alternation of a first stress period during cell division (DI1) and a second stress period during cell expansion (DI2). Significant differences are indicated by red bold, italic and underlined fonts for $P < 0.05$ (Two-way ANOVA test and Kruskal-Wallis test).

(dry matter basis). LA1420 fruits were the poorest in all compounds except acids and β-carotene. On the contrary, Stupicke fruits were the richest in all compounds except phytoene. Similar results were observed in control fruits of the S2 lot of fruits (data not shown).

Data of fruit quality traits are presented on a dry and fresh matter basis for the two lots S1 and S2 (Tab. 3 and 4 respectively and Supplementary data 3 and 4). Though not significant, the decrease in fruit size and fresh mass was more pronounced on S2 fruits than on S1 fruits. For instance on Levovil, the fruit fresh mass decreased by 42.8 % on S2 fruits and by 13 % on S1 fruits. Cervil S1 fruits were the less sensitive (Tab. 3).

On a dry matter basis, the variations in fruit composition due to the alternation treatment were significant for five genotypes (Cervil, LA1420, Stupicke, LA0147 and Levovil) (Tab. 3, lot S1). In the case of Cervil fruit, an increase in dry matter content (+8.9 %), total sugars (+20.3 %) and starch content (+56.3 %) was measured. Acid contents dropped in LA1420 (-19.8 % citric acid) and LA0147 (-30.6 % malic acid) fruits. Stupicke fruits were richer in dry matter content (+12.7 %), but the dry matter composition was unchanged under the alternation treatment. In Levovil fruits, the β-carotene content (-20.4 %) was significantly reduced. Interestingly the contents in lycopene and carotenoids increased in four genotypes (Criollo, LA1420, Plovdiv and LA0147) whereas total AsA content decreased in all genotype except LA1420; however these variations were not significant. Consistent results were observed on the second lot of fruits (S2, Tab. 4) except for LA0147 whose contents in lycopene, carotenoids and total AsA were hardly affected. The fruit dry matter content increased in all genotype (expect Ferum) and to a larger extend in Cervil (+21 %), LA1420 (+22.3 %) and Levovil (+33.7 %). Among soluble sugars, the sucrose content was more affected than glucose or fructose (+47.7 %, +61.8 %, +64.1 % in Cervil, Stupicke and Levovil respectively).

On a fresh matter basis (Supplementary data 3 and 4), sugars and quinic acid contents were significantly increased in Cervil fruits (both S1 and S2), in Levovil fruits (S2) and quinic acid only in Stupicke (S1). For the S2 treatment, reduced and total AsA were increased in Cervil fruits, in LA1420 fruits and in Plovdiv fruits. So, metabolic and dilution effects were cumulated for the compounds that increased both on a dry and fresh matter basis (mainly sugars and acids). Whereas compounds that decreased in a dry matter basis were compensated by dilution effects that maintained fruit quality.

	CERVIL	CRIOLLO	LA1420	PLOVDIV	STUPICKE	FERUM	LA0147	LEVOVIL
Fruit size	-1.5	-9.7	-7.4	-9.1	-6.7	-1.0	-7.0	-14.7
Fresh weight	-0.2	-21.0	-20.2	-23.7	-16.1	-0.4	-18.6	-42.8
Dry matter content	***21.0***	14.0	***22.3***	9.0	8.7	-9.5	3.6	***33.7***
Glucose	23.9	-2.7	5.4	3.7	16.4	0.8	-1.8	10.3
Fructose	21.6	-1.5	0.9	3.5	12.3	5.9	-1.6	8.0
Sucrose	***47.7***	102.2	42.3	-4.7	***61.8***	-14.5	15.0	64.1
Total sugar	23.6	-0.7	3.7	3.3	15.2	3.0	-1.2	9.9
Citric acid	-4.0	1.4	***-27.0***	-4.6	-6.4	5.2	2.1	-21.5
Malic acid	-4.7	-4.3	14.6	4.0	-4.1	-13.1	0.3	***-33.4***
Quinic acid	***22.2***	-3.2	-6.7	5.0	-0.1	10.7	-0.3	7.6
Total acid	7.5	-0.8	-17.6	1.1	-3.5	5.7	1.0	-11.5
Sugar/acid ratio	15.0	-1.1	24.5	1.8	20.8	-2.3	-2.7	23.3
Lutein	-3.3	8.8	-20.9	28.8	10.8	29.4	32.9	-7.9
Lycopene	-30.0	-11.8	-4.1	1.2	-11.8	41.3	8.8	-9.9
Beta-carotene	-15.8	3.8	-26.1	-2.5	-7.7	8.6	6.4	***-24.6***
Phytoene	-18.3	-11.5	1.1	-9.2	-3.1	44.2	3.8	-0.3
Total carotenoids	-27.5	-11.2	-4.6	-0.4	-10.7	40.9	8.6	-9.8
Reduced AsA	7.7	-10.9	11.7	31.8	-7.2	11.8	5.8	-9.1
Total AsA	2.6	-7.4	10.4	19.8	-9.8	9.9	4.3	-15.8
Reduced/total AsA	4.8	-3.6	-0.1	11.0	3.3	1.4	0.9	8.0
Starch	***86.7***							

Color scale -40 -30 -20 -10 0 10 20 30 40 50

Table 4: Relative differences in fruit metabolite contents between alternation treatment and control. Soluble sugars, organic acids, ascorbic acid (AsA) and carotenoids were measured on a dry mass basis on the 8 MAGIC TOM parents. Relative differences were calculated as described in legend of Table 2. Data are means of pools of 5 fruits ± SE ($n \geq 5$). Fruits S2 were harvested in DI3 (Cervil, Criollo, Plovdiv and Stupicke) and RP3 (Levovil, LA1420, LA0147 and Ferum) after the alternation of a first stress period during cell division (DI2) and a second stress period during cell expansion and ripening (DI3). Significant differences are indicated by red bold, italic and underlined fonts for $P < 0.05$ (Two-way ANOVA test and Kruskal-Wallis test).

3.3 Partial correlations network among leaf and fruit traits under control and alternation treatment

A partial correlation network was build based on the AUC calculated for the different leaf and fruit traits (Fig. 7). Partial correlations measure the degree of association between two variables, taking into account the effects of other variables. Thus, partial correlation network allows inferring functional relationships among variables. Under both conditions, none of the fruit traits correlated with leaf traits indicating rather independent response pathways.

Under control conditions, four independent leaf clusters were observed (Fig. 7A). Leaf composition in starch and malic and quinic acids correlated positively with the leaf dry matter content and $\Psi_{predawn}$, whereas leaf fructose and glucose correlated one with each other. The net photosynthesis rate, the transpiration rate and the stomatal conductance were positively correlated. The last cluster included chlorophyll *a* fluorescence parameters, PI, J_0^{RE1}/J^{ABS} and F_V/F_M. Concerning the fruit traits, five independent clusters were observed under control conditions. Fructose, glucose and quinic acid contents were positively correlated one to each other, as well as lycopene and phytoene. The fruit dry matter content negatively correlated with the leaf respiration, while the fruit fresh mass negatively correlated with the β-carotene content. Finally fruit citric acid content positively correlated with lutein content but negatively with the malic acid content.

The network density was much lower under the alternation treatment compared to control conditions (Fig. 7B) suggesting important physiological adaptation. At the leaf level, the net photosynthesis did not correlate any more with the leaf stomatal conductance and transpiration rate. Similarly the the quantum yield of the electron transport flux until PSI acceptors (J_0^{RE1}/J^{ABS}) did not correlate any more with the PI and F_V/F_M indexes. These results suggested regulations of the photosynthetic capacity. Concerning the leaf composition, sugars (starch, fructose, glucose) and acids (malic and citric acids) constituted two independent clusters. At the fruit level, the fruit dry matter content negatively correlated with the ASA content, while the phytoene content positively correlated with the lycopene content, as well as the lutein and β-carotene contents and the glucose and fructose contents.

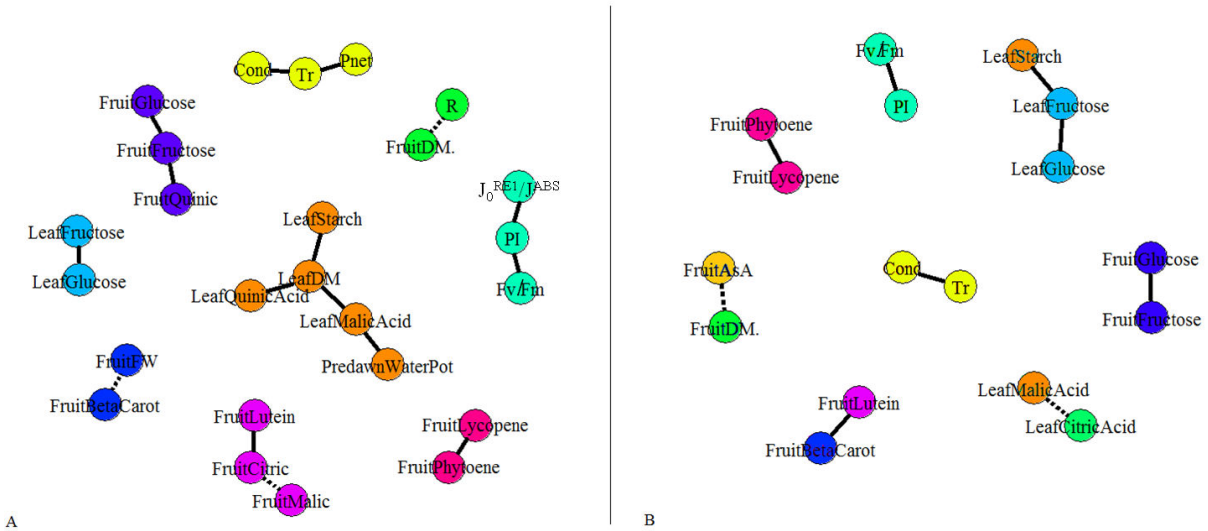

Figure 7: Partial correlation network among plant and fruit composition (dry matter content (DM), fruit fresh weight (FW), soluble sugars, organic acids, carotenoids and AsA contents) and physiological parameters (net photosynthesis rate (Pnet), leaf transpiration (Tr), stomatal conductance (cond), respiration (R), the maximum quantum efficiency of PSII Photochemistry on dark adapted leaves (F_V/F_M), the performance index (PI) and the quantum yield of the electron transport flux until PSI acceptors (J_0^{RE1}/J^{ABS}). Partial correlations were calculated on AUC for plant measurements and on S2 fruits (n = 5, 80 plants.) under control (A) and stress (B) conditions, using GGMselect, GeneNet and igraph packages on R. Solid lines indicated positive correlations between parameters whereas dotted lines indicated negative correlations.

IV- Discussion

In this study we intended to unravel the complex responses to repeated cycle of DI and RP periods in order to identify main mechanisms that could lead to interesting trade-offs between the expected negative impacts on plant growth and positive impacts on plant "memory effects" and fruit quality. Moreover the genetic variability in plant and fruit responses was assessed by investigating the 8 parents of the MAGIC TOM population which represent the highest allelic diversity in tomato accessions (Ranc, 2010). During each of the three DI periods, moderate WDs were applied which cumulated over the experiment with seasonal increase in temperature and decrease in air vapour pressure deficit, as they naturally occur in spring and summer commercial production. Overall, the alternation treatment consisted in a 25.6 % reduction of water supply compared to control plants. On their whole, results indicated that the responses to the alternation observed at the leaf and fruit levels were rather independent one from each other, independent on the species (*cerasiform* or *esculentum*), but highly dependent on the genotype. Although plant recovery after a period of DI was not trivial, a memory effect between two successive DI periods could be observed for some genotypes. Interestingly, the leaf photosynthetic activity could be maintained over the whole alternation treatment thanks to different adaptation mechanisms. Finally the expected improvement of fruit quality and reduction of fruit size in response to WD were only partial and dependant on stress intensity and genotype.

4.1 Recovery and plant memory effects during repeated cycles of DI and RP periods

Based on stem Ψ_{midday} (Fig. 3), which is a valuable indicator of the constraint imposed on plants by water availability in the root zone and by climatic conditions, genotypes could be distributed over three groups according to their response to the alternation treatment. Cervil, Ferum and Levovil were the most sensitive; Criollo and LA0147 were sensitive during DI1 only; LA1420, Plovdiv and Stupicke did not respond to the alternation treatment. Although the dynamics of Ψ_{midday}, net photosynthesis and leaf stomatal conductance highly varied among genotypes (Fig. 3), results suggested that a significant response to one DI/RP cycle coincided with the absence of response to the next cycle. This trend was observed for genotypes which were sensitive to the first cycle as well as for genotypes which were sensitive to the second DI/RP cycle. So the "memory effect" was not dependent on the genetic

level of sensitivity to WD. Whether this memory effect resulted from plant priming, is difficult to say.

Based on the three indicators presented on figure 3, plant recovery during RP periods was not as trivial as reported in the literature (Rahman *et al*., 1999; Xu *et al.*, 2010). This could be due to different plant developmental stage and or length and intensity of WD applied during the DI period but also to the cumulative effect of the 3 DI periods. Moreover in the present study, some genotypes exhibited a delayed response observed during the RP period which suggested a slow response or adaptation to the alternation treatment. Overall during the experiment the most intensive responses were observed during the first DI period although the WD intensity increased in DI2 and DI3. However this could be a bias due to the fact that control plants may have been stressed by the temperature increase at the end of the trial. Since control plants were not adapted to stress conditions on the contrary to plants of the alternation treatment, differences between the two treatments may have been attenuated.

4.2 Leaf responses to repeated cycles of DI and RP involved, hydric, osmotic and photosynthetic regulations

The cumulated effects of the alternation treatments were analysed through the differences in AUC between control and stressed plants (Tab. 3). Overall results are consistent with known responses to WD, involving important regulations of plant water relations (decrease in Ψ_{midday}, leaf stomatal conductance and transpiration rate), reduction of leaf growth, osmotic regulation (increase in acid, soluble sugars and starch) (Tardieu *et al*. 2006). Stomatal closure is one of the major and most well-documented effects of drought (Damour *et al.*, 2010). However this effect was not observed for two small-fruit genotypes (Cervil and Criollo). More generally the decrease in transpiration water loss associated to stomatal closure was essentially observed in large-fruit genotypes (Tab. 2) indicating that strategy to maintain water balance might be driven by the sink demand.

A general decrease in F_V/F_M and to a larger extend in PI was observed. The first indicated a reduction in maximum photochemical efficiency of light harvesting in photosystem II (Baker, 2008), but F_V/F_M ranged about 0.80 A.U. during WD and remained above the stress threshold of 0.75 A.U. defined for Arabidopsis (Stewart and Globig, 2011), suggesting that F_V/F_M is not highly sensitive to WD in tomato. PI is a global index of performance (expressed in analogy to the Nernst potential) which is composed of three components: the force due to the concentration of active reaction centres, the force of the light reactions which is related to the quantum yield of primary photochemistry and the force

related to the dark reactions (Živčák *et al*., 2008). PI has been defined as a "drought factor index" by Goltsev *et al*. (2012) during desiccation of beans, in accordance with the present results on tomato. Finally, the increase in chlorophyll content which indicates a low degree of photo-inhibition in leaves (Mäkelä *et al*., 2000) may have contribute to maintain the net photosynthesis rate as suggested by Stirbet and Govindjee (2011). Moreover, the increase in the quantum yield of the electron transport flux until PSI acceptors (J_0^{RE1}/J^{ABS}) could be explained by a return of electrons from PSI to PSII called the cyclic electron flow (Johnson, 2011). The cyclic electron flow is important in generating ATP and contributes to the balance of the ATP/NADPH output ratio (Johnson, 2011; Allen, 2003). The cyclic electron flow is also described as an orchestrator of the chloroplast energy budget, that increased under CO_2 limiting conditions and in response to environmental stressors such as high light, WD simulated by low CO_2 supply and extreme temperatures in higher plants (Livingston *et al*., 2010; Johnson, 2011; Walker *et al*., 2014). If these results were confirmed then the alternation treatment induced an adaptive strategy rarely expected in C3 plants (Johnson, 2005).

The analysis of leaf composition outlined an important increase in soluble sugars, starch, malic and quinic acids, except in Cervil which accumulated only quinic acid and in LA1420 which did not accumulate starch. Soluble sugars and starch were likely involved in osmotic regulations. The increase in malic acid content which was not correlated with citrate, let assume that reactions in the Krebs cycle promoted malic acid content. This increase in malic acid could supply the malate/aspartate shuttle, but further studies are needed including specific enzymes of the malate/aspartate shuttle. The malate/aspartate shuttle is a biochemical system involved in the translocation of electrons produced during the glycolysis and it has been reported to be the main route for re-oxidation of cytosolic NADH accumulating under stress conditions (Pastore *et al.,* 2003; 2007). Moreover, a significant increase in quinic acid was observed in all genotypes due to the alternation treatment. Quinic acid has been described as an inhibitor of ATP-synthesis which dissipated H^+-uptake and accelerated electron transport, like a non-classical decoupler on photophosphorylation (Barba-Behrens *et al*., 1993). It is also linked to the shikimate and phenylpropanoids pathways which are involved in the regulation of plant growth and act as antioxidants in lettuce (Yoshioka *et al*., 2004; Oh *et al*., 2010) and others species (see review of Ripoll *et al*., 2014).

4.3 Alternation treatment reduced fruit growth proportionally to stress intensity

Depending on its intensity, WD is expected to decrease fruit size and fruit water content, thus increasing the metabolite contents through dilution effects, and to stimulate the accumulation of osmotic and antioxidant compounds (Ripoll *et al*., 2014). Although the absence of significant response, fruit size was reduced by the alternation treatment (except in the cherry tomato Cervil with no impacts) with more pronounced effects in S2 fruits which is consistent with the idea that fruit yield decreases proportionally to the intensity of WD (Wang and Gartung, 2010). Moreover, competition for carbon was likely higher during development of S2 fruits compared to S1 fruits, due to the cumulative effects of the three DI periods on plant growth. Competition for carbon may explain that the alternation treatment had quite variable effects on fruit size among genotypes. Indeed in some studies, WD has been reported to have positive effects on fruit growth due to a negative regulation of fruit setting and thus an increase of carbon supply to the remaining fruits (Li *et al*., 1989; Vallverdu *et al*., 2012). This may explain for instance the opposite effects of alternation on fruit size measured on Ferum and Levovil two large-fruit genotypes (Tab. 4) although in the present experiment trusses were pruned to regulate fruit load and no significant differences were observed between control and alternation plants (data not shown).

4.4 Alternation treatment had beneficial effects on fruit sugar content despite low effects on primary metabolites except in cherry tomato

As discussed for fruit fresh weight, the increase in dry matter content was higher in S2 fruits than in S1 fruits, strengthening the idea that S2 fruits were submitted to higher stress intensity. The strongest increase in fruit dry matter content under alternation was observed on large-fruit genotype (Levovil) as well as on cherry tomato (Cervil). On the contrary, changes in dry matter composition were more pronounced in S1 fruits than in S2 fruits and responses were highly dependent on genotype. Variations in fruit composition in response to WD, may result either from dilution/dehydration effects (Etienne *et al.*, 2013; Guichard *et al.*, 2001), from active solute accumulation (Lo Bianco *et al.*, 2000; Hummel *et al.*, 2010), or from starch breakdown, as observed on tomatoes under salinity-induced WD (Balibrea *et al.*, 2003). In the present study we discussed variations reported on both dry and fresh matter basis to separate dilution and metabolic responses.

Soluble sugars and organic acids (primarily malic and citric acids) are major osmotic compounds that accumulate in fleshy fruits and that determine fruit taste. On a dry matter basis, sucrose was the most affected by the alternation treatment among soluble sugars, but it represents only a minor part (< 3 %) of total soluble sugars in these genotypes. Previous

studies on tomatoes reported an increase in fruit sugar content under WD depending on cultivar and timing of stress (Bertin *et al*., 2000, Veit-Köhler *et al*., 1999, Chen *et al*., 2014). In the present study, the total content in soluble sugars on a dry matter basis was not strongly affected by the alternation treatment except in Cervil fruits which also accumulated larger amount of starch and acids. Thus, in cherry tomato, important osmotic regulations through the accumulation of starch, soluble sugars and acids may explain that fruit fresh weight was not reduced by the alternation treatment. Other genotypes do not accumulate starch in ripe fruits which may appear as an interesting adaptive trait to maintain fruit growth under WD. On a fresh weight basis, the increase in fruit sugar content in response to WD was observed only in Cervil and Levovil (Supp. Data 3 and 4), which questioned the idea that WD conditions promote the final fruit taste (Ripoll *et al*., 2014) and suggested a strong genotype effect in this response. The effects of WD on fruit acidity are more conflicting (Etienne *et al*., 2013). In many species (peach, clementine, mandarin, pear, tomato), water supply has been shown to negatively correlate with organic acid content in ripe fruits, but in grapes, nectarines (Etienne *et al*., 2013) and tomatoes (Bertin *et al*., 2000; Mitchell *et al*., 1991; Veit-Köhler *et al*., 1999), this correlation has been shown to be positive. The present study pointed to a genotype-dependent response with an increase in acidity in cherry tomato only.

4.5 Effects of the alternation treatment on fruit carotenoid and ascorbic acid contents ranged from negative to nil to positive

Fruits supply a large range of health-promoting phytochemicals, of which secondary metabolites, primarily terpenoids (carotenoids, ABA and others) and phenolic compounds, are the largest group along with AsA. Of all of the environmental factors that play a stimulating role in the synthesis and accumulation of useful phytochemicals in fruits, moderate stress, and more specifically, controlled drought may influence the metabolism of these phytochemicals via at least two major mechanisms that are not mutually exclusive and that may even interact (Nora *et al.*, 2012; Poiroux-Gonord *et al.*, 2013b; Fanciullino *et al.*, 2014). First, drought typically induces a decrease in net photosynthesis rate which reduces the supply of primary metabolites to the fruits that are the major source of precursors for the biosynthesis of phenolic compounds, carotenoids and AsA. Second, drought may exacerbate oxidative stress and signalling which is known to directly and indirectly influence the biosynthetic pathways of these compounds (Fanciullino *et al.*, 2014). In the present study, the effects of the alternation treatment on fruit carotenoid content ranged from negative, to nil to positive depending on genotype and stress intensity (lots S1 or S2) on a dry matter basis. This is in

complete agreement with the range of responses to WD reported in the litterature (reviewed by Ripoll *et al*., 2014). Similarly, total AsA was reduced in all genotype but one (LA1420) in S1 fruits, whereas more variable effects were observed on S2 fruits. Many studies reported positive effects of WD on AsA (Favati *et al.*, 2009; Murshed *et al.*, 2013; Veit-Köhler *et al.*, 1999; Zushi and Matsuzoe, 1998), but also indicated variable effects depending on genetic and seasonal factors or the intensity and duration of the treatment. In S1 fruits, carotenoid accumulation (on dry matter basis) was increased in four genotypes and lessened in four other genotypes including cherry tomato and large-fruit genotypes. Alltogether, the present results refute the hypothesis that the supply of carbon to fruit could dertermine carotenoid or AsA synthesis. In tomato fruit, the absence of correlation between sugars and reduced AsA content also suggests that fruit AsA content is not limited by leaf photosynthesis or sugar availability (Gautier *et al.*, 2009). Thus variations in carotenoids and AsA content would merely result from the stress-induced cellular redox state (Fanciullino *et al.*, 2014). In tomato plant, AsA content has been suggested to correlate with resistance to WD (Garchery *et al.*, 2013; Zhang *et al.*, 2011). However in the present study, only one genotype (LA1420) exhibited an adaptive response at the fruit level through an increase in both carotenoid and AsA contents. On a fresh matter basis, the fruit health value was improved by the alternation treatment only in cherry tomato (Cervil) and small fruit size genotypes (LA1420 and Plovdiv) through an increase in reduced AsA.

V- Conclusion

In the present study, the alternation treatment which consisted of three successive cycles of WD and recovery periods during the plant reproductive period induced independent responses pathways at the leaf and fruit levels. In terms of photosynthesis, plant memory effects could be observed between two successive periods of WD. The cyclic electron flow (extrapolated from results of J_0^{RE1}/J^{ABS}), the quinic acid and the malate/aspartate shuttle were suggested to be involved in the energy-dissipation and oxidative stress regulation during the alternation treatment for some genotypes. Negative effects on fruit fresh weight were dependent on stress intensity, while beneficial effects on fruit taste (sugars and acids) were modest and obs erved on two genotypes only (on a fresh weight basis) and resulted from both metabolic and dilution effects. No clear increase in the accumulation of health compounds could be observed. Cervil, a cherry tomato was the less sensitive to the alternation treatment, hypothetically due to the accumulation of starch in fruits. However this hypothesis should be further investigated.

Acknowledgements

This paper was supported by the Federative Research Structure Tersys. The authors thank the team of the UR1052 Genetics and Improvement of Fruit and Vegetables (INRA, Montfavet) for their strong implication in this study. The authors thank also the laboratory of the unit "Plantes et Systèmes de culture Horticoles" (INRA, Avignon) for their help in the analysis of all these samples. Thanks to Béatrice Brunel, Alain Goujon and Jean-Claude l'Hôtel for technical assistance.

Beneficial effects of water deficit on tomato depend on fruit developmental stage and genotype

Dans le chapitre II, nous avons vu que l'alternance de WD et de RP conduits à des modifications de la masse fraiche et de la composition des fruits, qui sont dépendantes de la phase de développement affectée par les périodes de WD, de l'intensité du WD et du génotype.

Nous nous sommes donc interrogés sur les effets d'un WD d'intensité plus élevée que celles appliquées dans le chapitre II tout en restant modéré, sur la qualité des fruits, en fonction de la phase de développement du fruit qui subit le stress.

Nous avons donc appliqué un WD sur chacune des trois phases de développement du fruit: division cellulaire, expansion cellulaire et maturation. D'après le précédent chapitre, nous avons sélectionné deux génotypes Plovdiv et LA1420 parmi les huit parents de la MAGIC TOM, pour leur calibre de fruit semblable (Tab. 2, Chapitre II), mais aussi pour leurs sensibilités contrastées au WD et pour leurs vitesses de développement similaires qui ont permis l'application de stress simultanés.

Les effets des WD sur le fonctionnement des plantes ont été évalués au travers de la régulation des flux d'eau dans les feuilles (potentiels hydriques, potentiels osmotiques, conductance stomatique et transpiration) et de la croissance de la plante. La qualité des fruits récoltés a été évaluée en termes de croissance et de teneurs en sucres solubles, acides organiques, caroténoïdes et acide ascorbique.

Ce chapitre est présenté sous la forme d'un article en cours de préparation.

Résumé

De nombreuses études ont préconisé que le déficit hydrique (WD) peut exercer des effets bénéfiques sur la qualité des fruits mais avec des pertes de rendement. Cependant, la réponse des fruits en fonction de stades spécifiques de développement du fruit a été moins étudiée, bien que des mécanismes différents puissent être induits à chaque stade et induire des effets différents sur la qualité finale des fruits. Dans cette étude, un WD modéré de -60% d'apport en eau a été appliqué pendant chacune des trois étapes de développement du fruit, à savoir la division cellulaire (CD), l'expansion des cellules (CE) et la maturation (MT). Deux génotypes de tomate cocktail ont été utilisés, un tolérant au stress hydrique (LA1420), et l'autre sensible (PlovdivXXIVa, nommé Plovdiv). Des réponses contrastées ont été trouvées entre ces génotypes. Pour les deux génotypes, la masse fraiche des fruits et leur taille n'ont pas été réduites par le WD. La masse fraiche a même été augmentée pour LA1420 lors du traitement CD. Les fruits de LA1420 ont accumulé plus de sucres (à la fois en teneur en matière sèche et fraiche) et moins d'acides (en matière sèche) en conditions de WD, quel que soit le stade de développement affecté. Au contraire, la composition des fruits de Plovdiv n'a pas été affectée suite aux traitements CD et CE. De plus, les fruits du génotype Plovdiv ont eu une réponse négative avec globalement moins de sucres, moins d'acides et moins de caroténoïdes suite au traitement MT (à la fois en teneur en matière sèche et fraiche). Cependant, les teneurs totales en caroténoïdes et en acide ascorbique (AsA) n'ont pas été influencées par les déficits hydriques appliqués quel que soit le génotype et le stade de développement du fruit, principalement en raison de l'intensité modérée du WD. Fait intéressant, la qualité des fruits a été positivement influencé par un WD modérée dans le génotype de moins bonne qualité (LA1420) que dans le génotype de meilleure qualité mais plus sensible au WD (Plovdiv).

Mots clés: Déficit hydrique, Phase de développement des fruits, Qualité, Croissance, *Solanum lycopersicum* L.

Abstract

Many studies have advocated that water deficit (WD) may exert beneficial effects on fruit quality, but with yield losses. However, the fruit response to WD at specific developmental stage was seldom investigated, although different mechanisms could be involved at each stage and lead to different effects on final fruit quality. In the present study, a moderate WD of -60 % of water supply was applied during each of the three stages of fruit development, namely cell division (CD), cell expansion (CE) and maturation (MT). Two cocktail tomato genotypes have been studied, one tolerant to water stress (LA1420), and the other one sensitive (PlovdivXXIVa named Plovdiv). Contrasted responses were found between the two genotypes. For the two genotypes, fruit fresh mass and size were not reduced by WD. Also, fruit size of LA1420 fruits was increased with the CD treatment. LA1420 fruits accumulated more sugars (both on a dry and fres matter basis) and fewer acids (dry matter basis) in WD conditions, whatever the developmental stage affected. In contrast, the composition of Plovdiv fruits was not affected by the CD and CE treatments. Furthermore, Plovdiv fruits accumulated fewer sugars, acids and carotenoids during the MT stress (both on a dry and fresh matter basis). However, total carotenoids and total ascorbic acid (AsA) were not influenced by the WD treatment in either genotype and whatever the fruit developmental stage, mainly due to the moderate intensity of WD. Interestingly, fruit quality was positively influenced by a moderate WD in the genotype with poorer quality (LA1420) than in the genotype with higher quality but more sensitive to the WD (Plovdiv).

Key words: Deficit Irrigation, Fruit development phases, Quality, Growth, *Solanum lycopersicum* L.

I- Introduction

In the context of climate change, limitation of water resources is expected to exert an adverse impact on plant or crop growth and productivity (Shao *et al*., 2008). To cope with conditions characterized by water deficit (WD), different innovative irrigation strategies have been evaluated such as partial root zone drying (PRD) and regulated deficit irrigation (RDI), which both improve the soil exploration by roots and thus may increase water use efficiency (Stikic *et al*., 2003; 2014). However plant growth and crop productivity under PRD and RDI conditions may be adversely affected depending on intensity, timing and duration of WD, as well as on genotype, as observed in tomato (Davies *et al*., 2000; Marjanovic *et al*., 2012). Plant responses to WD have been extensively studied (Mitchell *et al*., 1991; Plaut *et al*., 2004; Costa *et al*., 2007). In terms of stomatal conductance and water potential regulations, these responses are species-specific and highly dependent on stress intensity and duration (Tardieu *et al*., 2011; Hetherington and Woodward, 2003). WD may induce a decrease in stomatal conductance and in water losses by transpiration (Blum, 1996). Unfortunately, more substantial decreases in stomatal conductance result in decreased photosynthesis, carbon gains, plant growth and crop productivity (Chaves *et al*., 1991; Xoconostle-Cazares *et al*., 2010). Aside from the negative impact on plant growth, beneficial effects on fruit quality may be expected, related to higher sugar accumulation and increased antioxidant activity (Ripoll *et al*., 2014; Tardieu *et al*., 2010). However, positive effects on fruit quality may be contradictory according to the developmental stage affected by stress and to the genotype under investigation (as resumed by Ripoll *et al*., 2014). As stressed by these authors, to take full advantage of irrigation management techniques we need to increase our understanding of the way WD impacts yield and quality criteria depending on genetic factors and on the stage of development of the considered plant or organ at the time WD is applied.

Most studies dealing with the effects of WD on fruit quality applied a constant WD over the whole fruit development. Few of them have investigated the effect of WD as a function of fruit development stage. Fleshy fruit development is divided in three phases: cell division, cell expansion and ripening. The duration of the cell division period varies according to genotypes (about 10 days for small tomato fruits and 25 days for big tomato fruits) (Gillapsy *et al*., 1993; Bertin *et al*., 2007). This phase strongly influences the final fruit size in

many species like tomato, through the number of cells and the sink activity of developing seeds (Varga and Bruinsma, 1976; Bertin *et al*., 2003; Bohner and Bangerth, 1988; Prudent *et al*., 2010). It has been suggested that WD applied during the cell division phase could induce carbon starvation and thus negatively regulate cell division and fruit tissue development in tomato (Prudent *et al*., 2010; Bertin *et al*., 2005; Baldet *et al*., 2006). However controversial studies suggest that WD applied at the cell division phase could improve carbon supply to the remaining fruits by a negative effect on fruit setting and fruit load. Regarding the fruit composition, moderate WD has been shown to improve peach fruit size and quality (sweetness and flavor) when applied during the stages of cell division and of rapid endocarp hardening (Li *et al*., 1989; Vallverdu *et al*., 2012). Furthermore, a low carbohydrate supply (i.e. in soluble sugars) during cell division was shown to promote the synthesis of carotenoids in clementine (Poiroux-Gonord *et al*., 2013).

Cell expansion is triggered by water entering in the cell. The water influx is dependent on the stem-to-fruit gradient of water potential generated by the gradient of osmotic potential which exists between source and sink tissues (Münch, 1930). Cell expansion is therefore related to sugar metabolism and sub-cellular compartmentalization (Lockhart, 1965; Ho, 1988). WD applied during cell expansion is expected to have the greatest impact on fruit growth considering the changes in water relationships and in cell wall properties occurring at that specific stage of development of the fruit (Schopfer, 2001). In peach, a negative impact on yield was observed associated to a decrease in water content (Li *et al.*, 1989; Girona *et al*., 2004).

Finally, fruit ripening consists in a rapid slowdown of cell expansion and an increase in metabolic changes. Several hormonal regulations and metabolic changes occur during this phase and WD may interact with the stress-induced increase in ethylene synthesis (Fray *et al*., 1994; Barry and Giovannoni, 2007). Furthermore, the accumulation of phytochemicals such as carotenoids or ascorbic acid (AsA) was reported to be ethylene-dependent (Iglesias *et al*., 2001). In peach, DI applied during ripening induces a yield reduction proportional to the reduction of water supply (Wang and Gartung, 2010).

In this study, a comprehensive experimental approach on tomato fruit was developed with the objective i) to better quantify the effect of WD on tomato fruit growth and quality traits as a function of the fruit developmental stage at the time WD is applied, and ii) to

evaluate the effect of genetic factors. Moderate DI (-60 % of water supply compared to the control) was applied during the three major phases of fruit development, namely cell division, cell expansion and maturation. Two cocktail tomato genotypes known for distinguishing themselves in terms of fruit quality traits and of sensitivity to WD were used. Fruit fresh and dry masses, cell pericarp number and size, seed weight, and the contents in soluble sugars, organic acids, ascorbic acid and carotenoids were measured along with indicators of plant and fruit water status. Our observations support the idea that fruit quality can be improved using moderate WD without fruit yield reduction. However, responses to WD depended on the fruit stages and responses were genotype dependant. Results complete conclusions achieved in similar studies on different plant species and contributes to a better understanding of the tomato plant and fruit responses under moderate WD.

II- Materials and Methods

2.1 Plant material & experimental conditions

Two indeterminate cocktail *Solanum lycopersicum* L. genotypes (fruit fresh weight between 20 and 70 g), were selected for their contrasted sensitivity to WD (unpub. data). The first genotype is PlovdivXXIVa (here called Plovdiv), a cultivated tomato whereas the second LA1420 is a wild tomato. LA1420 seeds were provided by the Tomato Genetics Resource Centre, Davis, California. Plovdiv seeds were provided by the Genetic Resource Centre in INRA, Avignon (France).

The trial was conducted during autumn 2012 in a glasshouse located near Avignon, France. Climate conditions (light intensity, temperature and humidity) in the glasshouse were measured every 30 minutes and recorded during the whole period. The mean daily photosynthetically active radiation (PAR) decreased over the trial period due to seasonal effects, whereas the air temperatures and relative humidity remained in relative narrow ranges (Tab. 1).

Plants were grown in 4L pots filled with compost (substrate 460, Klasmann, Champety, France) distributed among four rows (one row represented one irrigation treatment) of 18 plants each (9 per genotype and treatment) at a density of 1.3 plant m^{-2}. Plants were daily supplied with a nutrient solution (Liquoplant Rose, Plantin, Courthézon, France) (1.8 mS cm^{-2}, pH 6.8, mean for the whole period). Irrigation was monitored according to soil humidity measured every two days in all pots using water content sensors (WCM-control, Grodan, Roermond, The Netherlands) placed 90 degrees from drippers, and controlled using evapotranspiration values calculated according to Hamon (1963). In control plants, soil humidity and drainage were maintained at 70 % and 15 %, respectively.

The deficit irrigation (DI) treatments consisted in 3 periods of reduced water supply by 60 %. Each period corresponded to one of the three fruit developmental stages: cell division (from anthesis to 21 days after anthesis (DAA), called CD), cell expansion (from 21 to 45 DAA, called CE) and ripening (from 45 to 55 DAA, called MT). Different lots of plants were

Period	Start of DI period	End of DI period or trial	Mean temperature in °C		Mean air humidity in %		Mean Cumulative PAR in mol m-2 day-1
			day	night	day	night	
Before treatment	17/10/2012	24/10/2012	24.5	19.7	62.4	72.8	15.59
CD treatment	25/10/2012	20/11/2012	22.9	17.4	57.1	66.0	18.21
CE treatment	09/11/2012	06/12/2012	21.8	16.6	59.9	66.0	13.00
MT treatment and until the end of the trial	30/11/2012	11/01/2013	20.4	15.1	56.0	59.5	8.45

Table 1: Day and night mean temperatures and air humidity, and mean cumulated daily photosynthetically active radiation (PAR) for each considered fruit developing phase. CD treatment corresponds to the deficit irrigation (DI) applied during the cell division period, CE treatment to the DI applied during the cell expansion period, and MT treatment to the DI applied during the fruit maturation period.

used in order to apply WD during the same climatic period for the three fruit developmental stages of both genotypes. For this purpose, different trusses were prone to WD and the same trusses were observed on control plants, with truss 4 and truss 5 (CD treatment), truss 3 and truss 4 (CE treatment), and truss 2 and truss 3 (MT treatment), for Plovdiv and LA1420 plants respectively. Anthesis of a given truss was equated with the anthesis of the second flower. Preliminary experiments demonstrated that four days are necessary to reach stable soil humidity after the onset of a water restriction of about 60 % in the conditions of our trial. Therefore DI treatments were applied four days before anthesis of the target truss and stopped three days after the end of the considered development phase. Treatments were applied simultaneously on both genotypes. The substrate water content was maintained at 32 % in the CD treatment, at 35 % in the CE treatment, and at 39 % in the MT treatment. At the end of each period of water restriction the substrate water content was brought back to 70 % (data not shown). Plant and fruit measurements were made on 5 plants of each genotype and treatment (40 plants in total). All trusses were pruned to 6 set fruits per truss with the goal of ensuring a similar fruit load in all treatments. Flowers were pollinated three times a week using an electrical bee.

2.2 Plant measurements

The plant responses to DI were assessed just before and at the end of each DI treatment. Before the onset of each DI treatment, all parameters were homogeneous.

Stem water potential was measured using a pressure chamber (SAM Précis 2000 Gradignan, France) at predawn ($\Psi_{predawn}$) and around noon (Ψ_{midday}), before, during and after each DI period ($n \geq 5$). Leaves were bagged the day before, at the beginning of the night. After Ψ_{midday} was measured, leaves were frozen in liquid nitrogen and kept at -20 °C. Sap was extracted on thawed samples using a 500 µl syringe, and then filtered through cotton (as described in García *et al*. (2007). The leaf osmotic potential ($\Psi\pi$) was measured using a Vapro 5520 (Wescor Inc., Logan, U.S.A.). The stomatal conductance and the transpiration rate were measured at noon on 5 plants of each treatment and genotype, using a portable gas exchange measurement (ADC 4.0, ADC BioScientific Ltd., Hoddesdon, U.K.). The specific leaf area (SLA), which is defined as the ratio of leaf area to dry weight, was measured at the end of each DI trial at 11 a.m. ($n \geq 5$), using a planimeter (Li-3100 C Area Meter, LICOR, Lincoln, Nebraska, U.S.A.) and a ventilated oven (70 °C for 7 days). Four leaflets of adjacent leaves to the target truss were harvested at 11 a.m., at the end of each DI period, and dry

matter content was measured after lyophilisation (Virtis Genesis, SPScientific, Stone Ridge, U.S.A.).

2.3 Fruit measurements

All fruit measurements were made on fruits showing a uniform red colour. Fruits were harvested according to their position on the truss as specified below on all plants (72 plants in total). Fruit size and fresh weight were measured directly after harvest.

The third fruit of each considered truss was harvested 30 days after anthesis (DAA) for pericarp cell counting after tissue dissociation according to a method adapted from Bünger-Kibler and Bangerth (1983). Cells were counted using a microscope equipped with a camera (QImaging, Surrey, Canada) and the software Qcapture Pro 6.0 (QImaging, Surrey, Canada) (Bertin *et al*., 2002).

The second and the fourth fruit of each truss were frozen in liquid nitrogen and kept at -80 °C prior to biochemical analysis of pericarp soluble sugars, organic acids, carotenoids, ascorbic acid and dry matter content. Fruits harvested on all plants were pooled into 5 biological pools of 2 to 3 fruits per trusses for each treatment and genotype. Soluble sugars (i.e. glucose, fructose and sucrose) and organic acids (i.e. citric acid, malic acid and quinic acid) were extracted according to the method described in Gomez *et al*. (2002) and analysed by HPLC (Waters 410, Part WAT070390, Milford, U.S.A.). AsA content was measured according to the method described in Stevens *et al*. (2006), and the absorbance was read at 550 nm using a Multiscan Ascent MP reader (Labsystems, Thermo Fisher Scientific, Courtaboeuf, France). Carotenoids (i.e. lycopene, phytoene, β-carotene and lutein) were extracted according to the method described in Serino *et al*. (2009) and assayed by HPLC with a UV-visible detector (UV6000LP, Thermo Separation Products, Riviera Beach, U.S.A.). Totals sugar, total acid and total carotenoid contents corresponded to the sum of the individual metabolites.

Fruit water potential Ψ_F was measured on cut slices of the fifth and sixth fruits (pericarp + placenta) using a WP4C Dewpoint Potentiometer (Decagon devices, Pullman, U.S.A.) following the procedure of Léchaudel (2004). Fruit juice was extracted using a 500 µl syringe, filtered through cotton and then placed in a vapour pressure osmometer Vapro5520 (Wescor Inc., Logan, U.S.A.) to measure the fruit osmotic potential $\Psi\pi_F$.

2.4 Measurements performed on seeds

Seeds were extracted from 5 fruits of each treatment and put to soak in a solution of HCl (34 %, 1 L) with rapidase added (0.36 g L^{-1}) plus water (49 L). After 24 h the suspension was agitated and washed three times with water. Seeds were then bleached (9.6 %) for 30 min. and rinsed three times under water. Non-viable seeds floating on the surface at each wash were eliminated. Viable seeds were counted and the dry mass of 50 seeds per fruit was weighted after 5 days of drying.

2.5 Statistical analysis

Fruits traits (potentials, seeds characteristics, masses and composition) were analysed by two-analysis of variances (ANOVA) between genotypes and DI treatments, if the residue normality and the homoscedasticity of variances of the residues authorized this (Shapiro test, Royston, 1995; and Levene test, package car, Fox and Weisberg, 2011) and followed by multiple comparisons of means (lsmeans package; Lenth, 2014). Alternatively, the non-parametric Kruskal-Wallis test was applied (pgirmess package; Giraudoux, 2014).

All plants measurements were analysed using Duncan (package Laercio, Laercio, 2010) or Kruskal-Wallis test for comparison between control and DI values for each genotype.

Details of the applied parametric or non-parametric tests are given in the legendary of each figure. Then, a heat-map of the fruit response according to its composition was realized (gplots package; Warnes *et al*., 2014). All statistical analyses were performed using R 3.1.0 (R Core Team, 2014).

III- Results

3.1 Responses to DI treatments measured at the plant and leaf levels

Compared to the control plants, $\Psi_{predawn}$ was significantly lower in the CD treatment (LA1420: -0.27 vs. -0.13 MPa; Plovdiv: -0.34 vs. -0.17 MPa; $P < 0.05$) (Fig. 1A and 1C). Ψ_{midday} was lower in LA1420 plants of the CD treatment (-0.33 vs. -0.15 MPa; $P < 0.05$) and in Plovdiv plants of the CE treatment (-0.40 vs. -0.15 MPa; $P < 0.05$). $\Psi\pi$ was lower in LA1420 plants of the CD treatment (-1.08 vs. -0.77 MPa; $P < 0.05$) and $\Psi\pi$ was lower in Plovdiv plants of all DI treatments (-0.98 vs. -0.84 MPa, -0.98 vs. -0.82 MPa and -1.03 vs. -0.90 MPa, respectively; $P < 0.05$) (Fig. 1B and 1D).

There was a general trend for transpiration to decrease from CD to MT treatments (Fig. 2B and 2D), arguably as a consequence of the temperature and PAR-associated decrease in stomatal conductance (Fig. 2A and 2C). Compared to control, stomatal conductance was lower in the CE and the MT treatments for both genotypes (-57 % and -90 % in LA1420 plants; -76 % and -77 % in Plovdiv plants; $P < 0.05$). Transpiration was also substantially lower in LA1420 plants of all DI treatments (-28 % in CD, -45 % in CE and -75 % in MT; $P < 0.05$) as well as for Plovdiv plants (-14 % in CD, -72 % in CE and -68 % in MT; $P < 0.05$). Taken together our observations suggest that DI treatments had an impact which was more marked on stomatal conductance than on water status, which is consistent with the isohydric behaviour of tomato.

Leaf dry matter content (Fig. 3A and 3C) was higher in LA1420 plants of the CD and CE treatments (+37 % in CD and +9 % in CE compared to controls; $P < 0.05$) and in Plovdiv plants of CD treatment (+18 % compared to control; $P < 0.05$). SLA was lower in LA1420 plants of CD treatment due to higher leaf dry weight (1.36 g in control vs. 2.22 g in CD; $P < 0.05$) (Fig. 3B and 3D). On the contrary, no differences in SLA were found in any of the two genotypes as a consequence of CE and MT treatments.

3.2 Effects of DI treatments on fruit and seed characteristics

Fruit fresh and dry mass, seed number and weight, concentrations in soluble sugars, organic acids, AsA and carotenoids were measured at maturity along with fruit water and osmotic potentials (Tab. 2 and 3). Data about fruit composition are presented both on a dry and on a fresh weight basis (Tab. 3 and supplementary data).

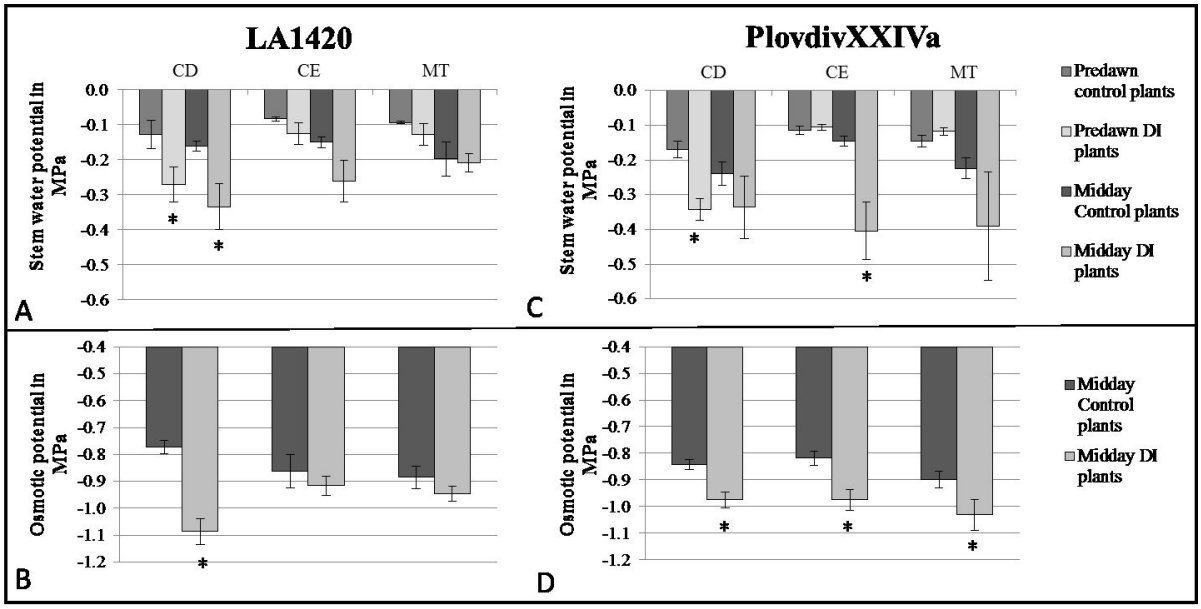

Figure 1: Predawn and midday stem water potential (A and C) and leaf osmotic potential (B and D) for LA1420 (left) and PlovdivXXIVa (right) tomato plants. Data presented correspond to the measurements made at the end of each deficit irrigation (DI) period. CD treatment was applied during the fruit cell division period, CE treatment was applied during the cell expansion period, and MT treatment was applied during the fruit maturation period. Data are means ± SE ($n \geq 5$). Significant differences between controls and treatments are indicated using * symbols for $P < 0.05$ (Duncan and Kruskal-Wallis tests performed for each genotype). Only the stem water potential at midday for PlovdivXXIVa was analysed using Kruskal-Wallis test.

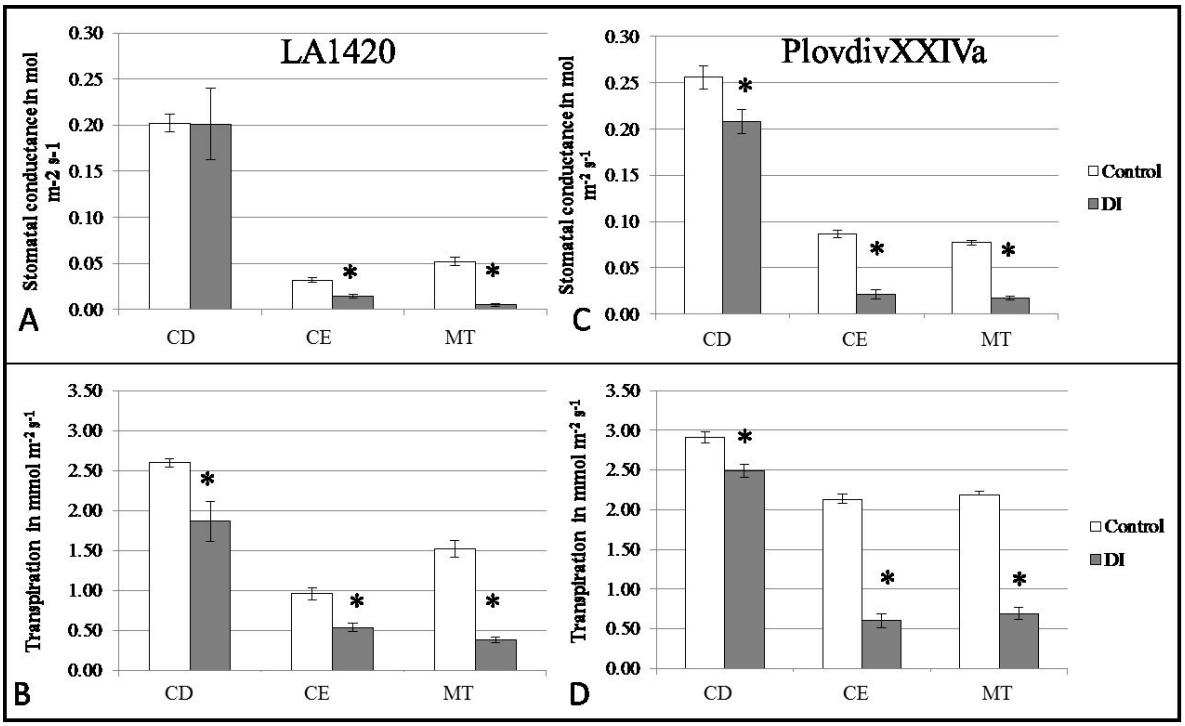

Figure 2: Plant stomatal conductance (A and C) and plant transpiration rate (B and D) for LA1420 (left) and PlovdivXXIVa (right) tomato plants. Data presented correspond to the measurements made at the end of each deficit irrigation (DI) period. CD treatment was applied during the fruit cell division period, CE treatment was applied during the cell expansion period, and MT treatment was applied during the fruit maturation period. Data are means ± SE ($n \geq 5$). Significant differences between controls and treatments are indicated using * symbols for $P < 0.05$ (Kruskal-Wallis tests performed for each genotype).

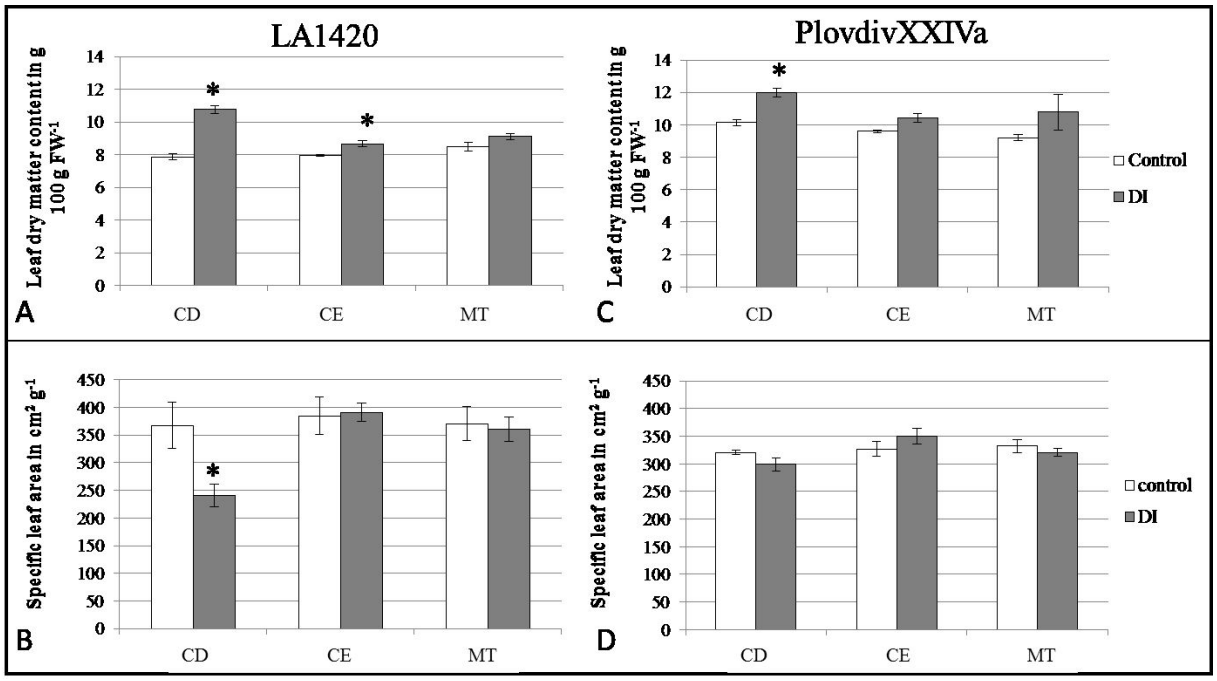

Figure 3: Leaf dry matter content (A and C) and specific leaf area (B and D) for LA1420 (left) and PlovdivXXIVa (right) tomato plants. Data presented correspond to the measurements made at the end of the whole trial. CD treatment corresponds to the deficit irrigation (DI) treatment applied during the fruit cell division period, CE treatment to DI applied during the cell expansion period, and MT treatment to DI applied during the fruit maturation period. Data are means ± SE ($n \geq 5$). Significant differences between controls and treatments are indicated using * symbols for $P < 0.05$ (Duncan tests performed for each genotype).

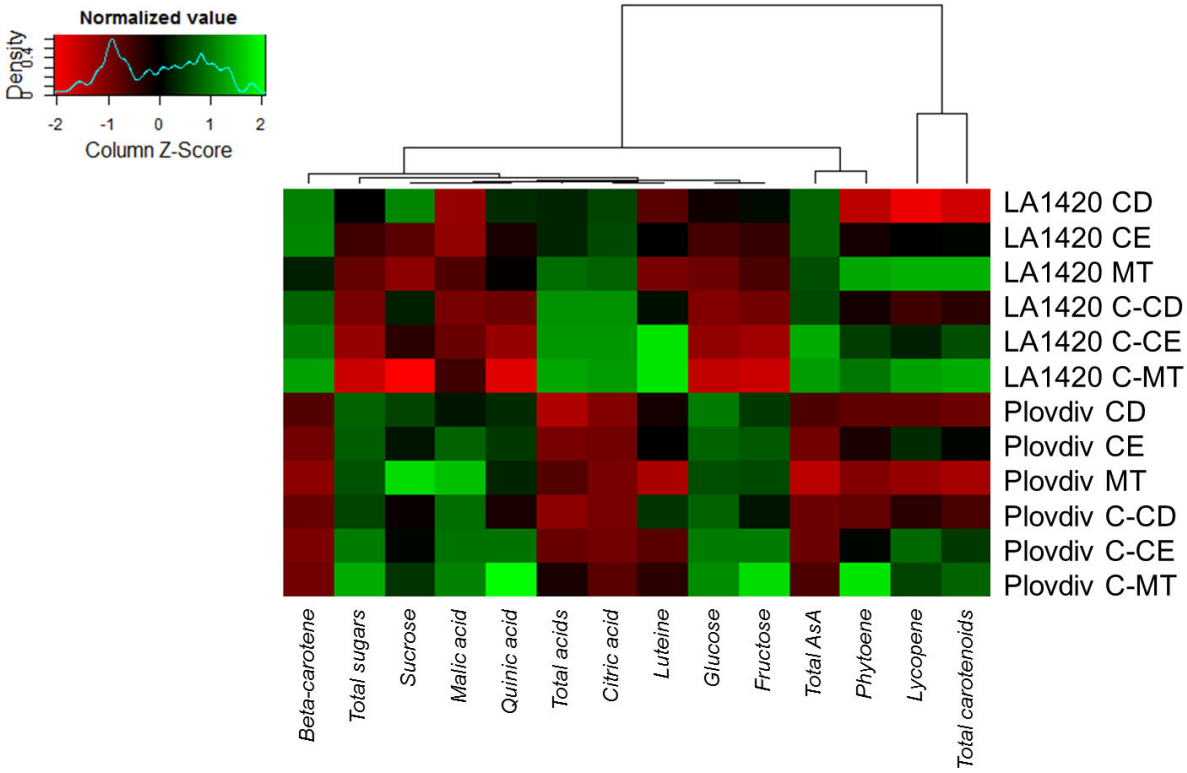

Figure 4: Heat map representation of the changes in fruit quality traits (contents in soluble sugars, in organic acids, in ascorbic acid (AsA) and in carotenoids) on a dry matter basis, in two tomato genotypes, LA1420 and PlovdivXXIVa (Plovdiv), grown under control conditions (C) or deficit irrigation applied during the fruit cell division period (CD), during the cell expansion period (CE), or during the fruit maturation period (MT). Data are centred and scaled by parameters ($n \geq 5$). These parameters are hierarchically classified according to Euclidean distances and ranged by colour (green for high and red for low).

Control fruits of the two genotypes had contrasted composition and fresh mass. LA1420 fruits had a final average fresh mass of 29.7 g and a dry matter content of 5.6 %. Ψ_F was -0.64 MPa and $\Psi\pi_F$ -0.71 MPa, whereas Plovdiv fruits had a final average mass of 40.8 g, a 8.1% dry matter content, a -1.08 MPa Ψ_F and -1.18 MPa $\Psi\pi_F$ (Tab. 2). We observed in Plovdiv a decrease in fruit fresh mass as a function of truss position along the stem, suggesting that competition for resources among fruits was high in Plovdiv plants. Control Plovdiv fruits were richer than LA1420 fruits in lycopene (+44.7 %), in soluble sugars (+82.4 %) and in malic acid (+307 %), but had lower concentrations in AsA (-17.6 %), in citric acid (-59.4 %) and in β-carotene (-58.1 %) (Fig. 4, and Supplementary data 1 and 2).

Also, a comparison between control plants and fruits grown at two different seasons (spring and autumn 2012) showed only differences for the transpiration rate, the fruit total sugar content and the total AsA content which were higher in spring than in autumn (Supplementary data 3).

Fruit fresh mass and fruit size (Tab. 2) were higher in LA1420 plants of the CD treatment (+40 % and +22 % respectively) than in control plants. Similarly, a significant increase in fruit fresh mass (not fruit size) was observed for Plovdiv fruits of MT (+22 %). On the contrary, the CE treatment did not impact fruit size or fresh mass for both genotypes.

The average mass of one seed (Tab. 2) was higher in the LA1420 plants of the CD and the CE treatments (+33.9 % and +23.4 % respectively, compared to control fruits), whereas there were no differences in Plovdiv plants due to DI treatments. The number of viable seeds per fruit was the lowest in Plovdiv C-CD control fruits (36 viable seed $fruit^{-1}$ vs. 90 viable seed $fruit^{-1}$).

The treatment effects on fruit dry matter content was genotype and stage dependent. Fruit dry matter content was increased in LA1420 plants of MT (5.17 vs 6.08 g 100 g FW^{-1}) and was unchanged in Plovdiv plants whatever the treatment. Fruit Ψ_F and $\Psi\pi_F$ potentials were lower in LA1420 plants of the MT treatment (-19.3 and -20.2 %, respectively, compared to control fruits) and in Plovdiv plants of the CD treatment (-14.8 and -12 %, respectively, compared to control fruits). In LA1420, the fruit $\Psi\pi_F$ was also reduced in the CD treatment (-18.8 % compared to control fruits), but not the fruit water potential.

LA1420	Control	CD	Control	CE	Control	MT
Fruit size (mm fruit^{-1})	41.4 ± 1.12 **a**	50.5 ± 1.05 **c**	40.68 ± 1.66 **a**	39.85 ± 0.70 **a**	45.27 ± 1.16 **b**	45.47 ± 1.28 **b**
Fresh fresh mass (g fruit^{-1})	29.22 ± 1.45 **ab**	42.67 ± 2.90 **c**	26.38 ± 1.89 **a**	27.53 ± 1.09 **ab**	32.9 ± 1.78 **b**	32.36 ± 2.21 **b**
Dry matter content (g 100 g FW^{1})	5.74 ± 0.25 **ab**	6.58 ± 0.23 **a**	5.74 ± 0.26 **bc**	5.86 ± 0.09 **cd**	5.17 ± 0.09 **d**	6.08 ± 0.16 **bc**
Water potential (MPa)	-0.63 ± 0.01 **bc**	-0.74 ± 0.10 **ab**	-0.62 ± 0.02 **bc**	-0.60 ± 0.02 **c**	-0.67 ± 0.01 **bc**	-0.83 ± 0.03 **a**
Osmotic potential (MPa)	-0.69 ± 0.02 **a**	-0.85 ± 0.08 **bc**	-0.70 ± 0.03 **a**	-0.80 ± 0.02 **ab**	-0.75 ± 0.04 **ab**	-0.94 ± 0.03 **c**
Seed number per fruit	124 ± 7.9 **a**	157 ± 7.9 **a**	118 ± 4.2 **a**	99,5 ± 17.3 **a**	145 ± 10.8 **ab**	140 ± 25.6 **ab**
Seed weight (mg)	1.70 ± 0.10 **de**	2.28 ± 0.10 **ab**	1.89 ± 0.09 **cd**	2.34 ± 0.07 **a**	2.02 ± 0.06 **bc**	2.03 ± 0.08 **bc**
Plovdiv XXIVa						
Fruit size (mm fruit^{-1})	41.32 ± 0.53 **a**	41.94 ± 0.45 **a**	40.47 ± 0.37 **a**	42.06 ± 0.33 **a**	39.20 ± 0.59 **a**	41.25 ± 0.42 **a**
Fresh fresh mass (g fruit^{-1})	40.48 ± 1.02 **a**	43.59 ± 1.30 **a**	41.5 ± 0.89 **a**	43.88 ± 0.89 **a**	34.03 ± 1.43 **b**	41.51 ± 0.81 **a**
Dry matter content (g 100 g FW^{1})	7.86 ± 0.17 **a**	8.25 ± 0.19 **a**	8.17 ± 0.17 **a**	7.85 ± 0.15 **a**	8.24 ± 0.12 **a**	8.58 ± 0.12 **a**
Water potential (MPa)	-1.09 ± 0.03 **a**	-1.28 ± 0.02 **b**	-1.09 ± 0.02 **a**	-1.10 ± 0.03 **a**	-1.07 ± 0.02 **ab**	-1.17 ± 0.02 **ab**
Osmotic potential (MPa)	-1.17 ± 0.03 **a**	-1.33 ± 0.05 **b**	-1.17 ± 0.03 **a**	-1.12 ± 0.03 **a**	-1.21 ± 0.06 **a**	-1.21 ± 0.03 **ab**
Seed number per fruit	36 ± 6.9 **bc**	108 ± 3.9 **a**	90,4 ± 5.9 **ab**	93 ± 6.6 **ab**	92,7 ± 1.6 **ab**	98,8 ± 4.6 **ab**
Seed weight (mg)	1.08 ± 0.07 **c**	0.95 ± 0.07 **c**	1.57 ± 0.09 **b**	1.45 ± 0.09 **b**	1.56 ± 0.08 **b**	1.65 ± 0.13 **ab**

Table 2: Fruit fresh mass and size, dry matter content, water potential (Ψ_F) and osmotic potential ($\Psi\pi_F$), seed number per fruit and averaged seed weight (for one seed) in LA1420 (upper part) and PlovdivXXIVa (lower part) tomato fruits. CD treatment corresponds to the deficit irrigation (DI) applied during the cell division period, CE treatment to the treatment applied during the cell expansion period, and MT to the treatment applied during the fruit maturation period. Data are means ± SE ($n \geq 5$). Different letters for a given parameter correspond to significant differences between all conditions (control and DI) at the $P < 0.05$ threshold (Two-way anova with Tukey's test and Kruskal-Wallis test). Only the dry matter content and the seed number was analysed using Kruskal-Wallis test.

	Dry matter basis						Fresh matter basis					
	LA1420			PlovdivXXIVa			LA1420			PlovdivXXIVa		
	CD	CE	MT	CD	CE	MT	CD	CE	MT	CD	CE	MT
Glucose	***27,5***	***20,5***	***24,1***	4,1	-4,1	***-9,3***	***27,5***	***20,5***	***24,1***	4,1	-4,1	***-9,3***
Fructose	***15,8***	***13,3***	***16,6***	3,7	-3,3	***-12,3***	***24,1***	11,6	***37,0***	5,4	-4,7	-6,9
Sucrose	16,0	-8,0	31,9	13,7	2,4	24,5	23,5	-8,8	55,6	15,2	0,9	***31,6***
Total sugars	***20,7***	***15,7***	***20,1***	4,1	-3,5	***-10,0***	***25,6***	***15,2***	***30,9***	5,1	-4,3	-7,0
Citric acid	***-18,1***	***-18,0***	***-13,1***	-5,6	0,6	-15,5	***-12,0***	-19,2	2,0	-3,6	-0,7	-10,4
Malic acid	-33,3	-37,4	-10,7	-26,4	-3,9	17,6	-28,4	-38,8	4,4	-24,2	-4,0	24,5
Quinic acid	***12,3***	10,2	***18,5***	5,4	-3,9	***-13,3***	***20,2***	8,6	***39,4***	7,3	-5,5	-8,2
Total acids	***-11,6***	***-12,6***	-6,3	-6,4	-2,2	-7,5	-5,1	-13,9	10,1	-4,4	-3,3	-2,1
Lycopene	-27,6	-4,2	1,8	-8,0	-7,5	-28,8	-21,9	-5,4	19,4	-6,6	-10,4	***-24,5***
Beta-carotene	8,0	1,6	***-27,9***	8,8	4,7	-11,6	***16,9***	0,2	-15,3	10,9	1,6	-6,3
Phytoene	-38,3	-16,3	7,9	2,0	-8,4	***-52,1***	-33,9	-17,4	26,5	3,3	-11,2	***-49,3***
Lutein	-18,3	***-28,1***	***-43,6***	-12,0	18,7	-24,1	-11,6	***-29,0***	***-34,1***	-10,0	15,6	-19,3
Total carotenoids	-27,2	-6,8	0,9	-5,4	-7,2	-35,0	-21,6	-8,0	18,3	-4,0	-10,1	***-31,1***
Total AsA	4,4	-11,9	-12,7	11,4	-1,0	-32,2	12,7	-13,0	2,9	13,0	-5,4	-28,1
Total sugars / total acids	***37,1***	***32,2***	***28,2***	10,7	-2,3	-2,3						

Color scale: -50 | -25 | -10 | 0 | 10 | 25 | 50

Table 3: Relative differences in metabolite contents (soluble sugars, organic acids, ascorbic acid (AsA) and carotenoids) on a dry matter (left) and on a fresh matter (right) basis observed for LA1420 and PlovdivXXIVa tomato fruits. CD treatment corresponds to the deficit irrigation (DI) applied during the cell division period, CE treatment to DI applied during the cell expansion period, and MT treatment to DI applied during the fruit maturation period. The difference was calculated as:

$$x\ (\%) = \frac{(\text{Mean DI [glucose]} - \text{Mean Control [glucose]})}{\text{Mean control [glucose]}} \times 100$$

Data are means ± SE ($n \geq 5$). The percentages were scaled by color (green for high and red for low value). Significant differences between control and stress values are indicated by using bold, italic and underlined fonts for $P < 0.05$ (Two-way anova with Tukey's test and Kruskal-Wallis test). Only, the content in β-carotene was analysed using Kruskal-Wallis test.

Differences in fruit composition between LA1420 and Plovdiv are summarized in figure 4. Clusters indicate fruit traits that co-vary in response to DI. Hierarchical clustering analysis showed that soluble sugars, organic acids, lutein and β-carotene could be pooled together, as could be pooled together total AsA and phytoene contents, on the one hand, and lycopene contents and total carotenoids, on the other hand. This figure highlights the contrast in fruit composition between the two genotypes already described above.

The relative differences in metabolite contents on a dry and fresh matter basis are given in table 3. On a dry matter basis, total sugar content was higher in LA1420 plants as a consequence of DI treatments (+20.7 % for CD, +15.7 % for CE and +20.1 % for MT) due to higher accumulation of both glucose and fructose. On the contrary, total organic acid content was lower in LA1420 fruits under DI conditions (-11.6 % in CD, -12.6 % in CE and -6.3 % in MT (N.S.)) thanks to a decrease in citric acid content (-18 % in CD and in CE treatments, -13 % for MT). The sugar-to-acid ratio appears therefore higher as a consequence of DI for this genotype. The content in total carotenoids was unchanged in LA1420 plants of the DI treatments, in spite of the significant decrease in lutein content (-28 % in CE and -43.6 % in MT) and in β-carotene content (-27.9 % in MT). There were no differences in total AsA content as a consequence of any of the DI treatments.

The fruit composition of Plovdiv fruits was barely affected by the treatments except in the fruits of the MT treatment. Total sugar content was lowered by 10 % in Plovdiv plants of the MT treatment when compared to the controls thanks to a decrease in glucose content (-9.3 %) and in fructose content (-12.3 %). Quinic acid content was lower only in Plovdiv plants of the MT treatment (-13.3 %). A phytoene-associated decrease in total carotenoids (-34 %) was observed in Plovdiv plants of the MT treatment. As for LA1420 fruits there were no differences in total AsA content as a consequence of any of the DI treatments.

The global trends evidenced from data expressed on a dry matter basis were not substantially modified when data were expressed on a fresh matter basis (Tab. 3 and supplementary data 2). The decrease in total organic acids in LA1420 fruits was not significant for all DI treatments, whereas the increase in β-carotene was significant for CD treatment (+16.9 %). In Plovdiv fruits, the increase in sucrose was significant (+31.6 %) as well as the decrease in lycopene (-24.5 %). Thus, metabolic effects are primarily involved in improving the fruit quality of LA1420 due to CD and CE treatments, while an accumulation

of metabolic and dilution effects are involved in the improvement of the final fruit quality of LA1420 in the MT treatment. The poor effect of dilution by WD on fruit traits should be explained by the recovery periods after the CD and the CE treatments. For Plovdiv, only low metabolic effects were observed in the MT treatment.

3.3 Effect of DI treatments on pericarp cell number and size

Pericarp cell number and the extrapolated mean cell volume (the ratio between pericarp volume and cell number) were measured during fruit development in fruits of the CD and CE treatments at 30 DAA after cell division stopped, but cell expansion still proceeded. At this stage LA1420 fruits of the CD treatment contained similar numbers of cells, but larger cells (2.96 vs. 1.91 nl) than control fruits. On the contrary Plovdiv fruits of the CD treatment contained less cells than control fruits ($3.19x10^6$ vs. $3.70x10^6$), but of similar size. However the measurement of cell size at maturity would be necessary to conclude. As expected the CE treatment did not affect the number of cells in both genotypes.

IV- Discussion

Many studies have advocated that WD may exert beneficial effects on fruit quality by stimulating the primary and the secondary metabolisms, therefore enhancing accumulation of compounds involved in fruit taste and nutritive value (Ripoll *et al*., 2014). At the fruit level, the response to WD results from interconnected processes involving trophic, hydric and hormonal regulations and signalling cascades, finely tuned according to WD intensity. However the fruit response at specific developmental stage was seldom investigated (Ripoll *et al*., 2014). In the present study, we investigated the effect on fruit quality criteria of a 60 % WD imposed at three different stages of fruit development followed by periods of recovery. This study was realized in autumn but different experiments conducted in spring and summer confirmed the main results (Chapter II). Also Nuruddin *et al.* (2003) compared the effects of two experiments of WD depending on season (summer and winter) and at different fruit developmental stage. They found small seasonal effects on fruit quality (evaluated by °Brix) and marketable yield, and no interactions between season and WD treatments.

4.1 Intensity of water stress

DI treatments consisted in reducing water supply by 60 % during the three targeted periods of WD. From a practical point of view 20 % of water supply was saved over the whole growing period in each DI treatment. Though the imposed WD may appear as substantial, the physiological observations suggested that the DI treatments were at the origin of only a moderate water stress. Plants closed their stomata, reduced their transpiration and generally maintained a high water status. It is likely that the seasonal decrease in climatic demand moreover attenuated the effects of the CE and the MT treatments.

4.2 Moderate WD applied during cell division had a positive impact on both fruit mass and sugar content in LA1420 but not in Plovdiv

Final size and mass of tomato fruit have been shown to be well correlated to the seed number (Varga and Bruinsma, 1986; Bertin *et al*., 1998), and to the number of pericarp cells (Bertin *et al*., 2003; Bohner and Bangerth, 1988) according to trophic and hormonal regulations. In the present study, under control conditions, the fruit fresh mass was higher in Plovdiv (40 g) than in LA1420 (30 g) and both genotypes contained similar number of cells.

A moderate WD applied during the period of cell division promoted final fruit size in LA1420, in relation to larger cells and bigger seeds without any significant effect on cell or seed number. On the contrary, the absence of effect on fruit size in Plovdiv was associated with a decrease in cell number and an increase in seed number without any significant effect on cell or seed size. The relationship between fruit weight and seed content has been attributed to the sink activity induced by developing seeds at an early stage of development rather that to the effect of seed-synthesized auxins on cell enlargement (Varga and Bruinsma, 1986). However Bertin *et al*. (1998) suggested that this positive correlation is genotype-dependent. Its intensity varies as a function of the plant source-sink ratio and the correlation coefficient sharply drops under conditions of high competition for carbohydrate-assimilates. Finally, our results suggested that cell expansion would be the most important process involved in the response to early WD for both genotypes. In accordance with this view, the absence of WD effects on cell division in fruit tissues has been also reported in grape berries (McCarthy *et al*., 2002; Ojeda *et al*., 2001), in olive (Gucci *et al*., 2009) and in pear fruits (Marsal *et al*., 2000), with the exception of severe stress (Gucci *et al*., 2009). In the present study, the reduction in cell number in Plovdiv-CD fruits was likely compensated by cell size, which was unfortunately not measured on mature fruits in this trial. In LA1420 fruits the increase in fruit size was not related to an increase in cell number but in cell size, hypothetically due to the longer period of fruit development (not shown) or to higher plasticity of cell expansion in this genotype. This is consistent with the hypothesis of Li *et al*. (1989), who attributed the increase in peach fruit size after an early WD to an increase in cell extensibility during recovery periods.

Regarding fruit quality, the CD treatment induced an increase in soluble sugar accumulation and a decrease in organic acid accumulation in LA1420 fruits, leading to an increase in the sugar-to-acid ratio, which is an essential quality criterion for consumers. Previous studies on tomatoes reported an increase in fruit sugar content under WD (applied during all fruit development phase), which is dependent upon the cultivar and the timing of stress (Bertin *et al*., 2000; Veit-Köhler *et al*., 1999)). In contrast, the effects of WD on fruit acidity are more conflicting. As resumed in Etienne *et al*. (2013), in many species (peach, clementine, mandarin, pear), WD has been shown to increase organic acid content in ripe fruits. However in grapes, nectarines (Etienne *et al*., 2013) and tomatoes (Mitchell *et al*., 1991; Bertin *et al*., 2000; Veit-Köhler *et al*., 1999), the effect of WD on organic acid content has been shown to be a negative one, as we observed here for CD treatment. Because the

variations in soluble sugar and organic acids in response to WD are often reported on a fresh weight basis, they may result either from dilution/dehydration effects (Etienne *et al*., 2013; Guichard *et al*., 2001) or from active solute accumulation (Lo Bianco *et al*., 2000; Hummel *et al*., 2010). The present data suggest that there was an effect of CD treatment on the metabolism, not only a dilution effect (tab. 2 and 3). The effects of WD on sugar-metabolizing enzymes in sink organs remain poorly documented. Although the literature on sugar-metabolizing enzymes is not specific of one fruit developmental stage, our result suggest that WD applied during a short period of the early fruit development stages impact its final composition in sugar and acids through metabolic regulations. On the contrary, the composition in lycopene, carotenoids and AsA were not affected by CD treatment. Many reports showed positive effects of WD on these compounds (Ripoll *et al*., 2014). However it is important to stress that these effects are highly dependent on genetic and seasonal factors, or on the intensity and duration of treatments.

4.3 Moderate WD during cell expansion influenced fruit concentration in primary metabolites in LA1420

Globally our results suggest that a moderate WD applied during cell expansion has no impact on fruit weight and size. This stays in contrast with observations on peach fruits for which the negative effect of WD on fruit weight and size was attributed to carbon limitations (Vallverdu *et al*., 2012; Girona *et al*., 2004) and decreased water content (Li *et al*., 1989). In pear and olive, DI was observed to decrease cell size depending on the intensity of WD (Gucci *et al*., 2009; Marsal *et al*., 2000). In the present study, WD was a moderate one which may explain discrepancies with existing literature. However in LA1420 the maintenance of fruit mass after CE conditions could also be attributed to osmotic regulation as illustrated by the decrease in fruit osmotic potential (Tab. 2) and increase in soluble sugar accumulation (Tab. 3). The fruit water influx via xylem and phloem tissues follows the stem-to-fruit gradient of water potential, which is generated by a gradient of osmotic potential between sources and sink tissues and that links cell expansion to sugar metabolism and subcellular compartmentalization (Guichard *et al*., 2001). Other turgor-independent processes may regulate fruit growth under WD. Among them, the cell sub-epidermal pH (Mingo *et al*., 2003; Thompson, 2001), and some modifications of the biochemical and physical properties of the cell wall (Boyer, 1988) have been reported. Several cell wall-loosening factors have been identified in plant cells, including numerous acid-induced or hormone-induced proteins (e.g. expansins hydrolases). Thus, the potential effect of WD on cell-wall loosening and water

balance is a complex process involving many factors that may have opposing effects on the final fruit size.

On the contrary to fruit size, the accumulation of sugars and acid were respectively, positively and negatively affected by CE treatment in LA1420. An increase in soluble sugar when WD was applied during cell expansion has been reported on peach (Li *et al*., 1989; Girona *et al*., 2004). Interestingly the relative variations in sugars and acid contents observed in CD and CE treatments were similar, suggesting that osmotic regulations were likely involved during both phases of fruit development (Tab. 2). A specific response to the CE treatment when compared to the CD treatment, was the decrease in lutein content (Tab. 3) which is synthesized from lycopene during ripening (Goodwin, 1971). This reaction is catalysed by the lycopene β-cyclase and the lycopene ε-cyclase (LCY-β and LCY-ε). The decreased expression of these two enzymes at the whole plant level is described as an adaptive mechanism to salt stress in potato and to water deficit induced by polyethylene glycol in carrot (Kim *et al*., 2014; Moreno *et al*., 2013). It may be interesting to test this hypothesis on tomatoes.

4.4 Moderate WD during ripening influenced the fruit content in primary metabolites and in phytonutrients in both genotypes

WD is often applied during ripening in order to accelerate fruit maturation in grapes (Castellarin *et al*., 2007), and to increase fruit quality in peach and tomato (Wang and Gartung, 2010; Wang *et al*., 2011). MT treatment was the only treatment that affected quality traits of Plovdiv fruits. However the two genotypes showed contrasted responses as sugar or quinic acid contents increased in LA1420 fruits and decreased in Plovdiv fruits. On the contrary, several carotenoids decreased in response to MT treatment in both genotypes, suggesting that this response was independent on sugar variations. In LA1420 the significant increase in fruit dry matter content (Tab. 2) requires to distinguish dilution and metabolic effects (Tab. 3). Cumulative effects were observed regarding sugar and quinic acid accumulation, whereas antagonistic effects were observed for citric acid, lutein or β-carotene. The reported effects of WD on carotenoid content in tomato, range from negative (De Pascale *et al*., 2007; Riggi *et al*., 2008), to non-significant (Krumbein *et al*., 2006), to positive (De Pascale *et al*., 2007; Favati *et al*., 2009; Krauss *et al*., 2006; Wu *et al*., 2004; Zushi and Matsuzoe, 1998). Similar findings have been reported for ascorbate (Ripoll *et al*., 2014). Most of current studies on WD and fruit quality stress the importance of experimental conditions

and intensity of stress. WD may influence the metabolism of these phytonutrients via at least two major mechanisms that are not mutually exclusive and that may even interact (Fanciullino *et al*., 2014). Drought typically induces a decrease in leaf stomatal conductance, resulting in a decrease in net photosynthesis which may result in a reduced transport of primary metabolites to the fruits. Primary metabolites are the precursors for the biosynthesis of phenolic compounds, carotenoids and AsA. Also, drought may exacerbate oxidative stress/oxidative signaling, and reactive oxygen species are known to directly and indirectly influence the biosynthetic pathways of secondary metabolites (Fanciullino *et al*., 2014). In the present study, the lack of carbon precursors was likely not involved in the decrease in carotenoids and AsA since sugar and carotenoid or ascorbate contents did not co-vary, at least in LA1420 fruits (Figure 4). This is consistent with the hypothesis that AsA content is not limited by leaf photosynthesis or sugar availability, but by environmental conditions such as light intensity (Gautier *et al*., 2009). The synthesis of carotenoids increases as chloroplasts turn into chromoplasts and the enzyme activities increase. Because lutein and β-carotene are sub-products of lycopene, it may be hypothesized that the activity of enzymes involved in their synthesis was slowed down by MT conditions, as it was suggested for the CE treatment. Moreover the stress imposed being a moderate one in our trial, oxidative stress which has been observed to be capable to drive synthesis of carotenoids in fruits (Poiroux-Gonord *et al*., 2013), was arguably not involved here in such mechanisms.

V- Conclusion

The present study supports the idea that moderate WD can enhance fruit quality (mainly by metabolic effects), in particular fruit taste which depends mainly on sugar content and on the sweetness/acidity balance, and this without entailing any reduction of fruit mass. Nutritional quality which depends on carotenoids and AsA contents appeared less affected by WD than expected, and sometimes even appeared negatively affected. When observations are put in perspective with the existing literature, they strongly suggest that the applied WD in this trial was *in fine* clearly a moderate one and that the pattern of fruit responses to moderate WD is profoundly different from the pattern of fruit responses to more severe WD. The less performing genotype (LA1420, in terms of fruits characteristics) was also the one that responded with the highest amplitude to the DI treatments. It could be that the potential for improving quality traits of already performing genotypes by moderate DI is much less than the potential of poor performers. This view, if confirmed, should be accounted for designing cultivars adapted to WD conditions by breeders.

Acknowledgments

This paper was supported by the *Federative Research Structure* TERSYS. The authors thank the team of the UR1052 Genetics and Improvement of Fruit and Vegetables (INRA, Montfavet) for providing the plant material.

Adaptive strategies of vegetative and reproductive plants to soil drying revealed by parameters derived from fluorescence induction curves

Nous avons vu dans les précédents chapitres que l'effet du WD sur les mécanismes d'adaptation et la qualité des fruits dépend du stade de développement de la plante (Chapitre I), du stade de développement du fruit (Chapitre III) et des génotypes (Chapitre II et III). Les mécanismes d'adaptation des plantes dépendent fortement de la gestion du stress oxydatif induit lors d'un WD en raison de la réduction de la quantité de CO_2 entrant (réduction de l'ouverture des stomates) et de la photosynthèse, créant des conditions favorables à la production de ROS. La gestion des dommages liés aux ROS dans les photosystèmes constitue un challenge pour la plante soumise à un WD.

Dans ce chapitre, nous avons cherché à quantifier et qualifier ces mécanismes d'adaptation à l'échelle des photosystèmes à deux stades de développement de la plante (végétatif et reproductif), lors d'un dessèchement complet du substrat. Cette étude a également été réalisée dans l'optique d'identifier des seuils de réponse au WD, repris dans la partie synthèse et discussion (Chapitre VI). Pour cela, nous avons réalisé des cinétiques de dessèchement du substrat de culture en fonction des stades de développement de la plante sur quatre génotypes sélectionnés parmi les huit parents de la MAGIC TOM, à savoir Cervil, LA1420, Plovdiv et Levovil, suite aux résultats contrastés observés dans les précédents chapitres.

L'accent a été mis sur l'utilisation de la fluorescence de la chlorophylle *a* et la méthode OJIP afin de caractériser les impacts du dessèchement sur les photosystèmes. Des paramètres classiques de réponse au WD ont également été étudiés, à savoir la conductance stomatique et la teneur en matières sèches des plantes. Pour les plantes reproductives, les flux d'eau entre plante et fruits ont été évalués au travers des mesures de masses fraiche et sèche des fruits et de leur potentiel hydrique.

Ce chapitre est présenté sous la forme d'un article en cours de préparation.

Résumé

Dans la littérature, des réponses contrastées au déficit hydrique (WD) ont été décrites en partie en raison de variations génotypiques, d'effets du stade de développement affecté par le stress et de l'intensité du stress. Dans cette étude, les réponses de différents génotypes à un dessèchement progressif du substrat ont été étudiées à l'échelle de la plante et du fruit aux stades de développement végétatif et reproductif. Pour cela, quatre génotypes de tomates sélectionnés parmi les huit parents de la population MAGIC TOM ont été étudiés. La conductance stomatique (g_s), la teneur en chlorophylles et la fluorescence de la chlorophylle *a* des feuilles ont été mesurées et les paramètres de la méthode OJIP calculés. Des réponses différentes au dessèchement du substrat ont été observées en fonction du stade de développement. Ces réponses ont été utilisées pour classer les quatre génotypes selon leur sensibilité et l'un d'entre eux (LA1420) s'est avéré moins sensible quel que soit le stade de développement affecté par ce WD. De fortes baisses des indicateurs de fluorescence suivants: le nombre de rotations (N), l'aire normalisée (Sm) et le flux de transport d'électrons des photosystèmes (PS) II jusqu'aux accepteurs primaires du PSI (J_0^{RE1}/RC) par centres réactionnels PSII, seraient caractéristiques de la réponse des plantes végétatives lors de ce WD. Les génotypes différaient dans la manière dont ils dissipaient l'énergie dans les processus autres que le piégeage (J_0^{DI}), par la fluorescence minimale (F_0) et par le rendement quantique de la photochimie primaire du PSII (F_V/F_0). En revanche, les plantes reproductives exposées à ce WD ont été caractérisées par une forte baisse de g_s, une forte augmentation de la teneur en chlorophylles, une diminution de l'indice de performance pour la conservation de l'énergie des photons absorbés par l'antenne PSII jusqu'à la réduction des accepteurs primaires du PSI (PI_{ABS}^{TOT}) et de ses composantes, et une baisse de la teneur en eau des fruits à l'exception du génotype LA1420. Dans l'ensemble, les résultats ont suggéré que les paramètres issus de la méthode OJIP sont de puissants outils pour analyser et distinguer les stratégies spécifiques entre génotypes de tomate soumis à un WD et que le génotype LA1420 aurait des traits adaptatifs intéressants.

Mot clés: Cinétique de dessèchement du sol, Fluorescence chlorophyllienne, méthode OJIP, Stade de développement, *S. lycopersicum* L.

Abstract

Contrasted responses of plants exposed to water deficit (WD) are reported in the literature, in part due to stress intensity, genotypic variations or developmental effects. In this study, tomato plant and fruit responses during progressive soil drying events were studied at two development stages, vegetative and reproductive. Four different tomato genotypes selected among the parents of the MAGIC TOM were investigated. Leaf stomatal conductance (g_s) chlorophyll content and chlorophyll fluorescence were measured and the parameters derived from OJIP transients were calculated. Different responses to soil drying were found according to the developmental stage. These responses were used to classify the four genotypes by their sensitivity. One genotype (LA1420) was found less sensitive whatever the developmental stage during soil drying. Strong decreases in the turnover number (N), the normalized area (Sm) and the electron transport flux until photosystem (PS) I acceptors per PSII reaction centres (J_0^{RE1}/RC) appeared as common features among vegetative plants submitted to WD. Genotypes differed by the way they dissipate energy in processes other than trapping (J_0^{DI}), by minimal fluorescence (F_0) and by quantum yield of primary PSII photochemistry (F_V/F_0). All reproductive plants exposed to WD were characterized by low g_s, high chlorophyll content, a decrease in the Performance Index for energy conservation from photons absorbed by PSII antenna until the reduction of PSI acceptors (PI_{ABS}^{TOT}) and all its components, and by lower fruit water content, with the exception of LA1420. Taken together our observations suggested that parameters derived from OJIP transients are powerful tools to analyse and distinguish specific strategies among tomato plants submitted to WD and that LA1420 may provide interesting adaptive traits.

Key words: Chlorophyll fluorescence, Developmental stage, Kinetics of soil drying, OJIP transients, *S. lycopersicum* L.

I- Introduction

In many parts of the world, the global climate change will result in an increase in the intensity, frequency or length of the periods of water deficit (WD). WD is known to impact negatively plant growth and yield but it has been recently suggested that WD could also be exploited to improve produce quality in fruit crops (Ripoll *et al*., 2014). To take advantage of this idea, it will however be necessary to increase our understanding of the way plants react to WD according to genetic effects and to development stages.

Contrasting effects of WD have indeed been observed depending on plant development stages. In germinated tomato plants, polyethylene glycol-induced WD was generally lethal, and the rare surviving germinated seeds produced either resistant or very sensitive plants (Kulkarni and Deshpande, 2007). The vegetative growth stage is highly sensitive to WD, and a major consequence of WD during this stage is a reduction of plant growth that may affect the entire reproductive period (Gladden *et al*., 2012). In grapes, WD applied during the vegetative stage (no irrigation until veraison) reduces the maximum rate of shoot elongation and node production, accelerates periderm development and decreases fruit growth (Matthews *et al*., 1987). In tomatoes, WD applied during the reproductive stage also reduces plant growth whereas fruit growth can be increased due to reduction in fruit set number; but it can also result in abnormal ovule development according to the intensity of WD (Chaves *et al*., 2003; Patanè and Cosentino, 2010; Rapoport *et al*., 2012). Moreover, the impact of WD on tomato yield depends on the fruit development stage affected by WD. When applied during cell division, WD has been observed to promote fruit size without any negative effect on fruit water content (Li *et al.*, 1989), whereas fruit water content appears to decrease when WD is applied during the cell expansion phase (Li *et al*., 1989). When WD is applied during ripening, a decrease in fruit yield can be observed that is proportional to the intensity of WD (Wang and Gartung, 2010). Clearly, WD applied during the reproductive stage has contrasted effects on fruit quality and yield depending on the specific phase of fruit development at the time of WD application.

The first tolerance mechanisms triggered by WD are the reduction in stomatal conductance (g_s) and in the associated transpirational losses, followed by osmotic adjustment

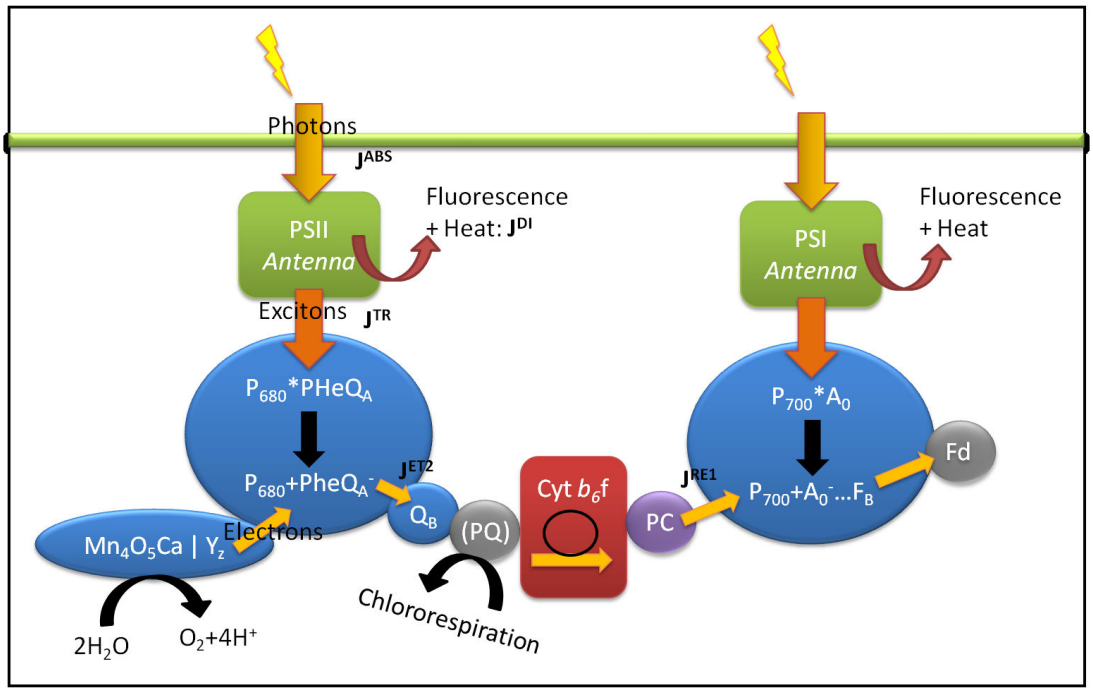

Figure 1: Schematic representation inspired of Stribet and Govindjee (2011) of the main energy pathways related to chlorophyll *a* fluorescence. Since most chlorophyll *a* fluorescence originates in photosystem II (PSII) antenna. J^{ABS} represents the rate of photon absorption by all PSII antenna pigments. The dissipated energy flux J^{DI} represents the part of the absorbed photon flux dissipated through direct fluorescence (F) and other non-radiative processes (heat), and the trapped exciton flux. J^{TR} represents the rate of exciton trapping by the PSII reaction centre P_{680}. The trapped energy is used for charge separation. The flux J^{ET2} represents the electron transport flux from Q_A to Q_B. Eventually, J_0^{RE1} represents the rate of electrons from Q_B to PSI acceptors. The use of PQ pool to chlororespiration was hypothetically represented according to Feild *et al.* (1998).

in leaves and fruits. A decrease in osmotic potential results in a decrease in plant water potential which is favourable to water uptake from the dehydrating soil. Reduction of turgor and growth are one of the most current adaptive responses to WD for tomato (Chaves *et al.*, 2003) to maintain water status. While very effective in reducing water losses and preserving the water balance in conditions of drought, stomatal closure has unfortunately also a very negative side-effect: it results in a decrease of the quantity of CO_2 entering the leaf and therefore of photosynthesis. A decrease in photosynthesis not only reduces growth but also creates conditions favourable to the production of reactive oxygen species (ROS) and to photodamages (see review of Damour *et al*., 2010). In addition to the usual parameters (water potential and leaf stomatal conductance) there is an increasing interest in the parameters derived from analysis of OJIP transients (Kalaji *et al*., 2014). The OJIP/OKJIP transient correspond to different steps (O, K, J, I and P) in the fluorescence kinetic profile, plotted in a logarithmic time scale from the initial fluorescence F_0 to the maximal fluorescence F_M (Strasser and Strasser, 1995). Step O corresponds to the fluorescence originally emitted, step K corresponds to a threshold around 300 µs which appears only in certain conditions of stress (like heat), step J to the fluorescence emitted at 2 ms, step I to the fluorescence emitted at 30 ms and the step P corresponds to the maximum fluorescence (Strasser *et al*., 2000). Each of these steps corresponds to a step of electron transfer of photosystems (PS) II and I (Fig. 1). Thus, the OJ section reflects the reduction of the acceptor side of PSII, JI section reflects the partial reduction of plastoquinone pool (PQ) and finally the IP section reflects the reduction of acceptor side of PSI. At each step corresponds one parameter described in the table 1 and different quantum yield are used to assess energy transfer from PSII to PSI. The idea behind is that WD necessarily impacts the energy and electron fluxes of the photosystems and must be reflected in the parameters derived from the analysis of the fluorescence induction curve.

Despite the evidence that all these parameters reflect plant adaptive responses to environmental stresses, their genetic variability has rarely been investigated and to our knowledge, their relationship with plant development stages was not investigated in tomato. The objective of this paper therefore is to evaluate their potential for analysing the specific strategies of different genotypes at different development stages in tomato plants exposed to soil drying. We decided to compare four genotypes at two different development stages (vegetative and reproductive) selected among the eight parents of the Multi-Parent Advanced Generation Inter-Cross population of tomato (MAGIC TOM) collection. The MAGIC TOM collection is the population with the highest rate of allelic variability in tomato (Ranc, 2010).

Parameters	Formulae	Comments
F_0	The first fluorescence value of OJIP curve, $F_{50\mu s}$	The first reliable fluorescence value after the onset of actinic illumination
$F_M \cong F_P$	The fluorescence value at the peak of OJIP curve	Maximum value under saturating illumination
F_K	The fluorescence at K step (300 µs), $F_{300\mu s}$	This step is usually hidden in the O-J rise, due to the established balance between the several electron transport reactions responsible for the fluorescence rise; thus k-step appearance is considered to result from a deviation of the usually established balance (Srivastava *et al*., 1997) and is related to the inactivation of the oxygen-evolving-complex (OEC) leaving to an imbalance between RC towards the acceptor side of PSII (Strasser, 1997)
F_J	The fluorescence at J step (2 ms), F_{2ms}	
F_V	$F_V = F_M - F_0$	Maximum variable Chlorophyll fluorescence
F_V/F_M		The maximum photochemical efficiency of light harvesting in PSII
F_V/F_0		Quantum yield of primary PSII photochemistry, represents the contribution to the PI of the light reactions for primary photochemistry
Sm	The normalized area Area/F_V	Sm corresponds to the energy needed to close all reaction centres (assumed proportional to the number of reduction and oxidation of one Q_A-molecule during the fast OJIP transient and therefore related to the number of electron carriers per electron transport chain)
N	Turnover number $N = Sm\, M_0\, (1/V_J)$	N represents the number of Q_A reduction events between F_0 and F_M (Vredenberg *et al*., 2006)
V_K	$V_K = (F_K - F_0)/(F_M - F_0)$	Variable fluorescence at K step
V_K/V_J	$V_K/V_J = (F_{300\mu s}-F_{50\mu s})/(F_{2ms}-F_{50\mu s})$	Time plot of the values of relative variable fluorescence where the K step occurs
$(1-V_J)/V_J$		Proportion of closed reaction centres. Corresponds to the probability that a trapped electron is transferred
RC/ABS	$RC / ABS = (RC / TR_0) \times (TR_0 / ABS) = [(F_J - F_0) / 4(F_K - F_0)] \times (F_V / F_M)$	Density of reaction centres on chlorophyll basis
J^{ABS}		Rate of photon absorption by total PSII antenna-denoted as absorbed photon flux express on a PSII RC basis (/ RC), or on cross-section basis (/CS)
J^{DI}		Rate of energy dissipation in all the PSIIs, in

		processes other than trapping, denoted as dissipated energy flux. Can be express on a PSII RC basis (/ RC) or on cross-section basis (/ CS)
J^{TR}		Rate of exciton trapping (leading to Q_A reduction) by all PSII RCs-denoted trapped exciton flux. At J_0^{TR}: maximum initial trapped exciton flux. Can be express on a PSII RC basis (/ RC) or on cross-section basis (/ CS)
J_0^{ET2}		Electron transport flux from Q_A to Q_B
J_0^{RE1}		Electron transport flux until PSI acceptors (defined at t = 30ms, corresponding to I-level)
J_0^{TR} / J^{ABS}	$J_0^{TR} / J^{ABS} = 1 - F_0 / F_M$	Maximum quantum yield of primary PSII photochemistry
J_0^{ET2} / J^{ABS}	$J_0^{ET2} / J^{ABS} = 1 - F_J / F_M$	Quantum yield of the electron transport flux from Q_A to Q_B
J_0^{RE1} / J^{ABS}	$J_0^{RE1} / J^{ABS} = 1 - F_I / F_M$	Quantum yield of the electron transport flux until the PSI electron acceptors
J_0^{RE1} / J_0^{ET2}	$J_0^{RE1} / J_0^{ET2} = (1 - V_I) / (1 - V_J)$	Efficiency/probability with which an electron from Q_B is transferred until PSI acceptors
PI	$PI = RC/ABS * (J_0^{TR} / J^{ABS} / (1- J_0^{TR} / J^{ABS})) * ((1-V_J) / (1-(1-V_J))$	Performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of Q_B.
PI_{ABS}^{TOT}	$PI_{ABS}^{TOT} = PI * (J_0^{RE1} / J_0^{ET2} / (1- J_0^{RE1} / J_0^{ET2}))$	Performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors
PI_{CSo}^{TOT}	$PI_{CSo}^{TOT} = F_M * PI_{ABS}^{TOT}$	Performance index on cross section basis

Table 1: Formulae and glossary of terms used in the analysis of the fluorescence transient OKJIP. Based on Strasser *et al*. (2004) and Stirbet and Govindjee (2011).

Moreover, different thresholds, if exist, with current parameters used to characterize plant response to soil drying were assessed. Water status was characterized at two levels, the plant and the soil. Stem and leaf water potentials were measured each day during the soil drying period, and periodic and concomitant measurements of soil humidity and g_s were realized. Parameters derived from the analysis of fluorescence induction curves were measured. Fruit response was analysed using fruit size, fresh mass, dry matter content and water potential before and at the end of the soil drying period. The different hypothesis tested here were the followings: i) the parameters measured as part of this trial can be exploited to characterize the differences in responses of tomato plants either at the vegetative or at the reproductive stage, when exposed to WD, ii) the parameters measured as part of this trial can be exploited to characterize the genotypic differences in responses of tomato plants exposed to WD.

II- Material and methods

2.1 Plant material & experimental conditions

Four indeterminate genotypes of *Solanum lycopersicum* L., Cervil, LA1420, Plovdiv and Levovil, provided by the Laboratory of Genetics and Fruit and Vegetable Breeding (GAFL) of the french National Institute of Agronomic Research (INRA), Avignon, France, were selected among the parents of the MAGIC TOM collection. We chose three small fruit size genotypes (Cervil, PlovdivXXIVa (here called Plovdiv) and LA1420) and one big fruit size genotype (Levovil). Levovil is close to the commercial tomato type Heinz, whereas Cervil, Plovdiv and LA1420 genotypes are close to the wild tomato *S. l. Pimpinellifollium* and show strong differences among them (Causse *et al*., 2013). The selected genotypes also distinguish themselves considering the growth rate criterion and can be classified as following; Levovil < Plovdiv < LA1420 < Cervil (unpublished data). LA1420 seeds were provided by the Tomato Genetics Resource Centre, Davis, California. Seeds of Cervil and Levovil were provided by Vilmorin Seed Company and Plovdiv seeds were from the Tomato Genetic Resource Centre of the GAFL lab, INRA, Avignon (France).

Two experiments were realized corresponding to the vegetative stage and to the reproductive stage, respectively. Sowing of 50 seeds by genotype for all trials was realized in January 2014 in a glasshouse located near Avignon, France. Vegetative plants were transferred in climatic chambers 30 days after sowing for the two trials (conducted in February 2014), one climatic chamber per experimentation also. These plant ages were selected according to the number of leaves available to realize the trial for vegetative plants (6 leaves) and to the fruit development stage of available trusses for reproductive plants (cell expansion stage). Climatic conditions imposed in climatic chambers were 11/13 h day/night photoperiod, at 400 µmol m^{-2} s^{-1} of photosynthetic photon flux, 23/18 °C day/night temperatures and 50/50 % day/night air humidity. Vegetative plants were grown in 1 L pots whereas reproductive plants were grown in 4 L pots, filled with a commercial substrate (Substrate 4, Klasmann France, Champety, France). Twelve vegetative plants of each genotype were transferred in climatic chambers and six plants by genotype for reproductive stage (i.e. 96 vegetative plants and 48 reproductive plants in total).

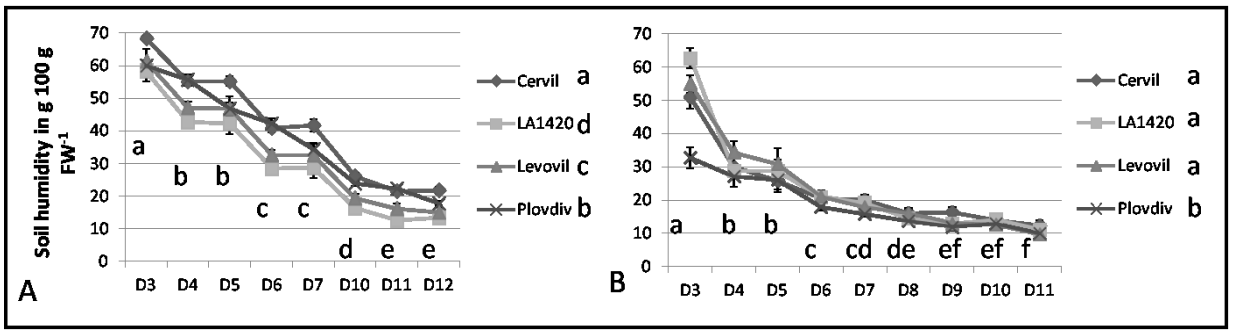

Figure 2: Kinetic curves for substrate humidity in vegetative (A) and reproductive plants (B) for 4 Tomato genotypes by day (D) of soil drying. Data are means of the two experiments ± SE ($n \geq 5$). Different letters for a given parameter correspond to significant differences between genotypes or between days of the kinetics of soil drying at the threshold $P < 0.05$ (Duncan test). Genotypic differences are indicated next to genotype legend. Interactions between genotypes and treatments were found only for reproductive plants.

Soil drying by stopping water supply started after three days of plant adaptation in the climatic chamber (and lasted until plant death). Substrate water content was used as an indicator of irrigation. Substrate humidity was 70 % at the onstart of soil drying and declined differently according to the developmental stage (vegetative or reproductive) (Fig. 2). Substrate water content for vegetative plants was estimated by weighting daily all the pots and using a calibration curve previously established (unpub. data). For trials on reproductive plants, soil humidity was assessed using water content sensors (WCM-control, Grodan, Roermond, The Netherlands).

2.2 Plant measurements

All plant measurements were realized daily on fully expanded leaves of 5 plants (vegetative plants) to 6 plants (reproductive plants). Stem and leaf water potential at predawn and noon ($\Psi_{predawn}$ and Ψ_{midday}), soil humidity and g_s were measured concomitantly each day during soil drying. Water potential was measured to assess the intensity of the stress only on reproductive plants for reliable water potential measurements cannot be made on developing tissues or organs. $\Psi_{predawn}$ and Ψ_{midday} were measured daily using a pressure chamber (SAM Précis 2000, Gradignan, France) on leaves bagged the day before for $\Psi_{predawn}$. Leaf g_s was measured using a porometer (AP4 Porometer, Delta T - Sols Mesures, Elancourt, France). Fluorescence emission of chlorophyll *a* was measured on 20 min. dark-adapted leaves using a fluorimeter (HANDY-PEA, Hansatech, King's Lynn, UK) in the morning. Light saturation was obtained by a 3000 µmol photons m^{-2} s^{-1} flash of 1 s. Parameters derived from OJIP fluorescence induction curves were calculated from the equations described in table 1. Chlorophyll content was evaluated using a chlorophyll meter (SPAD 502, Konica-Minolta, Osaka, Japan) and expressed as SPAD units.

Leaf dry matter content was measured only at the end of each stress period at 11 a.m., leaf dry weight was measured after 7 days in a ventilated oven at 70 °C. Two non-senescent leaves per plant were harvested at the end of the soil drying periods for measurements of the specific leaf surface area (SLA) on vegetative plants. Leaf area was measured with a Planimeter (Li-3100 C Area Meter, Li-Cor, Lincoln Nebraska, USA).

2.3 Fruit measurements

The second and the third fruits of the second truss (for Plovdiv, LA1420 and Levovil genotypes) and of the third truss (for Cervil) were harvested around noon for measurements of fruit size, of fresh weight, of dry matter content (using a ventilated oven, 70 °C for 7 days) and of water and osmotic potentials ($n \geq 6$). Fruit water potential Ψ_F was measured on cut slices of green fruits (pericarp with placenta) using a WP4C Dewpoint Potentiometer (Decagon devices, Pullman, U.S.A.) following the procedure of Léchaudel (2004).

2.4 Statistical analysis

All statistical analyses were performed using R3.1.0 (R Core Team, 2014). Soil drying effects were analysed by comparison with the initial measurements before irrigation was stopped.

Effects of soil drying and genotypes were analysed for all parameters using two-way analysis of variances (ANOVA) or the Kruskal-Wallis test (pgirmess package; Giraudoux, 2014), according to the residue normality tested using the Shapiro-Wilk test (Royston, 1995). The Levene test was used to check the homoscedasticity of variances of the residues (Car package; Fox and Weisberg, 2011). When authorized, two-way ANOVA was performed and followed by multiple comparisons of means (Tukey and Duncan tests, lsmeans and multcompView packages; Lenth, 2014, Graves *et al*., 2012 and Laercio package; Laercio, 2010). Correlations between physiological parameters were calculated using Pearson's test (Hmisc package, Harrell *et al*., 2014).

III- Results

Whatever the plant developmental stage, all plants wilted after 3 to 5 days of soil drying. Older leaves of all genotype at the vegetative stage displayed necrosis symptoms. Furthermore, Levovil's fruits plants at the reproductive stage were wilted (Fig. 3).

The soil drying was more gradual for seedlings than for reproductive plants. Significant differences between genotypes were observed due to morphological differences between them but they did not affect drying curves which were similarly shaped in all genotypes (Fig. 2).

3.1 Stress perception of vegetative plants

Leaf g_s was reduced in stressed vegetative plants, by 53.5 % in Cervil, 35.2 % in LA1420, 66.1 % in Plovdiv and 45.6 % in Levovil (Tab. 2). Considering dry weight and surface area increases, growth of vegetative plants was not affected during soil drying, on higher leaves (supplementary data).

Relationships between three fluorescence parameters F_V/F_M, PI and J_0^{RE1}/J^{ABS} and substrate humidity in figure 4 outlined that the observed decrease was well correlated with J_0^{RE1}/J^{ABS} index (64 %, Pearson coefficient) and F_V/F_M (46 % Pearson coefficient) in a low range of value (between 0.78 and 0.84 A.U.).

Soil drying resulted in an increase in F_0 in vegetative plants of Cervil, LA1420 and Levovil (+15 %, +21.6 % and +10.3 %, respectively, tab. 2). J_0^{TR}/RC decreased also in WD-stressed vegetative plants of all genotypes with the exception of Plovdiv (-7.8 % in Cervil, -15.5 % in Levovil and - 7.2 % in Plovdiv). This decrease in J_0^{TR}/RC was not associated with an increase in J_0^{DI}/RC in Cervil, Levovil and Plovdiv but to a (non-significant) decrease in J_0^{ABS}/RC. By contrast, there was a 56.8% increase in J_0^{DI}/RC in stressed LA1420 vegetative plants associated to a decreased of the electron trapping probability (F_V/F_0) of 24.8 %.

Stressed vegetative plants of all genotypes were characterized by a decrease in both Sm (-71.6 % in Cervil, -32.9 % in LA1420, -34.2 % in Plovdiv and -28.2 % in Levovil) and N (-74.2 % in Cervil, -34.9 % in LA1420, -38.9 % in Plovdiv and -39.4 % in Levovil). Similarly all stressed vegetative plants of all genotypes were characterized by a decrease in J_0^{RE1}/RC (-46.4 % in Cervil, -40.7% in LA1420, -47.4 % in Plovdiv and -37.3 % in Levovil) as well as in J_0^{RE1}/J^{ABS} (-42.6 % in Cervil, -38.5 % in LA1420, -43.3 % in Plovdiv and -26.7 % in Levovil)

Figure 3: Photographs of the experimental conditions at the vegetative stage (B) and at the reproductive stage (D) of Cervil, LA1420, Plovdiv and Levovil tomato genotypes (all mixed) in climate chambers. During soil drying, vegetative plant showed necrosis on older leaves (A) and Levovil's fruits wilted (C).

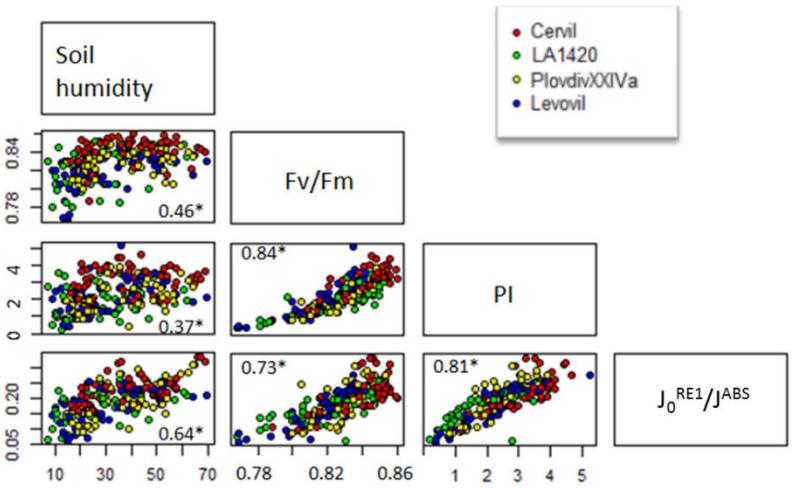

Figure 4: Binary relationships and Pearson's correlation coefficients between soil humidity, F_V/F_M, PI and J_0^{RE1}/J^{ABS} (see text for details) for 4 tomato genotypes exposed to WD at the vegetative stage. All data are represented here by genotype. Each colour corresponds to one genotype during soil drying ($n \geq 5$). The genotype Cervil is in red, LA1420 in green, Plovdiv in yellow and Levovil in blue. Significant differences are indicated using * symbols at the threshold $P < 0.05$ (Pearson correlation).

	Vegetative plants				Reproductive plants			
	Cervil	LA1420	Plovdiv	Levovil	Cervil	LA1420	Plovdiv	Levovil
Stomatal conductance	***-53,5***	***-35,2***	***-66,1***	***-45,6***	***-88,9***	***-66,4***	***-76,0***	***-65,9***
SPAD units	***13,6***	0,1	***8,2***	***6,7***	***14,3***	***20,5***	***12,5***	***8,5***
Fo	***15,0***	***21,6***	-1,6	***10,3***	-4,8	-0,5	2,0	3,2
Fm	8,9	-6,9	-3,3	7,5	-9,8	2,3	-6,8	-6,1
Fv/Fm	-1,2	-6,9	-0,2	-0,7	-1,3	0,6	-1,7	-1,7
Sm	***-71,6***	***-32,9***	***-34,2***	***-28,2***	-1,6	17,9	-9,3	0,4
N	***-74,2***	***-34,9***	***-38,9***	***-39,4***	2,3	11,9	-9,0	1,4
F_K	11,2	9,0	0,8	-0,1	4,1	1,5	10,0	5,6
V_K	0,8	21,7	3,9	-13,6	29,0	1,5	19,6	25,9
V_K/V_J	-7,6	1,6	-7,2	-15,5	4,9	-4,4	1,0	1,3
PI	-11,2	24,2	-14,8	10,0	***-33,4***	-1,3	***-33,4***	***-38,8***
RC/ABS	-1,0	-7,4	-4,1	16,4	-20,4	-0,7	-17,3	***-21,6***
Fv/Fo	-6,1	***-24,8***	-2,0	-3,1	-6,7	3,2	-10,2	-10,5
$(1-V_J)/V_J$	-12,8	-15,1	-19,6	-3,1	***-26,9***	-9,4	-24,2	***-30,1***
J_0^{ABS}/RC	-6,5	10,3	-6,8	-15,0	6,3	-5,1	2,0	2,7
J_0^{DI}/RC	-1,7	56,8	-5,8	-13,1	12,0	-7,3	11,7	12,9
J_0^{TR}/RC	***-7,8***	1,7	***-7,2***	***-15,5***	5,1	-4,6	0,4	0,8
J_0^{ET2}/RC	-13,3	-17,1	-16,2	***-17,0***	-10,0	-8,6	-10,1	-12,2
J_0^{RE1}/RC	***-46,4***	***-40,7***	***-47,4***	***-37,3***	-19,1	-10,6	***-29,2***	***-23,7***
J_0^{DI}/J^{ABS}	5,9	34,0	1,5	2,5	7,0	-3,2	9,9	9,3
J_0^{TR}/J^{ABS}	-1,2	-6,9	-0,2	-0,7	-1,3	0,6	-1,7	-1,7
J_0^{ET2}/J^{ABS}	-6,9	-18,3	-9,7	-1,8	-14,4	-3,1	-11,6	***-14,3***
J_0^{RE1}/J^{ABS}	***-42,6***	***-38,5***	***-43,3***	***-26,7***	***-22,3***	-7,0	***-30,2***	***-23,1***
J_0^{RE1}/J_0^{ET2}	***-38,8***	***-27,7***	***-37,9***	***-24,5***	-11,7	-3,4	-21,5	-11,5
PI_{ABS}^{TOT}	***-66,6***	-45,9	***-65,2***	-30,8	-40,0	-5,1	***-53,0***	***-50,1***
PI_{CSo}^{TOT}	2,2	-12,7	-17,6	21,1	-36,1	-0,3	-30,9	***-37,6***

Colour scale -90 -40 0 30 70

Table 2: Relative differences for stomatal conductance, leaf chlorophyll content extrapolated from SPAD units and chlorophyll *a* fluorescence measurements observed for vegetative plants (left) and reproductive plants (right) of Cervil, LA1420, Plovdiv and Levovil tomatoes exposed to WD. These differences were calculated on the means of initial values vs. the means of stressed values for the two experiments. The percentages were scaled by colour (green for high and red for low value). Significant differences are indicating by using bold, italic and underlined fonts (Tukey or Kruskal-Wallis). Tukey test was used for SPAD units, F_0 and F_M for vegetative plants; and for SPAD units, J_0^{ABS}/RC, J_0^{RE1}/J^{ABS} and PI for reproductive plants.

Genotype	Treatment	Fruit size (mm)	Fresh mass (g)	Dry mass (g)	Dry matter content (g 100 g FW^{-1})	Water potential (MPa)	Osmotic potential (MPa)
Cervil	Initial values	25.18 ± 1.18 **a**	7.43 ± 1.87 **a**	0.81 ± 0.028 **a**	10.39 ± 0.13 **a**	-1.41 ± 0.07 **a**	-1.10 ± 0.02 **a**
	Trial 1	23.71 ± 2.02 **a**	6.46 ± 1.48 **a**	0.64 ± 0.062 **a**	11.20 ± 0.19 **b**	-1.17 ± 0.04 **b**	-1.24 ± 0.05 **b**
	Trial 2	24.12 ± 1.47 **a**	6.85 ± 3.30 **a**	0.73 ± 0.039 **a**	11.32 ± 0.07 **b**	-1.35 ± 0.05 **a**	-1.36 ± 0.04 **b**
LA1420	Initial values	37.08 ± 0.32 **a**	22.16 ± 0.24 **a**	1.76 ± 0.198 **a**	6.71 ± 0.09 **a**	-0.92 ± 0.06 **a**	-0.85 ± 0.04 **a**
	Trial 1	46.81 ± 0.19 **b**	38.08 ± 0.17 **b**	2.12 ± 0.162 **a**	7.04 ± 0.22 **a**	-0.56 ± 0.03 **b**	-0.82 ± 0.02 **a**
	Trial 2	48.21 ± 0.30 **b**	37.71 ± 0.26 **b**	2.56 ± 0.363 **a**	6.86 ± 0.18 **a**	-0.74 ± 0.02 **b**	-0.89 ± 0.05 **a**
Plovdiv	Initial values	41.83 ± 2.04 **a**	43.98 ± 2.19 **a**	4.99 ± 0.185 **a**	7.39 ± 0.21 **a**	-0.87 ± 0.09 **a**	-0.86 ± 0.07 **a**
	Trial 1	25.81 ± 2.73 **b**	36.41 ± 5.28 **a**	5.22 ± 0.108 **a**	8.46 ± 0.15 **b**	-1.02 ± 0.10 **a**	-1.04 ± 0.02 **b**
	Trial 2	38.32 ± 2.28 **b**	32.38 ± 4.82 **a**	4.43 ± 0.113 **a**	7.80 ± 0.14 **b**	-0.81 ± 0.07 **a**	-0.91 ± 0.07 **a**
Levovil	Initial values	68.80 ± 1.87 **a**	133.05 ± 7.72 **a**	2.68 ± 0.328 **a**	4.82 ± 0.09 **a**	-0.78 ± 0.03 **a**	-0.69 ± 0.03 **a**
	Trial 1	50.72 ± 2.43 **b**	55.25 ± 6.41 **b**	2.41 ± 0.800 **a**	8.35 ± 0.22 **b**	-1.05 ± 0.03 **b**	-1.17 ± 0.04 **b**
	Trial 2	50.16 ± 1.39 **b**	57.72 ± 3.67 **b**	2.56 ± 0.256 **a**	8.24 ± 0.21 **b**	-1.25 ± 0.04 **b**	-1.16 ± 0.05 **b**

Table 3: Fruit size, fresh mass, dry mass, dry matter content and water potential measured in Cervil, LA1420, Plovdiv and Levovil tomato fruits. Trials 1 and 2 correspond to the measurements of the last day of soil drying for each experiment on reproductive plants. Measurements were realized on green fruits (under cell expansion process). Data are means ± SE ($n \geq 5$). Different letters for a given parameter correspond to significant differences between initial and stressed values at the threshold $P < 0.05$ for a given genotype (Tukey test, interactions between genotypes and treatments were found for all parameters).

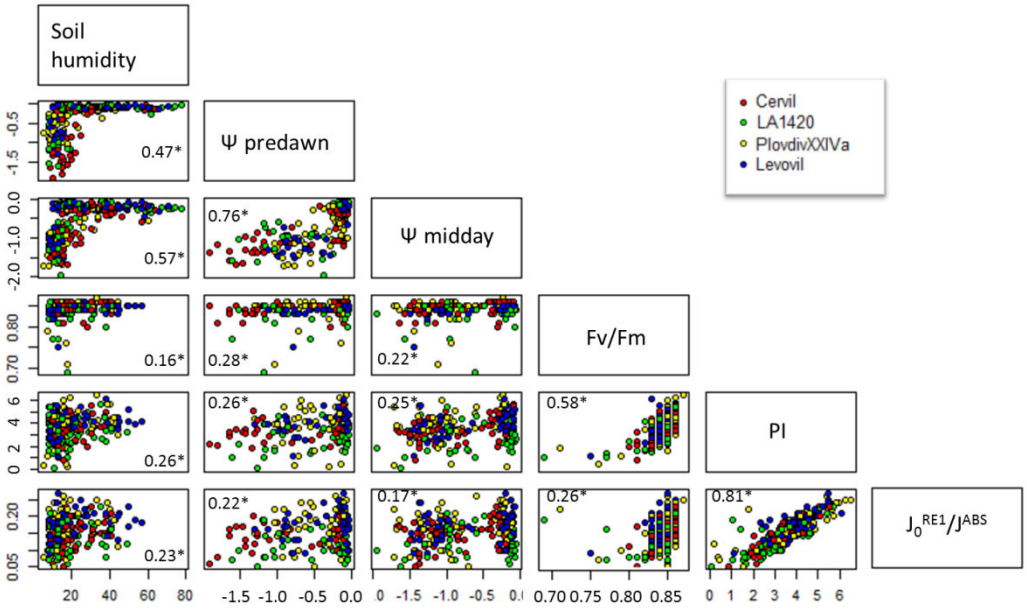

Figure 5: Binary relationships and Pearson's correlation coefficients between soil humidity, predawn water potential, leaf water potential at midday, F_V/F_M, PI and J_0^{RE1}/J^{ABS} (see text for details) for 4 tomato genotypes exposed to WD at the reproductive stage.
All data are represented here by genotype. Each colour corresponds to one genotype during soil drying ($n \geq 5$). The genotype Cervil is in red, LA1420 in green, Plovdiv in yellow and Levovil in blue. Significant differences are indicated using * symbols at the threshold $P < 0.05$ (Pearson correlation).

and in J_0^{RE1}/J_0^{ET2} (-38.8 % in Cervil, -27.7 % in LA1420, -37.9 % in Plovdiv and -24.5 % in Levovil). Consistent with these observations, the performance index for energy conservation from photons absorbed by PSII antenna until the reduction of PSI acceptors (PI_{ABS}^{TOT}) decreased in all genotypes, non-significantly in LA1420 and Levovil, and significantly in Cervil (-66.6 %) and in Plovdiv (-65.2 %).

3.2 Stress perception of reproductive plants

Leaf g_s was substantially reduced in stressed vegetative plants whereas in stressed reproductive plants it was strongly reduced by 88.9 % in Cervil, 66.4 % in LA1420, 76.0 % in Plovdiv and 65.9 % in Levovil (Tab. 2). The decrease in stomatal conductance was positively correlated with the decrease in soil humidity (74 %, Pearson coefficient) and in water potentials of leaves at noon and at predawn (57 % each, $P < 0.01$). The relationships between plant water potentials and substrate humidity (Fig. 5) observed a rapid decrease (in three days) to the threshold of 20 % of substrate humidity where after plant water potentials decreased (Fig. 2). Also, after this threshold of 20 % of substrate humidity (i.e. 0.2 g g^{-1} FW), the three indexes of fluorescence F_V/F_M, PI and J_0^{RE1}/J^{ABS} decreased. This relationship between predawn or midday water potentials and soil humidity was a log correlation (47 and 57 %, Pearson coefficient, fig. 4). Concerning the three fluorescence parameters used to determine the relationship with substrate humidity, PI was the parameter that was the best correlated with soil humidity (26 %) when compared to Fv/Fm and J_0^{RE1}/J^{ABS}.

Concerning all fluorescence parameters derived from the OJIP transient (Tab. 2), the performance index for energy conservation from photons absorbed by PSII antenna (PI) to the reduction of Q_B decreased in Cervil (-33.4 %), in Plovdiv (-33.4 %) and in Levovil (-38.8 %). This decrease could be explained by the decrease in the density of reaction centres (RC/ABS) in Levovil (-21.6 %) which was accompanied by a reduction in $(1-V_J)/V_J$ in Levovil (-12.8 %) and in Cervil (-13.8 %). Furthermore, J_0^{ET2}/J^{ABS} decreased in stressed plants of Levovil (-14.3 %, $P < 0.10$). Also, PI_{ABS}^{TOT} decreased in Plovdiv (-53 %) and in Levovil (-50.1 %). These decreases are linked to the reduction of electron transport flux until PSI acceptors (J_0^{RE1}/RC) in Plovdiv (-29.2 %) and in Levovil (-23.7 %). Similarly the quantum yield of the electron transport flux until the PSI electron acceptors (J_0^{RE1}/J^{ABS}) was found to be lower in Cervil, Plovdiv and Levovil (-22.3 % in Cervil, -30.2 % in Plovdiv and -23.1 % in Levovil).

It is interesting to note that the fruit water potential and the dry weight of reproductive plants were little affected during soil drying (Tab. 3). Ψ_F increased in Cervil and LA1420

fruits (from -1.41 MPa to -1.35 MPa in Cervil and from -0.92 to -0.74 MPa in LA1420). Ψ_F was stable in Plovdiv and decreased in Levovil fruits (from -0.78 to -1.25/-1.05 MPa).

Fruit dry masses during soil drying were stable whereas fruit fresh mass decreased in Levovil (-57.6 %), increased in LA1420 (+71 %) and were stable in Cervil and Plovdiv. These results suggest that LA1420 fruits were still growing during soil drying and that their dry matter content was stable. In Cervil, Plovdiv and Levovil, the dry matter content increased likely due to a decrease of water influx in fruits (+8.4 %, +10 % and +72 %, respectively). In Levovil, the decrease in fresh mass might be due to water efflux from fruits to the plant, as fruit wilting would suggest (Fig. 3).

IV- Discussion

The monitoring of four tomato genotypes selected according to their allelic and origins differences (Causse *et al*., 2013), at two developmental stages, constitute a good basis to characterize the impacts of soil drying and plant strategy to cope with stress. After assessing the constraints perceived by plants in terms of g_s and water potentials, we assessed damages perceived in photochemistry metabolisms by oxidative stress induced during soil drying. Damages can be more or less reversible. We shall consider here damage indicators from the OJIP-test model (Strasser *et al*., 2004). OJIP-test model can be linked with the efficiencies of electron transfer in photosystems (PSII and PSI) and between them. All these measurements permitted to differentiate different strategies to cope with WD depending on the developmental stages of tomato plants.

4.1 Effects of soil drying applied during the vegetative period

Vegetative plants exposed to soil drying were characterized by senescence of older leaves and a reduction of g_s. Surprisingly, the decrease in g_s was not associated with any decrease in leaf growth as evaluated from measurements of leaf dry mass, leaf dry matter content and leaf surface. This can only be explained by a concomitant increase in leaf photosynthetic capacity. We observed indeed an increase in the leaf dry mass-to-area ratio and in SPAD units (Supplementary data 1). Leaf dry mass-to-area ratio is related to leaf nitrogen per unit leaf area, which is correlated to photosynthetic capacity (Urban *et al*., 2003; Urban and Léchaudel, 2005). Light-driven shifts in leaf dry mass-to-area ratio have been observed in many species. Leaf dry mass-to-area ratio may also increase as a consequence of drought in some species (Perez-Martin *et al*., 2009). SPAD units can be correlated with leaf chlorophyll content on a surface basis (Urban *et al*., 2008) and can therefore also be considered as an indicator of photosynthetic capacity.

The so-called turnover number of Q_A reduction and reoxidation, N, was lower in all 4 genotypes exposed to WD. $N = Sm. J_0^{TR}/RC$ where Sm represents the area of the OJIP curve until F_M normalized by F_V, and J_0^{TR}/RC the maximum trapped exciton flux per PSII. Sm was found to be strongly lower, as a consequence of soil drying in all 4 genotypes. With the exception of LA1420, J_0^{TR}/RC, was also lower in plants during soil drying. Sm is arguably an indicator of the pool size of the electron carriers, i.e. of the size of the plastoquinone pool (Yordanov *et al*., 2008). The plastoquinone pool may decrease as a consequence of stress

(Bishop, 1961; Shavit and Avron, 1963) but then probably only when stress is severe. Christen *et al.* (2007) observed that moderate drought did not affect Sm in grapevine. The observations made about Sm suggest that soil drying was clearly a severe WD in our trial.

Lower values of J_0^{TR}/RC in Cervil, Plovdiv and Levovil plants exposed to WD are attributable either to a decrease in the average absorbed photon flux per PSII reaction centres (or the apparent antenna size of an active PSII), J^{ABS}/RC, or to an increase in the rate of dissipation of energy flux by processes other than trapping per PSII reaction centres, J_0^{DI}/RC, Although the differences were not significant, trapping decreased in Cervil, Plovdiv and Levovil plants exposed to WD apparently as a consequence of lower photon absorption, not of an increase in heat dissipation. Whereas trapping was unchanged in LA1420 plants exposed to WD, despite a ca. 10 % increase in photon absorption, because of increased heat dissipation. This at least when trapping is expressed per PSII reaction centres. The picture is different when trapping is considered on a photon absorption basis. The 25 % decrease in F_V/F_0 in LA1420 plants exposed to WD suggests that energy trapping probability in the sense of quantum yield of primary PSII photochemistry, was indeed reduced (Krause and Weis, 1991). This is surprising since it is generally observed that the maximum yield of primary photochemistry of PSII is not much affected by drought (Oukarroum *et al*., 2007). Interestingly, F_0, the initial value of fluorescence, was higher in Cervil, LA1420 and Levovil plants exposed to WD. F_0 is the level of fluorescence emission when all the primary quinone acceptors Q_A are in the oxidised state. An increase in F_0 may be caused by the release of free chlorophyll from protein-pigment complexes, which results in blocked energy transfer to the PSII traps (Armond *et al*., 1978, 1980; Sundby *et al*., 1986). An increase in F_0 may not be reflected in a decrease in F_V/F_M when there is a concomitant decrease in F_M, which was the case for Cervil and Levovil, albeit it was not a significant one, As for Sm, our observations about F_0 suggest that the stress we imposed in this trial was severe, possibly leading to photodamage. It must be kept in mind that drought stress may induce a loss of the D1 and D2 proteins of PSII (He, 1995).

By contrast to other studies (Livingston *et al*., 2010; Johnson, 2011) about the effect of drought, we observed that the electron transport flux until PSI acceptors expressed either per PSII reaction centres (J_0^{RE1}/RC), per cross section (J_0^{RE1}/CS_0) (not shown) or on a photon absorption basis (J_0^{RE1}/J^{ABS}) was strongly reduced, and this in all four genotypes of our trial. Consistent with these observations, the probability with which an electron from Q_B is transferred until PSI acceptors (J_0^{RE1}/J_0^{ET2}) appears also much reduced in all genotypes exposed to WD. We may speculate that impaired electron flow from intermediate electron

carriers to final acceptors of PSI is compensated by alternative electron routes such as chlororespiration. As hypothesized by Rumeau *et al*. (2007), plastid terminal plastoquinone oxidase (PTOX) may limit electron pressure on PSI acceptor and prevent PSI photoinhibition in conditions of severe stress.

4.2 Effects of soil drying applied during the reproductive period

Leaf g_s was found to decrease dramatically in all genotypes exposed to WD. As usually observed in drought-stressed plants, the dry matter content was higher and dry mass was lower, with the exception of Cervil (Supplementary data 2). SPAD units were higher, reflecting higher leaf chlorophyll content like in vegetative plants.

The relationships between plant water potentials and substrate humidity illustrate the rapid decrease in substrate humidity to 20 % (in three days) than in vegetative plants it was more progressive (more than seven days to 20 % of substrate humidity) that explained the linear relations found for the fluorescence parameters in vegetative plants. The logarithmic relationship in reproductive plants was previously observed between Ψ_{midday} and g_s in orange trees (Gomes *et al*., 2004), with a threshold at 50 mmol m^{-2} H_2O s^{-1} of g_s before the decrease in water potentials. Similar value of g_s was found in this trial (Supplementary data 1 and 2). Also, Coupel-Ledru *et al.* (2014) have observed this log relationship between $\Psi_{predawn}$ and soil water content in vines with a higher threshold of 0.6 g g^{-1} than the 0.2 g g FW^{-1} found in this study, probably by differences in soil composition or plant water use. Moreover, among the three selected parameters of chlorophyll *a* fluorescence, PI was the best correlated with substrate humidity in reproductive plants, while in vegetative plants it was J_0^{RE1}/J^{ABS}. The log relationship was also observed on beans in WD with a decrease of PI index after the threshold of 20 % of leaf relative water content (RWC) and a linear relationship between leaf RWC with J_0^{RE1}/J^{ABS} in a range of 60 to 20 % of RWC (Goltsev *et al*., 2012).

The way tomato plants at the reproductive stage organize energy and electron fluxes around PSII and PSI was completely different from tomato vegetative plants. The major strategies evidenced in vegetative plants exposed to WD were just absent, namely the decreases in N and Sm, the decreases in J_0^{TR}/RC or increases J_0^{DI}/RC, the increases in J_0 or decreases in F_0/F_M, with the notable exception of the parameters related to electron transport flux until PSI acceptors (J_0^{RE1}) that were lower, albeit less than in vegetative plants, in all genotypes except LA1420. By contrast to the observations made on vegetative plants, the

composite Performance Index on an absorption basis (PI) of Strasser (Srivastava and Strasser, 1999; Strasser and Srivastava, 1995; Strasser *et al*., 2004; Stirbet and Govindjee, 2011) was found to be reduced in all genotypes except LA1420, as a consequence of exposure to WD. In addition to RC/ABS, PI encompasses F_V/F_0 and $(1-V_J)/V_J$, an indicator of the performance of conversion of excitation energy to photosynthetic electron transport. This latter parameter was the one that provided the highest contribution to PI. PI is considered as a much more sensitive and discriminating stress indicator than F_V/F_M (Živčák *et al*., 2008; Johnson, 2011) a fact clearly confirmed by our data set. PI_{ABS}^{TOT} was found to be even more performing than PI in our trial which is understandable since PI_{ABS}^{TOT} is the performance index for energy conservation from photons absorbed by PSII antennae until the reduction of PSI acceptors.

The reproductive stage is characterized by important fluxes of water and metabolites entering into fruits. Fruit water influx via xylem and phloem tissues follows the stem-to-fruit gradient of water potential and is generated by a gradient of osmotic potential between sources and sink tissues (Guichard *et al.,* 2001). WD applied during this stage is known to induce water losses in fruits with a reduction in yield according to WD intensity (Li *et al*., 1989). Indeed, fruit size and fresh mass were reduced in Plovdiv and Levovil fruits, and were stable in Cervil. However, LA1420 fruits were surprisingly bigger than initial fruits, which could be due to protection mechanisms aiming at maintaining fruit growth under conditions of stress and linked to higher photosynthetic activity. Dry matter content increased in Cervil, Plovdiv and Levovil fruits due to reduced water fluxes, but was stable in LA1420. Wilting of Levovil fruits observed during the last days of soil drying could be explained by water efflux from the fruits to the rest of the plant, which is consistent with our observation of a decrease in fruit fresh mass during soil drying. Clearly contrasting responses were observed among genotypes: LA1420 was capable to maintain fruit growth whereas Levovil wasn't. These differences could be explained by differences in fruit size: Levovil fruits are bigger than LA1420 and Plovdiv fruits which are cocktail tomatoes, and Cervil fruits which are cherry-type tomato. We observed no reduction in growth of Cervil fruits arguably because fruit growth had come to an end in this genotype as the fact that Cervil fruits were at the breaker stage at the end of soil drying period would suggest.

4.3 At each plant developmental stage its strategy to soil drying

Clearly the impact of WD differed according to the plant developmental stage. Indeed, the monitoring of the soil kinetic during WD reveals different speed of soil drying according

to plant developmental stage (Fig. 2). In comparison, leaf g_s of reproductive plants was strongly reduced than in vegetative plants (74 % vs. 50 % in mean for all genotypes) and reproductive plants had a higher increase in chlorophyll content (14 % vs. 7 % in mean). An increase in chlorophyll content was described as an indicator of a low degree of photo-inhibition in leaves, due to the influence of carbohydrates and redox control in chloroplasts (Mäkelä *et al*., 2000; Murchie *et al*., 2009). Also, it was suggested that vegetative plants try to dissipate the excess of energy perceived in PSII by heat according to J_0^{DI} (for LA1420, Cervil and Levovil).

At both stages, the step between Q_B to PSI acceptors was the most affected by WD with a higher decrease in the electron transfer for vegetative plants. It appears that fluorescence of chlorophyll *a* parameters were very useful to characterize soil drying. For example, PI and J_0^{RE1}/J^{ABS} were more useful to characterize WD range and interestingly differed according to the plant developmental stage. However, whatever the plant developmental stage affected by soil drying, the different quantum yields of photo-induced electron transfer, from P680 to Q_A (J_0^{TR}/J^{ABS}), from Q_A– to PQ (J_0^{ET2}/J^{ABS}), and from PQ to the PSI electron acceptors (J_0^{RE1}/J^{ABS}), can be arranged according to their decreased sensitivity to WD in the sequence $J_0^{RE1}/J^{ABS} > J_0^{ET2}/J^{ABS} > J_0^{TR}/J^{ABS}$ as it was found on beans (Goltsev *et al*., 2012). So, the quantum yields of the reactions close to the PSII reaction centres were more tolerant to WD than the reaction close to the PSI reaction centres. Only LA1420 at the reproductive stage had no reduced quantum yields. Vegetative plants of Cervil and Plovdiv genotypes were globally the more sensitive to WD with the higher decrease in PI_{ABS}^{TOT}, whereas at the reproductive stage, Plovdiv and Levovil were the most sensitive to WD according to PI_{ABS}^{TOT}, followed by Cervil. Only LA1420 was the less sensitive to WD whatever the plant developmental stage affected by soil drying. To conclude on the selection of useful parameters of chlorophyll *a* fluorescence, at the vegetative stage in tomato, the most useful parameters were: F_0, Sm, N, J_0^{ET2} (expressed either on an absorption basis (ABS) or per reaction centres RC), J_0^{RE1} (expressed on an absorption basis (ABS), per reaction centres (RC) or by J_0^{ET2}) and PI_{ABS}^{TOT}. And at the reproductive stage, parameters were: F_M, PI (and its three components F_V/F_0, RC/ABS and $(1-V_J)/V_J$), J_0^{ET2} (expressed either on absorption basis), J_0^{RE1} (expressed either on ABS, RC and J_0^{ET2}) and PI_{ABS}^{TOT}.

On their whole, results permitted to distinguish the plant developmental stage responses using water status and chlorophyll fluorescence measurements in relation to substrate humidity. The genotypic differences observed before soil drying were maintained

during WD. Furthermore different sensitivity to WD was found among genotypes with decreasing sensitivity from Cervil > Plovdiv > Levovil > LA1420 at the vegetative stage, whereas on reproductive stages sensitivity decreased from Levovil > Plovdiv > Cervil > LA1420, according to results in table 2. In reproductive plants, these differences could be explained by the sink demand in carbon according to fruit size, with lower demand for small fruits. LA1420 origins could explain its different responses because it was the closest to *S.l. pimpinellifollium* L. which is known to be resistant to WD (Causse *et al*., 2013; Foolad *et al*., 2003).

V- Conclusion

Different strategies were found according to the developmental stage in tomato during soil drying kinetic. Vegetative plants used preferentially mechanisms to dissipate energy in excess around PSII while reproductive plants reduced their water losses by a higher decrease in g_s and leaf water regulations. Also, reproductive plants had higher chlorophyll content. Furthermore, genotypic differences can be assessed with the OJIP transient parameters. A selection of useful parameters to distinguish genotypic effects and effects of plant development stage was given, namely PI_{ABS}^{TOT} and J_0^{RE1}/J^{ABS} that appeared very useful to characterize WD conditions. These results offer useful tools to characterize WD around the photosystems II but they could be completed by studying the effects of soil drying in photosystems I with others parameters to complete our understanding of plant response to WD. Furthermore, the hypothesis that LA1420 could improve its photosynthesis activity and the inverse water flux between Levovil plant and fruits needed more investigations.

Acknowledgement

This paper was supported by the *Federative Research Structure* Tersys. The authors thank the team of the UR1052 Genetics and Improvement of Fruit and Vegetables (INRA, Montfavet) for providing the plant material.

Impacts du déficit hydrique sur la sensibilité de la tomate au pathogène *Botrytis cinerea* - Implications du métabolisme primaire et des voies de régulations hormonales

Lors des précédents chapitres, nous avons évalué la stimulation de la réponse adaptative des plantes lors de l'application de WD modéré en termes d'activité photosynthétique et des métabolismes à l'échelle de la plante et du fruit en fonction du stade de développement (plante ou fruit) et de la variabilité génétique. Dans ce chapitre, nous nous sommes interrogés sur les effets stimulants que pouvaient engendrés un WD modéré lors d'interaction avec d'autres stress. En effet, il a été vu que les interactions entre stress ne sont pas forcément additives (Mittler, 2006) et peuvent être bénéfiques à la plante notamment lors d'interactions entre un stress abiotique et un stress biotique (Atkinson and Urwin, 2012).

Nous avons donc étudié les effets de l'interaction d'un WD modéré avec un pathogène majeur de la tomate de type nécrotrophe. Les pathogènes nécrotrophes induisent la mort cellulaire des tissus infectés. Il existe peu de traitements contre ce type de pathogène, notamment pour *Botrytis cinerea*. L'interaction entre un stress azoté et *B. cinerea* sur laitue a révélé un lien entre les teneurs en saccharose et la pathogénicité (Lecompte *et al*., 2013). Les sucres étant des constituants basiques des défenses de la plante, il apparait intéressant de combiner le WD avec ce pathogène au vue des résultats précédemment observés.

Ainsi, l'objectif principal de ce chapitre était de voir si les effets d'un WD sur les teneurs en sucres de la plante avaient un impact sur le développement du pathogène. Deux niveaux de WD ont été appliqués (un modéré et un plus intense). Deux types d'infection ont également été réalisées: i) des infections sur tige comme il en est souvent observé dans les cultures suite à des effeuillages, ii) des infections sur feuilles détachées. Nous avons choisi d'utiliser deux variétés de tomate commerciale (Momor et Monalbo) connues pour leurs différents niveaux de sensibilité.

Les effets des WD et des infections à l'échelle des feuilles et des tiges ont été caractérisés en termes de potentiels hydriques, de conductance stomatique, de la fluorescence de la chlorophylle *a*, de croissance foliaire et de développement du pathogène, des teneurs en matières sèches, sucres solubles, acides organiques, carbone et azote des tissus, de régulations hormonales (voies de l'acide abscissique et de l'acide salicylique) et de dépôts de callose. Ce chapitre est présenté sous la forme d'un article en cours de préparation.

Résumé

Botrytis cinerea est un agent pathogène nécrotrophe. Les pratiques culturales telles que l'effeuillage facilitent la pénétration de *Botrytis* dans la plante, son développement, et l'émission de spores propageant l'agent pathogène dans les serres. Il a récemment été montré sur laitue que le contenu en sucres solubles de la plante hôte était corrélé à l'intensité des symptômes observés après infection. Les stress abiotiques sont connus pour impacter les métabolismes primaires et secondaires des plantes. Deux intensités de WD ont été appliquées sur des plants de tomate, correspondant à des diminutions de 60% et 80% d'apport d'eau par rapport à un contrôle, pendant 21 jours. Les plantes ont été cultivées en serre puis transférées en chambres de culture dans un environnement contrôlé après inoculation de *B. cinerea* sur des plaies d'effeuillage. Parallèlement, des inoculations sur feuilles détachées ont été réalisées. La croissance des chancres de *B. cinerea* sur les tiges de tomate est apparue plus forte sur les plantes ayant subi un stress hydrique. A contrario, les symptômes sur feuilles détachées étaient plus forts sur les témoins bien alimentés en eau. Les analyses biochimiques ont révélé des différences importantes de teneurs en sucres solubles et en acides organiques entre feuille et tige (avec des teneurs plus importantes en sucres dans les tiges). Sur l'ensemble des données, le contenu relatif en fructose dans le pool de sucres solubles (fructose / sucres totaux) est corrélé négativement aux symptômes observés sur les tiges, à l'inverse du glucose. Les régulations hormonales observées pour les voies de l'acide salicylique (SA) et de l'acide abscissique (ABA) mettent à jour une forte stimulation de la voie SA par les stress seuls (WD ou infection) et combinés, et de l'ABA uniquement par les WD. L'accroissement de la sensibilité observé sur les tiges est attribué à des modifications du métabolisme primaire favorisant la propagation du pathogène dans les tiges et des régulations hormonales antagonistes avec la voie de l'acide jasmonique et de l'éthylène, voie de réponse aux pathogènes nécrotrophes. Un effet systémique entre infections sur tige et infections foliaires a révélé une réduction des infections foliaires en condition de WD, offrant de nouvelles perspectives de recherche.

Mot clés: *Botrytis cinerea*, Déficit hydrique, Interactions entre stress, Métabolisme primaire, Régulations hormonales, *Solanum lycopersicum* L.

Abstract

Botrytis cinerea is a necrotrophic pathogen. Cultural practices such as thinning out of leaves facilitates the penetration of *Botrytis* in plant, the development of fungal tissue and spores synthesis spreading the pathogen in greenhouses. It has recently been shown in lettuce that modifications of solubles sugars contents appeared related to a decrease of infected areas. Abiotic stresses are known to impact on primary and secondary metabolisms in plants. Two intensities of water deficit (WD) have been applied before infestation, by a reduction of 60 % and 80 % of water supply for 21 days. Plants were grown in a greenhouse and infected with *B. cinerea* on wounds of thinning out of leaves, in controlled environment. A deleterious effect of the interaction WD - *B. cinerea* was observed for disease symptoms on stem, on the contrary to leaf infections. On their whole, results reveal significant differences in the levels of soluble sugars and organic acids between leaf and stem (with higher sugar content in stems). Out of all data, the ratio fructose on total sugars spring negatively correlated with the disease symptoms on stem, on the contrary to the ratio glucose on total sugars. Hormonal regulation observed for salicylic acid (SA) and abscisic acid (ABA) pathways in this preliminary study updates a strong stimulation of SA pathway by stresses alone (WD or infection) and combined, and ABA pathway only by WD. Thus, our observations suggested a deleterious effect for WD and *B. cinerea* interaction, due to changes in sugar content that promote sugars necessary for spread of the pathogen in stems, with antagonistic regulations of jasmonic acid and ethylene pathways induced in responses to necrotroph pathogens. Also, a systemic effect of stem infections for foliar infections has been updated with new research perspectives.

Key words: *Botrytis cinerea*, Deficit irrigation, Hormonal regulations, Interactions, Primary metabolism, *Solanum lycopersicum* L.

I- Introduction

Au cours de leur développement les plantes sont soumises à des stress multiples entraînant différentes réactions défensives selon les combinaisons de stress biotique et abiotique subies. Les interactions entre stress abiotiques ont souvent des effets additifs délétères comme la combinaison déficit hydrique (WD) avec de fortes températures (Mittler et Blumwald, 2010). Cependant, les interactions entre stress abiotique et biotique peuvent entraîner des effets contradictoires selon les combinaisons de stress, en augmentant ou diminuant la sensibilité de la plante aux pathogènes en fonction de l'intensité, la durée et la nature du stress abiotique (Achuo *et al*., 2006). Ces interactions entre stress abiotique et biotique sont orchestrées par les voies de régulations hormonales induites par chacun des stress.

La régulation hormonale et le signal induit par les contraintes abiotiques et biotiques impliquent généralement la voie de l'acide abscissique (ABA) pour les stress abiotiques et les voies antagonistes de l'acide salicylique (SA) et de l'acide jasmonique (JA) associées aux voies de l'éthylène (ET) pour les stress biotiques. Cependant les interactions entre ces différentes voies sont complexes (Fig. 1). L'ABA, qui a un rôle central dans la réponse des plantes aux stress abiotiques en déclenchant des changements majeurs dans l'expression des gènes et les réactions physiologiques d'adaptation au stress (résumé par Danquah *et al*., 2014), est aussi impliqué dans les processus de réponse aux agents pathogènes (champignons ou bactéries). Ce rôle de l'ABA lors de stress biotique concerne la fermeture des stomates pouvant possiblement limiter l'entrée du pathogène dans la plante (Ton *et al.*, 2009), la formation de dépôts de callose (polymère dérivé de la cellulose de type β-1,3-glucan) permettant de limiter la propagation du pathogène, ou en interagissant avec les autres voies hormonales de défense, en réprimant la voie de signalisation de SA qui est connue pour réprimer celles de JA/ET (Flors *et al*., 2005). Les voies de JA/ET régulent les réponses immunitaires de la plante contre les pathogènes nécrotrophes comme *Botrytis cinerea*. La voie de SA est impliquée contre les pathogènes biotrophes et augmente la sensibilité aux pathogènes nécrotrophes de par son antagonisme avec la voie JA via la protéine NPR1 et l'inhibition de la signalisation de l'auxine (Blanco-Ulate *et al*., 2013). Cependant dans certains cas, l'ABA peut aussi inhiber la production de formes réactives de l'oxygène (ROS), augmentant la sensibilité de la plante aux pathogènes chez la tomate (Asselbergh *et al*., 2007). L'ABA peut également réprimer l'activité de la PAL, enzyme clé de la voie de biosynthèse

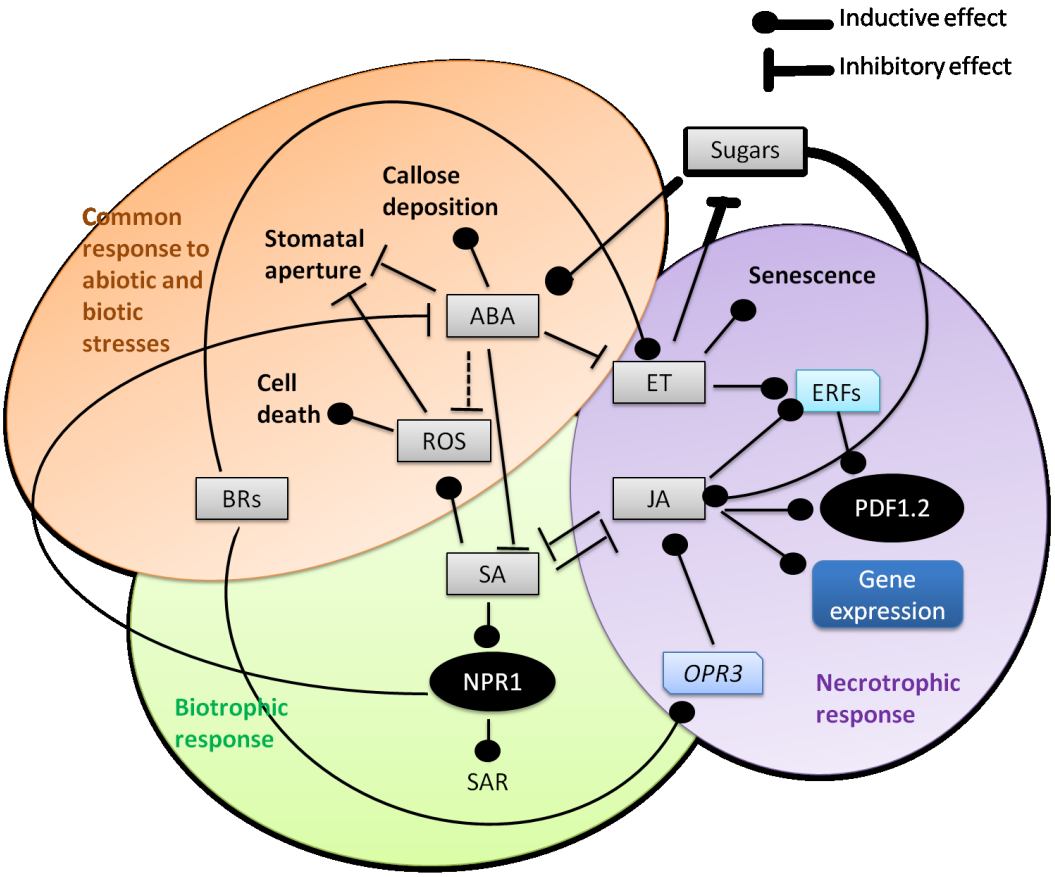

Figure 1: Illustration des principales voies de réponses hormonales aux stress biotiques biotrophes et nécrotrophes, avec les réponses communes aux stress abiotiques chez les plantes en général, au vue de la littérature citée. La voie de l'acide abscissique (ABA), normalement induite en réponse aux stress abiotiques, va en réponse à un stress biotique induire la réduction de l'ouverture des stomates et la formation de dépôts de callose et dans certains cas (flèche en pointillé) réduire la production de composés réactifs de l'oxygène (ROS) (Ton *et al*., 2009). Les ROS à fortes doses induisent des phénomènes de mort cellulaire, qui lors de stress biotiques permettent aussi de réduire la propagation du pathogène (Mur *et al*., 2013). Certains ROS, tel l'oxyde nitrique, sont impliqués dans des interactions positives avec les voies de biosynthèse de l'acide salicylique (SA), de l'acide jasmonique (JA) et de l'éthylène (ET) (Mur *et al*., 2013), non représentées sur ce schéma. Les brassinostéroïdes (BRs) favorisent la voie de JA au travers de l'activation du gène *OPR3* ainsi que la synthèse d'ET et ont donc un potentiel pour influencer les réponses aux stress biotiques (Bajguz et Hayat, 2009). La voie SA est induite lors de stress issus de pathogènes biotrophes, permettant la synthèse de la protéine NPR1 et la mise en place de la résistance systémique active (SAR). Les voies SA et ABA sont antagonistes aux voies JA/ET induites lors d'infection par des pathogènes nécrotrophes. La synergie entre les voies JA/ET confère à la plante une résistance aux pathogènes nécrotrophes, en induisant l'expression d'une centaine de gènes de défense et la stimulation de l'émission de composés organiques volatils (Devoto et Turner, 2003). Il a également été observé des interactions positives entre les sucres et les hormones ABA et JA et des interactions négatives avec l'ET (Tauzin et Giardina, 2014).

des phénylpropanoïdes à l'origine de métabolites secondaires dont certains présentent des propriétés antimicrobiennes (Ward *et al*., 1989).

Lors d'infections par un pathogène, le WD peut avoir des effets variables selon le pathogène (Atkinson et Urwin, 2012). A notre connaissance, une seule étude s'est intéressée aux effets d'un WD (par arrêt des apports d'eau, trois fois consécutives) sur la propagation du pathogène *B. cinerea* chez la tomate. Cette étude d'Achuo *et al*. (2006) a mis en évidence une interaction positive pour la plante avec une diminution des symptômes lors d'infections sur feuilles détachées. Le rôle de l'ABA dans cette interaction n'est pas univoque. Sur feuilles détachées, les mutants ABA de la tomate *sitiens* sont plus résistants lors d'infections par des pathogènes nécrotrophes (Audenaert *et al*., 2002; Achuo *et al*., 2006; Asselbergh *et al*., 2008). Sur plante entière, aucune différence entre mutants *sitiens* et plantes non mutées n'a été retrouvée (Beyers *et al*., 2014), de même pour les mutants ABA *flacca* (Vicedo *et al*., 2009). Lors d'infection sur tige, les mutants *sitiens* sont plus résistants que les plantes non mutées (Beyers *et al*., 2014). Cette résistance des mutants *sitiens* serait reliée à un renforcement de la paroi anticlinale des cellules en contact avec le pathogène en lien avec une accumulation d'H_2O_2 (Asselbergh *et al*., 2008). De plus, la mutation ABA de *sitiens* engendre une altération de la structure et de la composition de la surface des feuilles (déposition irrégulière de cutine, hydrophobie réduite, méthyl-estérification plus importante de la pectine) accentuant sa résistance au pathogène (Curvers *et al*., 2010). Des applications d'ABA exogène restaurent la sensibilité des mutants *sitiens* et *flacca* au pathogène (Achuo *et al*., 2006; Asselbergh *et al*., 2008) pouvant même dans certains cas rendre les mutants plus sensibles que les plantes non mutées (Audenaert *et al*., 2002). La baisse des symptômes observée par Achuo *et al.* (2006) lors d'un WD semblerait donc liée à une meilleure défense de la plante qu'à un possible rôle de l'ABA.

Il est généralement admis que l'activité métabolique primaire des plantes module la défense contre les agents pathogènes (Berger *et al*. 2007; Bolton, 2009). Une hypothèse d'explication est que les voies métaboliques primaires fournissent les squelettes carbonés et les ressources énergétiques pour la mise en place de la défense (notamment via la glycolyse, la respiration, la voie des pentoses phosphates et la voie du Shikimate) (Scharte *et al*. 2009), mais également que certains composés primaires constituent des signaux pour l'activation de gènes de défense (Ferri *et al*., 2011; Solfanelli *et al*., 2006). Par exemple, l'activité de protéines antifongiques est accrue avec l'apport de sucres (Herbers *et al*., 1996; Salzman *et*

al., 1998). L'existence d'une relation entre teneur en sucres des plantes et sensibilité aux champignons pathogènes a été suggérée de longue date (Horsfall et Dimond 1957). Cependant, elle a été peu exploitée à des fins de protection des plantes probablement parce que cette relation n'est pas univoque et qu'elle n'implique pas globalement le pool de sucres solubles (Lecompte *et al.*, 2013). De plus, l'hypothèse selon laquelle les métabolites primaires, et notamment les sucres, « alimentent » la défense n'a jusqu'à présent pas été démontrée, ni les mécanismes et les sucres en jeu décryptés. En cas d'infection par un pathogène, il est généralement observé une augmentation de l'expression des invertases pariétales, enzymes qui libèrent des hexoses à partir du saccharose dont on peut penser qu'ils sont utilisés à des fins de défense (Berger *et al*., 2004; Kocal *et al*., 2008; Essmann *et al*., 2008). Cependant ces hexoses constituent également l'une des sources carbonées principales des champignons, qui pour certains sont capables de sécréter leurs propres invertases (Dulermo *et al*., 2009; Jobic *et al*., 2007), et l'on ignore si une disponibilité plus élevée en sucres dans la plante favorise les prélèvements de nutriments par les pathogènes. Par ailleurs, la disponibilité relative en carbone et en azote, pour de jeunes plants de tabac ou de tomate, influe sur leur métabolisme secondaire, notamment la voie des phénylpropanoïdes qui est stimulée par des déficiences en azote (Fritz *et al*., 2006; Le Bot *et al*., 2009). Cependant, la synthèse accrue de polyphénols observée en cas de faible nutrition azotée, chez la tomate et la pomme de terre, ne se traduit pas systématiquement par une moindre sensibilité aux champignons nécrotrophes (Lecompte *et al*., 2010; Mittelstrass *et al*., 2006).

Lors des précédents chapitres (I, II et III), il a été vu que le WD peut modifier les teneurs en métabolites primaires et secondaires dans les plantes, à l'échelle de la feuille et du fruit. Le WD pourrait donc influencer la stimulation des défenses de la plante lors d'une infection par un pathogène. Dans cette étude, les effets de deux WD (-60 et -80 % d'apport en eau) lors d'une infection par *B. cinerea* ont été étudiés à différentes échelles (biochimiques, moléculaires et histologiques). L'objectif était de vérifier si le WD génère des conditions physiologiques défavorables au pathogène, soit par une barrière "physique" (réduction de l'ouverture des stomates, changement de la structure des feuilles) soit par des modifications de l'allocation du carbone et donc des ressources primaires (activation de dépôt de callose plus rapide, sucres, acides, carbone, azote) ou par l'activation de signaux cellulaires en lien avec les régulations hormonales (ABA et SA).

II- Matériel et Méthodes

2.1 Matériel végétal

Deux génotypes de tomates commerciales à croissance indéterminée Momor et Monalbo, fournis par le GAFL (Laboratoire de Génétique et Amélioration des Fruits et Légumes, INRA Avignon, Domaine St Maurice), ont été choisis pour leur différente sensibilité à *Botrytis cinerea* (Monalbo étant plus sensible) (comm. pers.). Ces génotypes sont issus du même parent Moneymaker (sensible à *B. cinerea*; Cristescu *et al*., 2002; Audenaert *et al.*, 2002).

2.2 Protocole expérimental

Deux expérimentations identiques portant sur la caractérisation de la sensibilité à *B. cinerea* de feuilles et de tiges de tomate ont été réalisées au premier semestre 2014. Les analyses biochimiques et moléculaires des échantillons récoltés n'ont été réalisées que pour la première expérimentation. La seconde expérimentation a été réalisée afin de vérifier la répétabilité des tests pathologiques.

Les plants de tomates ont été cultivés en serre verre à Montfavet (France), dans des pots de 4 L remplis avec du compost (substrat TS3, Klasmann, Champety, France). Les plants étaient ferti-irrigués quotidiennement au moyen d'un système d'arrosage par goutte à goutte avec une solution d'engrais commerciale (Fertiplant 16 10 24, Plantin, Courthézon, France). L'irrigation était pilotée par pesée continue de deux plantes en pots disposées sur une balance (Mettler E22, Mettler, Viroflay, France). Le poids initial des pots a été homogénéisé pour toutes les plantes.

Deux niveaux de WD correspondant à des réductions d'apport d'eau de -60 % (WD-60) et -80 % (WD-80) par rapport à un traitement témoin ont été appliqués. Les apports en éléments minéraux ont été identiques dans chaque traitement, en compensant avec la concentration en minéraux la baisse de la quantité d'eau apportée. Le dispositif était arrangé en blocs, chaque bloc était constitué de 3 rangées de plantes comprenant chacune les deux génotypes, chaque rangée correspondant à un type d'irrigation. L'ensemble du dispositif a été randomisé. Les WD ont été appliqués 47 jours après le semis (sur des plantes possédant en moyenne neuf feuilles), pendant une durée de 20 jours. Au 67ème jour après semis, les plantes ont été transférées en chambre climatique (appelée ci-après phytotron) pour l'inoculation et l'incubation. Les traitements WD ont été poursuivis dans les phytotrons pendant 7 jours, avec

des consignes de température de 21/18 °C jour/nuit, et d'humidité de 80/90 % jour/nuit, et un flux lumineux de 400 µmol m^{-2} s^{-1} (sauf le jour de l'infection: 200 µmol m^{-2} s^{-1} pour faciliter la prise de l'infection). Les différences de traitement hydrique ont été maintenues dans les phytotrons à l'aide d'un système d'irrigation programmable de type goutte à goutte.

2.3 Suivi des WD

Les effets des WD ont été quantifiés par des mesures du potentiel hydrique de la tige, au moyen d'une chambre à pression de type Scholander (SAM Précis 2000, Gradignan, France), avant le lever du jour et aux alentours du midi solaire. Les feuilles étaient ensachées la veille de chaque mesure à la tombée de la nuit. Ces mesures ont été réalisées le jour avant le début des WD, 7 jours après le début du stress et le jour avant les inoculations. Seules les données mesurées le jour avant inoculations ont été sélectionnées dans la partie résultat pour tous les paramètres de suivi des WD. L'humidité du substrat a été mesurée tous les 2 jours à l'aide d'un humidimètre (WCM Control, Grodan, Roermond, The Netherlands). La conductance stomatique a été mesurée à l'aide d'un poromètre (AP4, Delta-T - Sols Mesures, Elancourt, France) le jour avant le début des WD, 7 jours après le début du stress et le jour avant les inoculations, après les mesures de potentiel hydrique minimum. La fluorescence chlorophyllienne a été mesurée à l'aide d'un fluoromètre (Handy-PEA, Hansatech, King's Lynn, UK) sur des feuilles adaptées à l'obscurité (pendant 30 min) à l'aide de clip, également le jour avant le début des WD, 7 jours après le début du stress et le jour avant les inoculations, dans la matinée. La teneur relative en chlorophylle des feuilles a été mesurée à l'aide d'un SPAD 502 (Konica-Minolta, Osaka, Japan), juste après les mesures de fluorescence chlorophyllienne. La surface spécifique des feuilles (SLA) a été mesurée le jour avant les inoculations, sur 2 feuilles par plante en croissance pendant la période d'application des WD, à l'aide d'un planimètre (Li-3100 C Area Meter, Li-Cor, Lincoln Nebraska, USA) pour la surface foliaire, puis les feuilles étaient mises à l'étuve à 70 °C pendant 7 jours pour la mesure de poids sec. Toutes les mesures sur plante ont été réalisées sur des feuilles de type mature non sénescentes situées à mi-hauteur de la plante.

2.4 Suivi des infections et récoltes d'échantillons de feuilles et de tiges

Les échantillons ont été récoltés à 3 dates différentes: le jour de l'inoculation (J0, plantes témoins et plantes WD), trois jours après inoculation (J3) et sept jours après inoculation (J7) (plantes témoins et WD infectées et non infectées). Les différents échantillons récoltés sont résumés sur la figure 2.

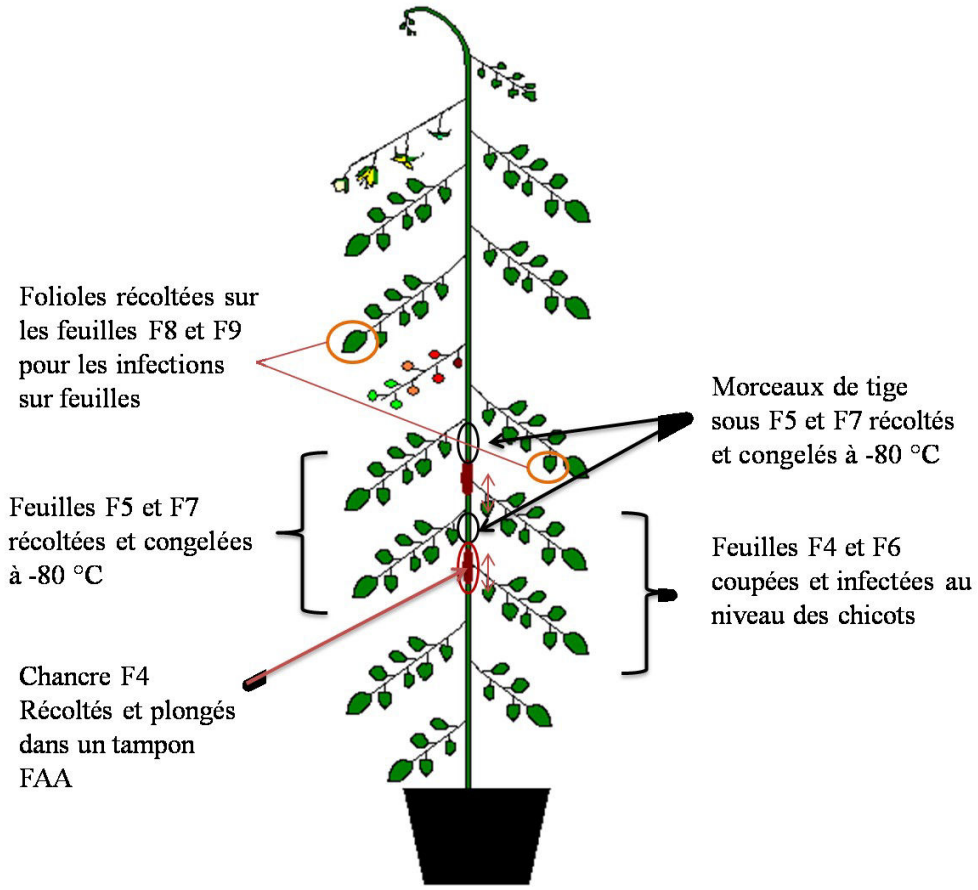

Figure 2: Détail des infections par *Botrytis cinerea* et des mesures réalisées sur les plants de tomate. Les feuilles F4 et F6 ont été coupées et les surfaces des chicots (d'une longueur d'environ 0.5 cm) ont été inoculées avec 10 µl d'une suspension de spores de BC1 à 10^7 spores mL^{-1}. Les chancres autour des feuilles F4 et F6 ont été mesurés 3 et 7 jours après infection. Le chancre développé sur la tige autour de la feuille F4 a été récolté et plongé dans un tube contenant du tampon formaldéhyde/acide acétique/alcool (FAA). Les feuilles F5 et F7 ont été récoltées dans de l'azote liquide et congelées à -80 °C pour des analyses biochimiques et moléculaires. Les segments de tige sous les feuilles F5 et F7 (sans tissus nécrotiques) ont également été récoltés et congelés à -80 °C pour les mêmes analyses. Des folioles des feuilles F8 et F9 ont été prélevés et infectés avec des pastilles de gel de dextrose de pomme de terre contenant du mycélium repiqué trois jours auparavant.

a) Agent pathogène et infection

La souche BC1 de *B. cinerea* a été mise en culture sur une gélose Difco^{TM}, à base de dextrose de pomme de terre, dans une chambre de culture à 21 °C avec une photopériode de 16 h à 162 µmol m^{-2} s^{-1}. La souche BC1 est une souche montrant une forte agressivité sur tomate (Lecompte *et al*., 2010). Deux types d'infection ont été réalisés. Des infections sur feuilles détachées ont été réalisées à l'aide de pastilles de gel contenant des fragments mycélien âgés de trois jours. Les pastilles, découpées à l'emporte-pièce dans la gélose, ont été déposées au contact de la feuille, la face contenant le mycélium entrant directement en contact avec le tissu folaire. Une foliole de la feuille 8 et une foliole de la feuille 9 ont été prélevées sur chaque plante. Les folioles ont été placées dans une boite hermétique humidifiée à l'aide d'un papier adsorbant, puis, une fois le mycélium déposé, laissées en incubation pendant 72 h à 21 °C. Des photos des lésions en développement sur chaque foliole ont été réalisées 48 h et 72 h après l'infection. Les photos des infections foliaires à 48 h ont été réalisées afin de contrôler la propagation du pathogène et les photos à 72 h ont été réalisées afin de comparer les intensités des infections entre traitements. La surface des lésions a été mesurée à l'aide du logiciel d'analyse d'image Image J (National Institute of Health, Bethesda, USA). Des infections sur tiges ont également été réalisées. Des cultures de *Botrytis* sur gel de dextrose de pomme de terre, identiques à celles décrites ci-dessus, ont été réalisées. Après 14 jours de culture in vitro, une suspension de spores à une concentration de 10^7 spores mL^{-1} a été préparée à partir du mycelium sporulant contenu dans les boites de pétri, suivant un protocole décrit par Lecompte *et al*. (2010). Les infections ont été réalisées après ablation avec un ciseau stérilisé des feuilles n°4 et n°6 en partant de la base de la plante, par injection de 10 µl de suspension de spores à la surface de chacun des chicots de pétiole. Les symptômes ont été évalués trois et sept jours après infections, en mesurant la longueur des chancres développés sur les tiges, suivant le protocole décrit par Lecompte *et al*. (2010).

b) Au jour J0

Trente plantes (5 plantes par niveau de WD et par génotype) en provenance de la serre ont été échantillonnées. Les feuilles 4 et 6 de ces plantes ont été utilisées pour les mesures de SLA. Les feuilles 5 et 7 et des morceaux de tiges autour de ces feuilles ont été congelés dans de l'azote liquide et conservés à -80 °C pour des analyses de biochimie et de biologie moléculaire. Deux folioles ont été prélevées afin de réaliser les infections sur feuilles détachées.

Les autres plantes (120 au total) ont été installées dans les 2 phytotrons sur 6 rangées de 10 plantes dans chaque chambre (1 rangée par traitement, selon un dispositif randomisé). Les feuilles 4 et 6 ont été coupées et la moitié du lot a été infectée selon le protocole détaillé ci-dessus. L'autre moitié du lot correspondait aux témoins non inoculés.

c) Au jour J3

Soixante des 120 plantes placées en phytotron à J0, soit cinq plantes par traitement (infection x génotype x WD) ont été échantillonnées. Les feuilles 5 et 7 (feuilles autour des zones infectées) et des segments de tige adjacents ont été coupés et immédiatement placés dans l'azote liquide, à des fins d'analyses biochimiques et moléculaires. La longueur des chancres sur tige a été mesurée, et les folioles des feuilles 8 et 9 ont été découpées pour une seconde série d'infections sur feuilles détachées. L'humidité du substrat dans chaque pot a été mesurée. Des segments de tige contenant les chancres en développement au niveau de la feuille 4 ont été récoltés et stockés dans des tubes contenant du tampon Formaldehyde/Acide acétique/Alcool (FAA), constitué de 80 ml d'éthanol à 95 %, 10 ml de formaldéhyde, 10 ml d'acide acétique glacial (pur), puis placés pour conservation à 4 °C. Soixante nouvelles plantes issues de la serre de culture ont été placées dans les phytotrons en remplacement des plantes échantillonnées, afin de ne pas modifier les conditions environnementales dans les phytotrons.

d) Au jour J7

L'autre moitié du lot de plantes placées dans les phytotrons à J0 (60 plantes) a été récoltée et a fait l'objet de mesures identiques à celles décrites à J3.

2.5 Analyses biochimiques, moléculaires et histologiques

a) Composition biochimique

Les feuilles et tiges congelées ont été broyées et une partie a été lyophilisée. Les sucres solubles (glucose, fructose et saccharose) et les acides organiques (acides citrique, malique et quinique) ont été extraits et analysés par HPLC (Waters 410, Part WAT070390, Milford, U.S.A.) selon le protocole de Gomez *et al*. (2002). L'amidon a été récupéré sur ces extraits et analysé selon la méthode de Gomez *et al*. (2007). Les variations des composés seuls n'étant pas toujours corrélées à des mécanismes de défense (Lecompte *et al*., 2013), les résultats des dosages des sucres et acides ont été présentés en fonction de leur contenu

respectif par rapport au total des sucres ou acides. Les contenus en azote et carbone libre ont été analysés à l'aide de la méthode de Dumas (1831) avec un auto-analyseur d'éléments (Flash EA 1112 series, Thermo Fisher Scientific, Courtaboeuf, France). L'ABA n'a été dosé que dans les poudres de feuilles fraiches des plantes Monalbo à J7, à l'aide d'un kit commercial Phytodetek ABA Test Kit (PDK, 09347/0096, Agdia, Elkhart, USA).

b) Expression de gènes dans les tiges

Les ARN ont été extraits à l'aide d'un kit commercial Tri Reagent solution (AM9738, ambion, applied biosystems) auquel des modifications ont été apportées, à savoir la quantité de poudre végétale utilisée pour les échantillons de tige lyophilisée qui a été fixée à 50 mg, l'ajout de billes de verre (de diamètre 0,5 à 0,75 mm) pour homogénéiser la matrice lors de la première étape d'extraction et les volumes des tampons chloroforme (400 µl) et isopropanol (700 µl) qui ont été modifiés comme indiqué entre parenthèses. Trois étapes de lavage à l'éthanol ont été réalisées.

La qualité et la quantité des ARN extraits ont été vérifiées à l'aide d'un dosage au nanodrop (Nanovue GE Healhcare, Life Sciences, Velizy-Villacoublay, France) et d'une migration sur gel d'agarose 0,8 % TBE 1 X avec dépôt d'environ 200 - 300 ng. par puits d'ARN totaux et additionnés de bleu de charge. Un traitement DNase a ensuite été appliqué à l'aide du kit commercial Turbo^{TM} DNAase treatment and removal reagents (AM1907, ambion, life technologies) pour une concentration initiale de 100 ng. µl^{-1}. La qualité et la quantité des ARN ont à nouveau été évaluées comme précédemment. Puis les ARN ont été rétrotranscrits en ADNc à l'aide d'un kit commercial Reverse Transcriptase Core Kit TM (Eurogentec). Les échantillons ont été traités à 50 ng. µl^{-1} pour 16 µl pour une concentration finale des ADNc à 20 ng. µl^{-1}.

Une qPCR (Thermocycler, Mastercycler®ep realplex) a ensuite été effectuée sur les gènes de référence et d'intérêt résumés dans le tableau 1, à l'aide du kit commercial Takyon ^{TM} No ROX SYBR® Core kit dtt Blue (Eurogentec), sur des échantillons d'ADNc dilués au tiers avec une concentration finale des primers de 200 nM. L'expression relative du gène d'intérêt a été normalisée à l'aide de l'actine comme gène de référence préalablement sélectionné entre Actine et Sand, selon la formule de Pfaff (2004):

$$Ratio = \frac{(\text{E cible})\ \Delta\ \text{Ct cible (témoin} - \text{traité)}}{(E'ref)\Delta\ Ct\ ref\ (témoin - traité)}$$

où E et E' sont respectivement les efficacités d'amplification du gène cible et du gène de référence et Ct le nombre de cycles après lequel la fluorescence atteint une valeur seuil fixée

N° accession	Gène	Sens et antisens 5'->3'	Taille de l'amplicon	TM°C
AY640378	NPR1	Fw: GCTATGGCAGGCGATGAT Rev: TCCACTGTTGTCCTCTGTGC	185 bp	59
AJ011520	PR1a)	Fw: CGTGCAATTGTGGGTGTC Rev: TCTCCAACCCAGTTGCCTA	195 bp	59
XM_004243304	Cals12	Fw:AGAACTGTTAGCCCTGGCAC Rev:ACTTGGTCGCGAGCACTACT	194 bp	59
FJ532351	Actine			60
SGN-U316474	Sand	Fw:TTGCTTGGAGGAACAGACG Rev: GCAAACAGAACCCCTGAATC	100 bp<	60

Tableau 1: Amorces utilisées (numéro d'accession, séquence, taille de l'amplicon et température de fusion de l'ADN (TM)) pour la quantification des gènes de NPR1et PR1a) liés à la voie JA, Cals12 lié à la synthèse de callose et des gènes de références *Actine* et *Sand* comme témoins.

par l'appareil. Le témoin utilisé correspond aux plantes non infectées, sans WD, récoltées à J0. Seuls les échantillons de tige du génotype Momor (moins sensible) ont été analysés.

c) Analyse des dépôts de callose

Le tampon de fixation a été retiré et les morceaux de tige ont été lavés trois fois avec de l'éthanol à 70 % puis stockés dans des piluliers contenant de l'éthanol à 70 % à 4 °C. Les tissus ont ensuite été déshydratés par immersion dans des bains successifs d'éthanol à 70, 80, 90, 100 %. Une infiltration avec un mélange alcool/résine (issue d'un kit commercial) a été réalisée avant l'inclusion dans la résine seule (Kit Technovit 7100, Kultzer, Wehrheim, Germany). Les tissus ont ensuite été sectionnés avec un microtome (Supercut 2065, Reichert-jung, Leica Instruments) en coupe fine de 3 µm et déposés sur lame. Deux zones de coupe ont été observées: une première zone nommée Zi (pour zone infectée) correspondant à la zone de jonction entre le tissu macéré et le tissu sans symptôme apparent et une zone Zs (pour zone saine) correspondant aux tissus situés 5 mm au-dessus de la zone Zi sur des tissus sans symptôme apparent. Les échantillons ont été révélés au bleu d'aniline à 0.01 % et les observations réalisées à l'aide d'un microscope à fluorescence (Leica DM 2000, camera Leica DFC 300FX) avec filtre UV. Les observations ont été réalisées aux longueurs d'ondes 340-380 nm pour l'excitation et 425 nm pour l'émission.

2.6 Analyses statistiques

Les analyses statistiques ont été réalisées à l'aide du logiciel R3 .1.0 (R Core Team, 2014). Tous les paramètres mesurés sur feuilles et tiges (réponses physiologiques, composition des échantillons récoltés, expression de gènes) ont été analysés comme suit. La normalité des résidus des analyses de variances (ANOVA) à deux facteurs a été vérifiée à l'aide du test de Shapiro (Royston, 1995). L'homoscédasticité des variances des résidus a été analysée à l'aide du test de Levene (package Car, Fox et Weisberg, 2011). Si les conditions requises pour l'ANOVA étaient validées, un test de comparaisons multiples a été appliqué (test de Tukey, packages lsmeans et multcompView; Lenth, 2014, Graves *et al*., 2012). Dans le cas contraire, le test non paramétrique de Kruskal-Wallis a été appliqué (package pgirmess; Giraudoux, 2014). Les corrélations partielles entre les paramètres ont été analysées sur les résidus de deux régressions linéaires (la première en fonction des effets des génotypes et traitements hydriques et la seconde en fonction des effets des génotypes et de l'infection sur tige) afin de voir les corrélations partielles qui sont influencées pour la première par l'infection sur tige et pour la seconde par les traitements hydriques (package GGMselect, GeneNet et igraph; Giraud *et al*., 2009; Schaefer *et al*., 2014; Csardi et Nepusz, 2006).

III- Résultats

3.1 Effets des deux niveaux de WD

Les réductions d'apport d'eau ont fortement abaissé l'humidité du substrat, tombée à moins de 20 % dans les deux traitements à la fin de la période de stress (Fig. 3A). Le potentiel hydrique de tige à midi a en conséquence fortement chuté (-22 % WD-60 et -47 % WD-80 pour Momor et -33 % WD-60 et -58 % WD-80 pour Monalbo). La conductance stomatique des plantes stressées a également diminuée (-13 % WD-60 et -55 % WD-80 pour Momor et -28 % WD-60 et -55 % WD-80 pour Monalbo) et de manière proportionnelle avec le potentiel hydrique de tige à midi, quel que soit le génotype (Fig. 3B).

Concernant la croissance des plantes, le nombre de feuilles total au jour J0 a seulement diminué pour les plantes Momor en WD-80 (-9.3 %; Tab. 2) et le poids sec moyen d'une feuille a diminué pour Monalbo en WD-80 (-21.5 %). Aucune différence significative de SLA n'a été observée entre les différents traitements. La teneur relative en chlorophylle exprimée en unités SPAD est elle aussi similaire entre les traitements (Tab. 2).

Des augmentations du F_V/F_M (WD-80 Monalbo), de PI (WD-60 et WD-80 pour les deux génotypes) et de $(1\text{-}V_J)/V_J$ (WD-60 et WD-80 Momor, et WD-60 Monalbo) ont été observées (Tab. 2). Ces augmentations suggèrent une amélioration de l'activité photosynthétique chez les plantes stressées via l'augmentation de la probabilité qu'un électron attrapé soit transféré (i.e. $(1\text{-}V_J)/V_J$). Cette augmentation du transfert des électrons a entraîné une augmentation du rendement de conservation de l'énergie absorbée par les photosystèmes (PS) II (i.e. PI) pour les deux génotypes. De plus, le flux cyclique d'électrons des PSII aux accepteurs primaires de PSI (J_0^{RE1}/J^{ABS}) a été conservé pour Momor quel que soit le WD et augmenté pour Monalbo en WD-60 (+25 %).

3.2 Suivi des infections sur tige et feuilles détachées

Les symptômes sur tige observés à J7, et les surfaces de lésions observées sur les feuilles infectées à J7 sont présentés dans la figure 4. Les données complètes des infections sur feuilles de l'expérimentation 1 aux dates J0, J3 et J7 sont fournies en annexe 1. Les WD ont fortement augmenté les symptômes de la maladie sur tige (+115.6 % en WD-60 et +138.8 % en WD-80 pour Momor, et +138.5 % en WD-60 et +119 % en WD-80 pour Monalbo). A l'inverse, les symptômes de la maladie sur feuilles détachées ont été identiques dans les différents WD chez les plantes non infectées sur tige (Annexe 1) et plus faibles dans le

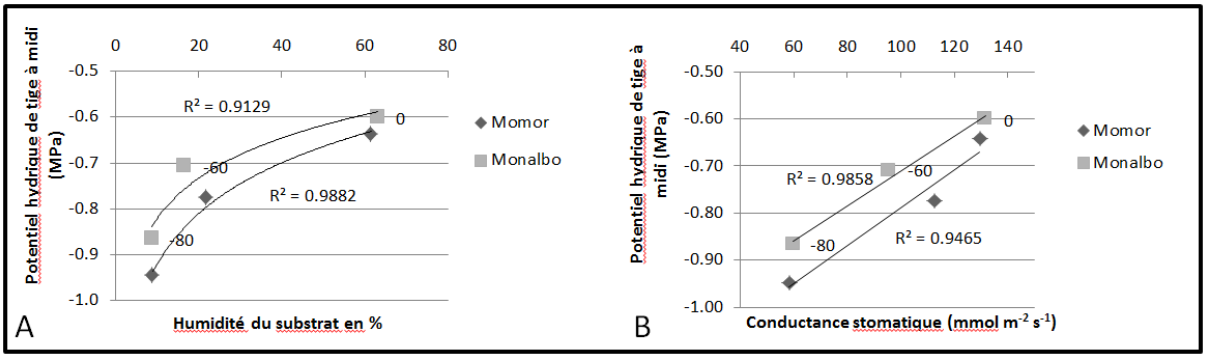

Figure 3: Relations entre l'humidité du substrat et le potentiel hydrique de tige à midi (A) et entre la conductance stomatique et le potentiel de tige à midi (B) en fonction des traitements hydriques appliqués (-60 et -80 % d'apport), pour les deux génotypes de tomate Momor et Monalbo, en fin de période de stress en serre avant transfert des plantes en chambre climatique. Les lignes sont des ajustements log en A (R^2 de 0.91pour Monalbo et de 0.99 pour Momor) et linéaire en B (R^2 de 0.99 pour Monalbo et de 0.95 pour Momor) des relations entre les paramètres.

Génotype	Momor			Monalbo		
Traitement	Témoin	WD-60	WD-80	Témoin	WD-60	WD-80
Nombre de feuilles	17,20 ± 0,20 **b**	16,40 ± 0,24 **ab**	15,60 ± 0,40 **a**	15,40 ± 0,24 **a**	15,00 ± 0,32 **a**	15,60 ± 0,24 **a**
Poids sec (g)	1,29 ± 0,19 **a**	1,44 ± 0,15 **a**	1,18 ± 0,11 **a**	1,77 ± 0,11 **b**	1,64 ± 0,16 **b**	1,39 ± 0,07 **a**
SLA ($cm^2\ g^{-1}$)	205,56 ± 18,83 **a**	217,36 ± 31,40 **a**	231,59 ± 26,72 **a**	199,44 ± 11,07 **a**	258,06 ± 27,96 **a**	274,27 ± 13,76 **a**
SPAD units (U.A.)	39,34 ± 0,76 **a**	41,60 ± 1,14 **a**	40,94 ± 0,78 **a**	40,22 ± 0,66 **a**	41,72 ± 1,00 **a**	41,04 ± 2,01 **a**
$(1\text{-}V_J)/V_{J\ (U.A.)}$	1,06 ± 0,03 **a**	1,26 ± 0,06 **b**	1,39 ± 0,08 **b**	1,12 ± 0,09 **a**	1,53 ± 0,16 **b**	1,33 ± 0,07 **ab**
Fv/Fm (U.A.)	0,84 ± 0,01 **a**	0,85 ± 0,00 **a**	0,85 ± 0,00 **a**	0,84 ± 0,00 **a**	0,85 ± 0,01 **ab**	0,86 ± 0,00 **b**
PI (U.A.)	2,67 ± 0,21 **a**	3,48 ± 0,18 **b**	4,03 ± 0,27 **b**	2,92 ± 0,44 **a**	4,31 ± 0,32 **b**	4,17 ± 0,28 **b**
J_0^{RE1}/J^{ABS} (U.A.)	0,18 ± 0,01 **a**	0,21 ± 0,00 **a**	0,20 ± 0,01 **a**	0,20 ± 0,01 **a**	0,25 ± 0,01 **b**	0,20 ± 0,01 **a**

Tableau 2: Nombre de feuilles et surface spécifique foliaire (SLA), composition foliaire (poids sec et teneur relative en chlorophylles exprimée en unité SPAD) et paramètres de fluorescence chlorophyllienne (F_V/F_M : rendement quantique de la photochimie du photosystème II, $(1\text{-}V_J)/V_J$: la probabilité qu'un électron piégé soit transféré et donc la proportion de centre réactionnel fermé, PI : indice de performance de Strasser *et al.* (2004) pour la conservation de l'énergie absorbée par l'antenne PSII jusqu'à la réduction par Q_B, et J_0^{RE1}/J^{ABS} défini comme le rendement quantique du flux de transport d'électrons jusqu'aux accepteurs du photosystème PSI) au dernier jour de déficit hydrique (WD) avant transfert des plantes de la serre vers les phytotrons. Les données sont les moyennes de 5 répétitions par traitement et par génotype ± SE. Les lettres indiquent des différences significatives entre traitements hydriques pour un génotype donné à $P < 0.05$ (Test de Tukey).

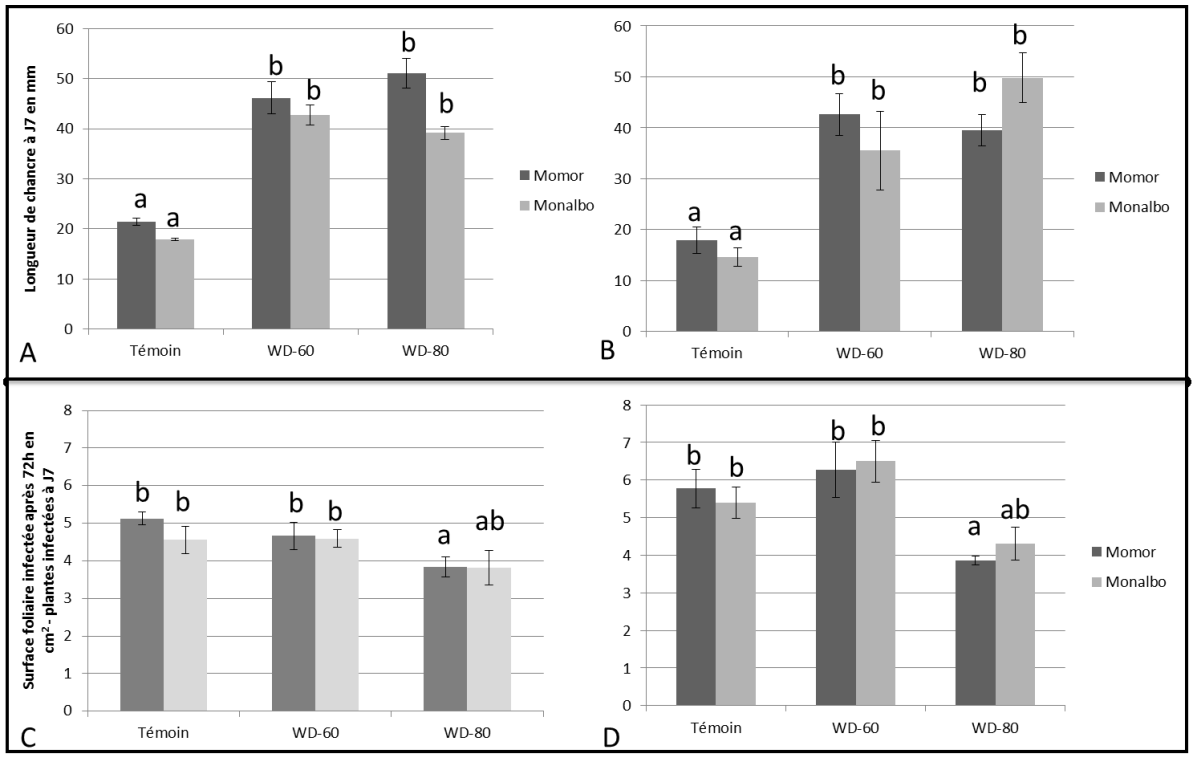

Figure 4: Intensité des symptômes causés par *Botrytis cinerea* sur tiges (expérimentation 1 : A ; expérimentation 2 : B) et sur feuilles détachées (expérimentation 1 : C ; expérimentation 2 : D), en fonction du déficit hydrique appliqué. Sur tige, la longueur moyenne des deux chancres, développés autour des points d'infection sur les chicots F4 et F6 observée 7 jours après infection, a été utilisée. Les barres correspondent aux moyennes et erreurs standards pour 5 plantes. Les symptômes sur feuilles correspondent à la surface des lésions observées 72 h après inoculation, sur les feuilles prélevées à J7 des plantes infectées sur tige. Les barres correspondent aux moyennes et erreurs standards pour 5 plantes. Les lettres indiquent les différences significatives à $P < 0.05$ (Tukey) entre traitements.

traitement WD-80 chez les plantes infectées sur tige (Fig. 3, Annexe 1). Des tendances similaires ont été observées chez les deux génotypes. Des résultats comparables ont été observés lors de la seconde expérimentation, même si la valeur absolue de l'intensité des symptômes sur feuille est apparue différente entre les deux expérimentations (Fig. 4C et 4D).

3.3 Impacts des WD et de l'infection sur les métabolites primaires pour les feuilles

Les ratios des teneurs en sucres, acides et C/N mesurés pour chaque traitement aux trois dates de récolte ont été résumés dans les tableaux 3 (feuilles) et 4 (tiges), les données brutes sont fournies en annexes (Annexes 2 et 3).

A J0, les WD ont impacté négativement le ratio C/N des feuilles de Monalbo (WD-60) et Momor (WD-60 et WD-80). De plus, le WD-80 a affecté les métabolites primaires des deux génotypes avec une baisse de la teneur en amidon (-32.8 % pour Momor et -42 % pour Monalbo) et de la teneur en matière sèche (-18.2 % pour Momor et -17.9 % pour Monalbo). Au niveau des sucres, le WD-80 a induit une baisse de la teneur en saccharose sur sucres totaux (-57.3 % pour Momor et -57 % pour Monalbo), en faveur d'une augmentation de la teneur en glucose sur sucres totaux pour Momor (+33.7 %) et une tendance similaire pour Monalbo (NS). Au niveau des acides, le WD-80 a induit une baisse de l'acide malique sur acides totaux pour Momor (-27.2 %) avec une tendance similaire pour Monalbo mais non significative, en faveur d'une augmentation de la teneur en acide quinique sur acides totaux pour les deux génotypes (+41.8 % pour Momor et + 29.1 % pour Monalbo). Au final, peu de différences entre plantes témoins et WD-60 ont été observées.

A J3, des différences entre plantes infectées et non infectées ont été observées. Les infections sur plantes témoins ont induit une baisse du ratio C/N pour les deux génotypes (-28.4 % pour Momor et -14.6 % pour Monalbo). Les infections ont également provoqué une baisse des teneurs en amidon pour les deux génotypes (-46.4 % pour Momor et -26.5 % pour Monalbo) et une baisse de la teneur en matière sèche pour Momor (-27.3 %). Les infections ont affecté les teneurs en acides des plantes Momor WD-60 avec une baisse de la teneur relative en acide citrique à J3 (-18 %) et à J7, avec une augmentation de la teneur relative en acide quinique (+41.1 %). Les infections ont également affecté les plantes Momor WD-80 à J7 avec une baisse de la teneur en amidon (-47.1 %) en faveur d'une augmentation du glucose sur sucres totaux (+26.8 %) et une baisse de la teneur en acide citrique (-33.5 %), en faveur d'une augmentation de l'acide quinique sur acides totaux (+58.7 %).

Feuilles	Génotype	Momor			Monalbo			Interaction
	Déficit hydrique	0	-60	-80	0	-60	-80	
Date de récolte	Infection sur tige							
J0								
Amidon (g 100 g-1 MF)	non	38.65 ± 2.49 **b**	36.45 ± 2.68 **b**	25.96 ± 1.99 **a**	35.42 ± 1.11 **b**	29.16 ± 2.66 **ab**	20.43 ± 3.48 **b**	NS
GLU/TOT	non	0.28 ± 0.02 **a**	0.26 ± 0.02 **a**	0.38 ± 0.03 **b**	0.35 ± 0.03 **ab**	0.31 ± 0.03 **a**	0.41 ± 0.02 **b**	NS
FRU/TOT	non	0.60 ± 0.03 **a**	0.62 ± 0.01 **a**	0.57 ± 0.02 **a**	0.55 ± 0.03 **a**	0.62 ± 0.02 **a**	0.55 ± 0.01 **a**	NS
SAC/TOT	non	0.11 ± 0.02 **b**	0.12 ± 0.01 **b**	0.05 ± 0.01 **a**	0.10 ± 0.01 **b**	0.07 ± 0.00 **ab**	0.04 ± 0.01 **a**	NS
MAL/TOT	non	0.28 ± 0.02 **b**	0.28 ± 0.03 **b**	0.21 ± 0.03 **a**	0.23 ± 0.01 **a**	0.24 ± 0.01 **a**	0.17 ± 0.02 **a**	NS
QUI/TOT	non	0.24 ± 0.01 **a**	0.26 ± 0.01 **a**	0.33 ± 0.03 **b**	0.29 ± 0.02 **a**	0.29 ± 0.01 **a**	0.37 ± 0.02 **b**	NS
CIT/TOT	non	0.48 ± 0.03 **a**	0.46 ± 0.02 **a**	0.46 ± 0.01 **a**	0.48 ± 0.02 **a**	0.47 ± 0.01 **a**	0.45 ± 0.00 **a**	NS
C/N	non	23.81 ± 1.26 **b**	21.15 ± 1.00 **b**	17.98 ± 0.45 **a**	22.91 ± 0.58 **b**	18.35 ± 0.65 **a**	16.48 ± 0.88 **a**	NS
Matières sèches (g 100 g-1 MF)	non	20.52 ± 1.49 **b**	20.34 ± 1.26 **b**	16.80 ± 0.45 **a**	18.40 ± 0.32 **b**	17.54 ± 0.59 **ab**	15.10 ± 0.74 **a**	NS
J3								
Amidon (g 100 g-1 MF)	non	42.05 ± 2.14 **c**	28.54 ± 1.42 **b**	19.73 ± 3.23 **a**	28.34 ± 1.25 **b**	14.17 ± 1.78 **a**	14.71 ± 2.23 **a**	NS
GLU/TOT	non	0.31 ± 0.04 **a**	0.32 ± 0.02 **ab**	0.40 ± 0.01 **b**	0.39 ± 0.02 **a**	0.39 ± 0.02 **a**	0.45 ± 0.02 **a**	NS
FRU/TOT	non	0.57 ± 0.04 **a**	0.60 ± 0.01 **a**	0.56 ± 0.01 **a**	0.53 ± 0.01 **a**	0.55 ± 0.01 **a**	0.51 ± 0.02 **a**	NS
SAC/TOT	non	0.11 ± 0.02 **b**	0.08 ± 0.01 **b**	0.04 ± 0.00 **a**	0.07 ± 0.01 **a**	0.06 ± 0.01 **a**	0.05 ± 0.00 **a**	NS
MAL/TOT	non	0.25 ± 0.00 **a**	0.24 ± 0.01 **a**	0.25 ± 0.01 **a**	0.17 ± 0.01 **a**	0.19 ± 0.02 **a**	0.17 ± 0.01 **a**	NS
QUI/TOT	non	0.21 ± 0.01 **a**	0.29 ± 0.01 **ab**	0.33 ± 0.01 **b**	0.35 ± 0.03 **a**	0.39 ± 0.03 **a**	0.35 ± 0.03 **a**	*
CIT/TOT	non	0.54 ± 0.00 **b**	0.46 ± 0.01 **ab**	0.42 ± 0.01 **a**	0.48 ± 0.04 **a**	0.43 ± 0.02 **a**	0.48 ± 0.03 **a**	*
C/N	non	25.84 ± 0.40 **b**	19.82 ± 0.41 **a**	17.92 ± 1.11 **a**	21.33 ± 0.85 **b**	15.58 ± 0.71 **a**	16.23 ± 0.63 **a**	NS
Matières sèches (g 100 g-1 MF)	non	19.65 ± 0.32 **b**	17.12 ± 0.48 **ab**	16.22 ± 0.64 **a**	15.72 ± 0.95 **b**	13.02 ± 0.90 **a**	15.62 ± 0.72 **b**	*
Amidon (g 100 g-1 MF)	oui	22.52 ± 1.96 **a**	22.76 ± 2.92 **a**	21.93 ± 1.33 **a**	20.83 ± 2.43 **b**	16.19 ± 0.64 **ab**	11.97 ± 1.62 **a**	NS
GLU/TOT	oui	0.34 ± 0.02 **a**	0.36 ± 0.02 **a**	0.38 ± 0.01 **a**	0.38 ± 0.03 **a**	0.38 ± 0.01 **a**	0.42 ± 0.02 **a**	NS
FRU/TOT	oui	0.57 ± 0.02 **a**	0.57 ± 0.01 **a**	0.55 ± 0.01 **a**	0.54 ± 0.02 **a**	0.58 ± 0.01 **a**	0.53 ± 0.02 **a**	NS
SAC/TOT	oui	0.09 ± 0.01 **a**	0.08 ± 0.01 **a**	0.07 ± 0.00 **a**	0.07 ± 0.01 **b**	0.05 ± 0.00 **a**	0.05 ± 0.00 **a**	NS
MAL/TOT	oui	0.21 ± 0.02 **a**	0.25 ± 0.02 **a**	0.23 ± 0.01 **a**	0.19 ± 0.01 **a**	0.20 ± 0.01 **a**	0.17 ± 0.01 **a**	NS
QUI/TOT	oui	0.27 ± 0.01 **a**	0.32 ± 0.02 **a**	0.33 ± 0.03 **a**	0.33 ± 0.02 **a**	0.31 ± 0.01 **a**	0.35 ± 0.02 **a**	NS
CIT/TOT	oui	0.52 ± 0.03 **b**	0.42 ± 0.02 **a**	0.44 ± 0.03 **ab**	0.48 ± 0.03 **a**	0.49 ± 0.02 **a**	0.48 ± 0.01 **a**	NS
C/N	oui	18.50 ± 0.75 **a**	18.47 ± 0.66 **a**	18.90 ± 1.03 **a**	18.20 ± 0.87 **b**	16.14 ± 0.20 **ab**	15.48 ± 0.27 **a**	NS
Matières sèches (g 100 g-1 MF)	oui	14.28 ± 0.76 **a**	15.34 ± 0.44 **a**	15.60 ± 0.36 **a**	14.06 ± 0.96 **a**	13.50 ± 0.36 **a**	14.06 ± 0.34 **a**	NS
J7								
Amidon (g 100 g-1 MF)	non	22.29 ± 4.86 **a**	18.88 ± 1.01 **a**	18.59 ± 2.05 **a**	10.03 ± 1.62 **a**	11.17 ± 2.44 **a**	9.69 ± 2.21 **a**	NS
GLU/TOT	non	0.30 ± 0.01 **a**	0.38 ± 0.02 **b**	0.35 ± 0.01 **ab**	0.41 ± 0.02 **a**	0.40 ± 0.02 **a**	0.41 ± 0.02 **a**	NS
FRU/TOT	non	0.58 ± 0.02 **a**	0.58 ± 0.01 **a**	0.59 ± 0.01 **a**	0.56 ± 0.01 **a**	0.56 ± 0.02 **a**	0.57 ± 0.02 **a**	NS
SAC/TOT	non	0.12 ± 0.01 **b**	0.04 ± 0.01 **a**	0.06 ± 0.00 **a**	0.04 ± 0.02 **a**	0.04 ± 0.01 **a**	0.03 ± 0.01 **a**	*
MAL/TOT	non	0.24 ± 0.03 **a**	0.28 ± 0.04 **a**	0.25 ± 0.01 **a**	0.22 ± 0.03 **a**	0.21 ± 0.03 **a**	0.20 ± 0.04 **a**	NS
QUI/TOT	non	0.27 ± 0.03 **a**	0.36 ± 0.03 **a**	0.34 ± 0.02 **a**	0.43 ± 0.04 **a**	0.36 ± 0.02 **a**	0.44 ± 0.05 **a**	NS
CIT/TOT	non	0.49 ± 0.05 **b**	0.36 ± 0.03 **a**	0.40 ± 0.02 **ab**	0.35 ± 0.05 **a**	0.43 ± 0.03 **a**	0.36 ± 0.02 **a**	*
C/N	non	16.91 ± 1.54 **a**	17.52 ± 0.46 **a**	16.83 ± 1.03 **a**	14.90 ± 0.39 **a**	14.43 ± 1.07 **a**	14.12 ± 1.02 **a**	NS
Matières sèches (g 100 g-1 MF)	non	13.78 ± 1.38 **a**	15.28 ± 0.30 **a**	15.18 ± 0.59 **a**	11.30 ± 0.40 **a**	12.46 ± 0.46 **a**	12.75 ± 0.47 **a**	NS
Amidon (g 100 g-1 MF)	oui	18.42 ± 1.66 **b**	12.49 ± 2.89 **ab**	9.74 ± 2.04 **a**	7.39 ± 2.04 **a**	6.28 ± 1.13 **a**	6.50 ± 0.99 **a**	NS
GLU/TOT	oui	0.29 ± 0.02 **a**	0.31 ± 0.02 **ab**	0.37 ± 0.03 **b**	0.46 ± 0.02 **a**	0.39 ± 0.01 **a**	0.40 ± 0.02 **a**	*
FRU/TOT	oui	0.58 ± 0.02 **a**	0.61 ± 0.01 **a**	0.55 ± 0.02 **a**	0.50 ± 0.04 **a**	0.54 ± 0.01 **a**	0.57 ± 0.02 **a**	NS
SAC/TOT	oui	0.13 ± 0.02 **a**	0.08 ± 0.02 **a**	0.08 ± 0.03 **a**	0.04 ± 0.04 **a**	0.07 ± 0.01 **a**	0.03 ± 0.01 **a**	NS
MAL/TOT	oui	0.27 ± 0.04 **a**	0.31 ± 0.01 **a**	0.30 ± 0.07 **a**	0.34 ± 0.06 **a**	0.22 ± 0.01 **a**	0.21 ± 0.02 **a**	NS
QUI/TOT	oui	0.24 ± 0.01 **a**	0.33 ± 0.02 **b**	0.37 ± 0.02 **b**	0.42 ± 0.03 **a**	0.42 ± 0.01 **a**	0.35 ± 0.03 **a**	*
CIT/TOT	oui	0.49 ± 0.03 **b**	0.36 ± 0.02 **ab**	0.33 ± 0.08 **a**	0.24 ± 0.05 **a**	0.35 ± 0.02 **ab**	0.44 ± 0.02 **b**	*
C/N	oui	17.34 ± 0.86 **a**	16.26 ± 0.51 **a**	18.23 ± 0.55 **a**	14.14 ± 0.70 **a**	14.47 ± 0.46 **a**	14.35 ± 0.30 **a**	NS
Matières sèches (g 100 g-1 MF)	oui	13.50 ± 0.59 **a**	12.84 ± 0.64 **a**	13.36 ± 0.64 **a**	11.80 ± 0.58 **a**	11.28 ± 0.34 **a**	11.78 ± 0.26 **a**	NS

Tableau 3: Teneurs des tissus foliaires en amidon, en glucose sur sucres totaux (GLU/TOT), en fructose sur sucre totaux (FRU/TOT), en saccharose sur sucres totaux (SAC/TOT), en acide malique sur acides totaux (MAL/TOT), en acide quinique sur acides totaux (QUI/TOT),

en acide citrique sur acides totaux (CIT/TOT), le ratio carbone/azote et la teneur en matières sèches pour les génotypes Momor et Monalbo, aux trois dates de récolte en fonction des traitements hydriques et des infections sur tige. Les données sont les moyennes ± SE de 5 répétitions (plantes) par traitement et par génotype. Les lettres indiquent des différences significatives entre traitements hydriques pour un génotype donné à $P < 0.05$ (Test de Tukey). Les interactions entre génotype et traitement sont indiquées à l'aide du symbole *, dans le cas contraire elles sont non significatives (NS).

Les infections ont également affecté les plantes Monalbo WD-60 à J3 avec une baisse des teneurs en amidon, en saccharose sur sucres totaux et du ratio C/N (-42.5 %, -35 % et -14.9 % respectivement) et à J7 avec une augmentation de la teneur en acide citrique (+85.2 %) par rapport aux témoins infectés.

3.4 Impacts des WD et de l'infection sur les métabolites primaires des tiges

Les traitements hydriques (observés à J0) n'ont pas impacté les teneurs en métabolites primaires des tiges quel que soit le génotype.

Par contre les infections sur tige ont induit à J3 une baisse du ratio C/N chez les deux génotypes (-9 % pour Momor et -11.1 % pour Monalbo) et à J7 pour Monalbo (-18.5 %). Les infections ont également affecté les teneurs en métabolites primaires des tiges de Monalbo principalement, avec une baisse de la teneur en amidon (-29 %), une baisse du fructose sur sucres totaux (-9 %) et une baisse de l'acide malique sur acides totaux (-17.7 %), en faveur d'une augmentation de la part d'acide quinique sur acides totaux (+10.2 %). A J7, les infections ont induit l'augmentation de la teneur en fructose sur sucres totaux des tiges des deux génotypes (+29.4 % pour Momor et +40.2 % pour Monalbo) et une baisse de la part d'acide quinique sur acides totaux pour Momor (-10.0 %).

Les infections ont affecté les teneurs en métabolites primaires des plantes WD Monalbo à J3 avec une augmentation de la teneur en amidon (+38.2 % WD-60 et +26.6 % WD-80), une augmentation du saccharose relatif (+35.8 % WD-80), une augmentation du fructose relatif (+21.3 % WD-60 et +15 % WD-80) et une baisse du glucose relatif (-20.7 % WD-60 et -20.2 % WD-80), par rapport aux plantes témoins J3 infectées. Au niveau des teneurs en acides, les infections ont impacté les plantes WD-80 Monalbo à J3 par une baisse de l'acide quinique (-13.7 %) en faveur d'une augmentation de l'acide citrique (+27.9 %).

Pour les plantes Momor en WD, les infections à J3 ont induit une augmentation du saccharose sur sucres totaux (+40.5 % WD-60 et +53.8 % WD-80), en défaveur du glucose (-20.8 % WD-80) par rapport aux plantes témoins J3 infectées. Les infections ont également impacté les teneurs en acides des plantes Momor J3 WD-80 avec une augmentation de l'acide malique (+66.4 %) en défaveur de l'acide quinique (-16.8 %).

Tiges	Génotype	Momor			Monalbo			Interaction
	Déficit hydrique	0	-60	-80	0	-60	-80	
Date de récolte	Infection sur tige							
J0								
Amidon (g 100 g-1 MF)	non	9,00 ± 0,24 **a**	10,17 ± 1,22 **a**	9,49 ± 0,77 **a**	17,56 ± 1,23 **a**	19,74 ± 2,22 **a**	13,60 ± 2,02 **a**	NS
GLU/TOT	non	0,60 ± 0,01 **a**	0,59 ± 0,01 **a**	0,61 ± 0,02 **a**	0,55 ± 0,02 **a**	0,52 ± 0,02 **a**	0,52 ± 0,02 **a**	NS
FRU/TOT	non	0,09 ± 0,00 **a**	0,09 ± 0,00 **a**	0,09 ± 0,00 **a**	0,08 ± 0,00 **a**	0,08 ± 0,00 **a**	0,08 ± 0,00 **a**	NS
SAC/TOT	non	0,31 ± 0,02 **a**	0,32 ± 0,01 **a**	0,30 ± 0,02 **a**	0,37 ± 0,02 **a**	0,40 ± 0,02 **a**	0,40 ± 0,02 **a**	NS
MAL/TOT	non	0,09 ± 0,00 **a**	0,11 ± 0,01 **a**	0,11 ± 0,01 **a**	0,16 ± 0,01 **a**	0,20 ± 0,02 **a**	0,17 ± 0,01 **a**	NS
QUI/TOT	non	0,73 ± 0,02 **a**	0,74 ± 0,01 **a**	0,70 ± 0,02 **a**	0,63 ± 0,01 **a**	0,59 ± 0,02 **a**	0,60 ± 0,02 **a**	NS
CIT/TOT	non	0,18 ± 0,02 **a**	0,15 ± 0,01 **a**	0,19 ± 0,01 **a**	0,21 ± 0,01 **a**	0,22 ± 0,02 **a**	0,23 ± 0,02 **a**	NS
C/N	non	34,84 ± 0,93 **a**	30,60 ± 1,02 **a**	32,70 ± 2,03 **a**	40,14 ± 1,01 **a**	31,94 ± 0,96 **a**	32,11 ± 0,87 **a**	NS
J3								
Amidon (g 100 g-1 MF)	non	11,06 ± 0,93 **a**	11,63 ± 1,62 **a**	9,89 ± 1,04 **a**	21,87 ± 1,16 **b**	18,41 ± 1,31 **ab**	15,77 ± 1,67 **a**	NS
GLU/TOT	non	0,63 ± 0,02 **b**	0,60 ± 0,01 **b**	0,50 ± 0,03 **a**	0,61 ± 0,01 **b**	0,54 ± 0,02 **ab**	0,48 ± 0,02 **a**	NS
FRU/TOT	non	0,08 ± 0,01 **a**	0,08 ± 0,00 **a**	0,09 ± 0,00 **a**	0,09 ± 0,01 **a**	0,08 ± 0,00 **a**	0,07 ± 0,00 **a**	*
SAC/TOT	non	0,28 ± 0,02 **a**	0,32 ± 0,01 **a**	0,41 ± 0,03 **b**	0,30 ± 0,01 **a**	0,39 ± 0,03 **b**	0,45 ± 0,03 **b**	NS
MAL/TOT	non	0,14 ± 0,01 **a**	0,14 ± 0,01 **a**	0,15 ± 0,02 **a**	0,26 ± 0,01 **b**	0,19 ± 0,01 **a**	0,19 ± 0,02 **a**	*
QUI/TOT	non	0,69 ± 0,03 **b**	0,69 ± 0,02 **b**	0,61 ± 0,01 **a**	0,55 ± 0,01 **a**	0,61 ± 0,02 **a**	0,56 ± 0,01 **a**	*
CIT/TOT	non	0,17 ± 0,02 **a**	0,16 ± 0,01 **a**	0,24 ± 0,02 **b**	0,20 ± 0,01 **a**	0,20 ± 0,01 **a**	0,25 ± 0,02 **a**	NS
C/N	non	41,02 ± 0,76 **c**	36,78 ± 1,05 **b**	29,64 ± 1,27 **a**	43,03 ± 1,36 **b**	36,77 ± 0,87 **a**	34,35 ± 0,21 **a**	NS
Amidon (g 100 g-1 MF)	oui	9,17 ± 0,93 **a**	7,79 ± 0,62 **a**	11,81 ± 0,81 **a**	15,35 ± 1,04 **a**	21,21 ± 1,88 **b**	19,43 ± 1,94 **b**	NS
GLU/TOT	oui	0,68 ± 0,01 **b**	0,56 ± 0,02 **a**	0,54 ± 0,02 **a**	0,61 ± 0,03 **b**	0,48 ± 0,02 **a**	0,49 ± 0,01 **a**	NS
FRU/TOT	oui	0,08 ± 0,00 **a**	0,09 ± 0,00 **a**	0,09 ± 0,00 **a**	0,08 ± 0,00 **a**	0,10 ± 0,01 **b**	0,09 ± 0,01 **b**	NS
SAC/TOT	oui	0,24 ± 0,01 **a**	0,34 ± 0,02 **b**	0,37 ± 0,02 **b**	0,31 ± 0,03 **a**	0,42 ± 0,02 **b**	0,42 ± 0,01 **b**	NS
MAL/TOT	oui	0,12 ± 0,01 **a**	0,13 ± 0,01 **ab**	0,21 ± 0,00 **b**	0,21 ± 0,01 **a**	0,23 ± 0,01 **a**	0,24 ± 0,02 **a**	NS
QUI/TOT	oui	0,74 ± 0,02 **b**	0,69 ± 0,02 **ab**	0,62 ± 0,02 **a**	0,60 ± 0,02 **b**	0,55 ± 0,01 **ab**	0,52 ± 0,02 **a**	NS
CIT/TOT	oui	0,13 ± 0,02 **a**	0,17 ± 0,02 **a**	0,17 ± 0,02 **a**	0,19 ± 0,02 **a**	0,21 ± 0,01 **ab**	0,24 ± 0,01 **b**	NS
C/N	oui	37,32 ± 1,01 **b**	30,95 ± 1,84 **ab**	31,13 ± 0,93 **a**	38,07 ± 0,98 **b**	32,70 ± 0,91 **a**	30,07 ± 0,96 **a**	NS
J7								
Amidon (g 100 g-1 MF)	non	14,29 ± 1,62 **a**	14,69 ± 1,56 **a**	14,21 ± 1,66 **a**	22,02 ± 1,06 **ab**	25,21 ± 1,82 **b**	18,65 ± 2,70 **a**	NS
GLU/TOT	non	0,67 ± 0,01 **b**	0,53 ± 0,01 **a**	0,49 ± 0,02 **a**	0,62 ± 0,01 **b**	0,46 ± 0,02 **a**	0,47 ± 0,02 **a**	NS
FRU/TOT	non	0,10 ± 0,01 **a**	0,11 ± 0,00 **a**	0,10 ± 0,01 **a**	0,11 ± 0,01 **a**	0,12 ± 0,01 **a**	0,10 ± 0,01 **a**	NS
SAC/TOT	non	0,23 ± 0,01 **a**	0,36 ± 0,01 **b**	0,40 ± 0,02 **b**	0,27 ± 0,01 **a**	0,42 ± 0,02 **b**	0,43 ± 0,02 **b**	NS
MAL/TOT	non	0,16 ± 0,02 **a**	0,28 ± 0,03 **b**	0,22 ± 0,01 **ab**	0,23 ± 0,02 **a**	0,37 ± 0,02 **b**	0,27 ± 0,03 **a**	NS
QUI/TOT	non	0,71 ± 0,03 **b**	0,47 ± 0,03 **a**	0,52 ± 0,02 **a**	0,63 ± 0,02 **c**	0,38 ± 0,01 **a**	0,50 ± 0,04 **b**	NS
CIT/TOT	non	0,13 ± 0,02 **a**	0,25 ± 0,01 **b**	0,26 ± 0,01 **b**	0,15 ± 0,01 **a**	0,25 ± 0,02 **b**	0,23 ± 0,01 **b**	NS
C/N	non	27,43 ± 0,73 **a**	29,62 ± 2,27 **a**	32,73 ± 1,90 **a**	31,01 ± 1,18 **a**	34,75 ± 1,50 **a**	30,39 ± 1,93 **a**	NS
Amidon (g 100 g-1 MF)	oui	12,07 ± 1,02 **a**	9,36 ± 1,40 **a**	10,84 ± 0,88 **a**	17,85 ± 0,74 **a**	14,37 ± 1,53 **a**	16,39 ± 1,24 **a**	NS
GLU/TOT	oui	0,67 ± 0,00 **b**	0,64 ± 0,01 **b**	0,58 ± 0,02 **a**	0,61 ± 0,01 **b**	0,56 ± 0,01 **a**	0,53 ± 0,01 **a**	NS
FRU/TOT	oui	0,13 ± 0,00 **b**	0,11 ± 0,01 **b**	0,10 ± 0,00 **a**	0,15 ± 0,00 **b**	0,13 ± 0,01 **a**	0,12 ± 0,01 **a**	NS
SAC/TOT	oui	0,20 ± 0,00 **a**	0,25 ± 0,01 **a**	0,32 ± 0,02 **b**	0,24 ± 0,01 **a**	0,31 ± 0,02 **b**	0,35 ± 0,01 **b**	NS
MAL/TOT	oui	0,20 ± 0,01 **a**	0,21 ± 0,01 **a**	0,24 ± 0,01 **b**	0,23 ± 0,01 **a**	0,24 ± 0,01 **a**	0,27 ± 0,01 **b**	NS
QUI/TOT	oui	0,64 ± 0,01 **b**	0,67 ± 0,01 **b**	0,59 ± 0,02 **a**	0,61 ± 0,02 **b**	0,61 ± 0,02 **b**	0,53 ± 0,00 **a**	NS
CIT/TOT	oui	0,16 ± 0,01 **b**	0,12 ± 0,01 **a**	0,17 ± 0,01 **b**	0,16 ± 0,02 **a**	0,15 ± 0,01 **a**	0,20 ± 0,00 **b**	NS
C/N	oui	24,94 ± 0,64 **a**	26,39 ± 0,77 **a**	28,04 ± 1,24 **a**	25,27 ± 1,26 **a**	25,16 ± 0,99 **a**	29,18 ± 1,44 **b**	NS

Tableau 4: Teneurs des tiges en amidon, en glucose sur sucres totaux (GLU/TOT), en fructose sur sucre totaux (FRU/TOT), en saccharose sur sucres totaux (SAC/TOT), en acide malique sur acides totaux (MAL/TOT), en acide quinique sur acides totaux (QUI/TOT), en acide citrique sur acides totaux (CIT/TOT) et le ratio carbone/azote pour les génotypes Momor et Monalbo, aux trois dates de récolte en fonction des traitements hydriques et des infections sur tige. Les données sont les moyennes ± SE de 5 répétitions (plantes) par traitement et par génotype. Les lettres indiquent des différences significatives entre traitements hydriques pour un génotype donné à $P < 0.05$ (Test de Tukey). Les interactions entre génotype et traitement sont indiquées à l'aide du symbole *, dans le cas contraire elles sont non significatives (NS).

A J7, les infections ont affecté les teneurs en sucres des plantes Monalbo en WD avec une augmentation du saccharose (+30.9 % WD-60 et +46.1 % WD-80) en défaveur du glucose (-8.4 % WD-60 et-12.2 % WD-80) et du fructose (-14.3 % WD-60 et -22.7 % WD-80). Les infections à J7 ont également affecté les plantes Momor en WD avec plus de saccharose sur sucres totaux (+59.6 % WD-80), moins de glucose sur sucres totaux (-12.9 % WD-80) et moins de fructose sur sucres totaux (-25.5 % WD-80). Au niveau des acides, les infections ont induit une baisse de la teneur en acide citrique sur acides totaux (-23.8 % pour Momor WD-60), une augmentation de l'acide malique (+21.5 % pour Momor WD-80 et +21.8 % pour Monalbo WD-80) en faveur d'une diminution de la teneur en acide quinique sur acides totaux (-8 % pour Momor WD-80 et -14.4 % pour Monalbo WD-80).

3.5 Relations entre les teneurs en métabolites primaires et les infections observées

Des réseaux de corrélations partielles (à $P < 0.05$, Fig. 5) ont été réalisés sur les résidus pour deux types de régressions, en ôtant l'effet génotype pour chacun de façon à ne garder que les effets souhaités pour la visualisation: effet infection pour la première et WD pour la seconde. L'infection sur tige est corrélée négativement avec le contenu relatif en saccharose dans les tiges (Fig. 5A, Tab. 4). Une diminution du saccharose dans les tiges (lors de fortes infections) est également reliée positivement à une augmentation du glucose dans les tiges. Par ailleurs, il y a moins d'amidon dans les tiges et les feuilles des plantes infectées que dans les tiges et les feuilles des plantes non infectées (Tab. 3 et 4). C'est probablement la raison pour laquelle on observe à J7 une corrélation partielle positive entre contenu en amidon des tiges et des feuilles et contenu en saccharose des tiges, même si cette corrélation ne traduit pas la tendance de transformation de l'amidon en saccharose du fait de l'infection. D'autre part, l'augmentation de glucose chez les plantes infectées est liée à une diminution des acides citrique et malique et à une augmentation de l'acide quinique (Fig. 5A). Les corrélations partielles pour les WD (Fig. 5B) montrent une relation négative entre l'humidité du substrat et l'intensité des symptômes des tiges, ce qui est conforme aux observations de la figure 3, mais aussi une corrélation positive entre l'humidité du substrat et la teneur en fructose sur sucres totaux dans les tiges (lien positif). Ceci est également visible dans le tableau 4: la diminution du saccharose chez les plantes infectées se traduit par une évolution plus forte de la teneur relative en fructose chez les plantes non stressées, et une évolution plus forte de la teneur en glucose chez les plantes stressées. On observe par ailleurs sur cette régression, comme on

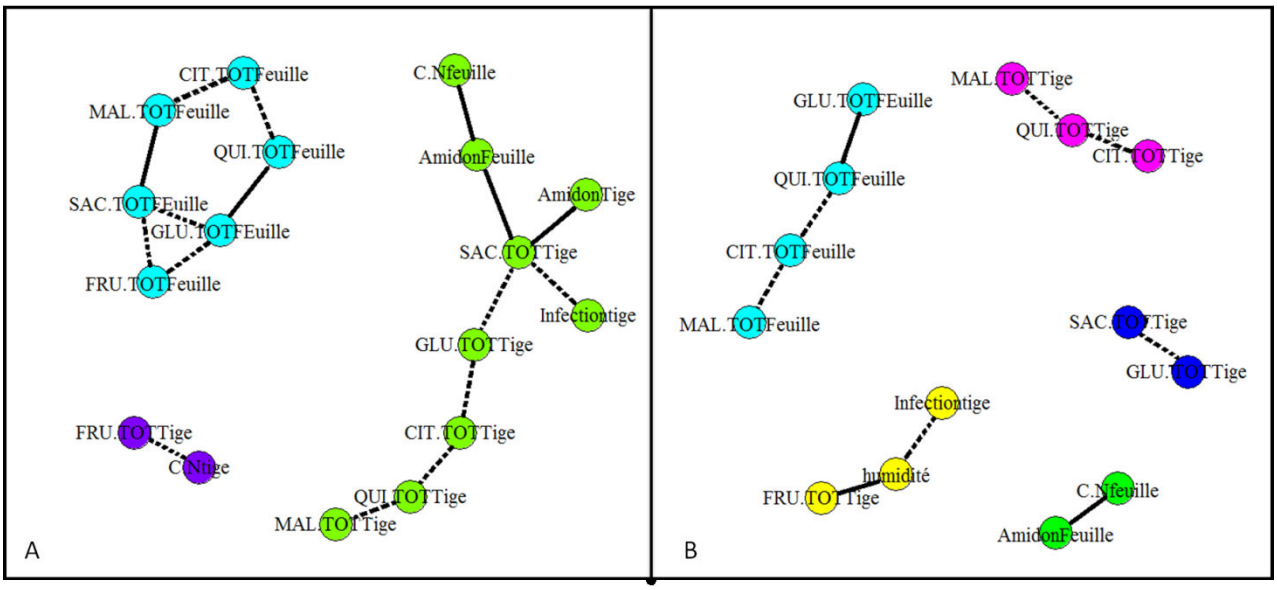

Figure 5: Corrélations partielles entre teneurs en métabolites primaires (sucres, acides, azote, carbone et amidon) des feuilles et tiges entre plantes infectées et non infectées sur tige (Infectiontige) avec traitements hydriques (humidité) en fonction des effets de l'infection seule en A et des effets des traitements hydriques seuls en B. Les teneurs sont exprimées en ratio des sucres solubles (glucose, fructose et saccharose) par rapport au total de ces sucres (GLU/TOT, FRU/TOT, SAC/TOT), en acides organiques (acide malique, quinique et citrique) par rapport au total de ces acides (MAL/TOT, QUI/TOT, CIT/TOT) et par le ratio carbone/azote (C/N). Les corrélations partielles ont été calculées sur l'ensemble des données à J7 pour les deux génotypes (n = 5) à l'aide des packages GGMselect, GeneNet et igraph. Les lignes pleines représentent des corrélations positives et les lignes en pointillés les corrélations négatives.

l'avait observé sur la première, une corrélation négative entre les acides du cycle de Krebs et l'acide quinique.

3.6 Régulation hormonale mise en jeu lors de ces interactions

Les teneurs en ABA ont été mesurées à J7, sur les feuilles des plantes Monalbo. Des différences significatives entre les plantes stressées (WD-60 et WD-80) et les témoins ont été observées, à la fois chez les plantes inoculées sur tige et les non inoculées (Fig. 6) avec une augmentation des teneurs en ABA dans les feuilles des plantes WD.

Des différences significatives d'expression de NPR1 et PR1a) entre les plantes infectées et non infectées sur tige ont été observées à J3 et à J7 (Fig. 7). A J3, chez les plantes infectées l'expression de ces deux gènes était significativement plus forte chez les plantes en WD. Les différences entre témoin et WD se sont estompées à J7, surtout pour PR1a).

Une tendance à un niveau d'expression plus fort de Cals12 chez les plantes infectées a été observée, mais les différences ne sont pas significatives (Fig. 7). Aucun effet du WD sur l'expression de ce gène n'a été observé, à l'exception des plantes WD-60 non infectées à J0.

3.7 Effets de l'infection sur la formation de dépôts de callose

Une méthode de quantification fiable n'a pas encore pu être mise au point afin de comparer les effets des traitements hydriques (plantes WD vs. plantes témoins). Aucune interprétation fiable ne peut être donnée en conséquence. Cependant, à l'oeil nu une augmentation des dépôts de callose a été observée entre plantes infectées et non infectées (Fig. 8).

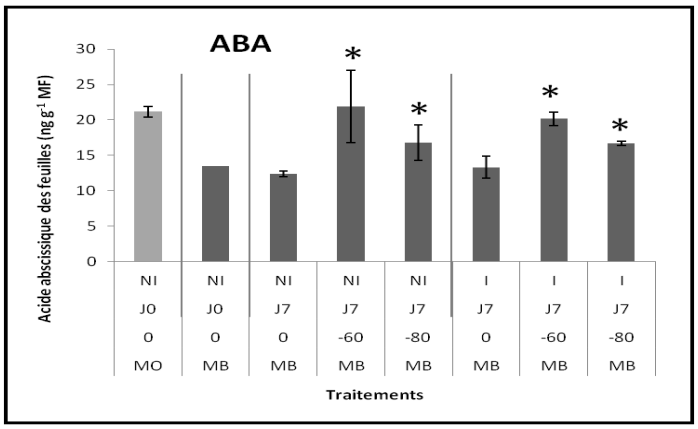

Figure 6: Teneurs en acide abscissique dans les feuilles de Monalbo (des plantes à J7) et Momor (témoin uniquement). Les données sont exprimées par les moyennes ± SE (n = 3). Les symboles * indiquent les différences significatives à P < 0.05 (Tukey) entre le témoin et les déficits hydriques de chaque catégorie (séparation par des barres).

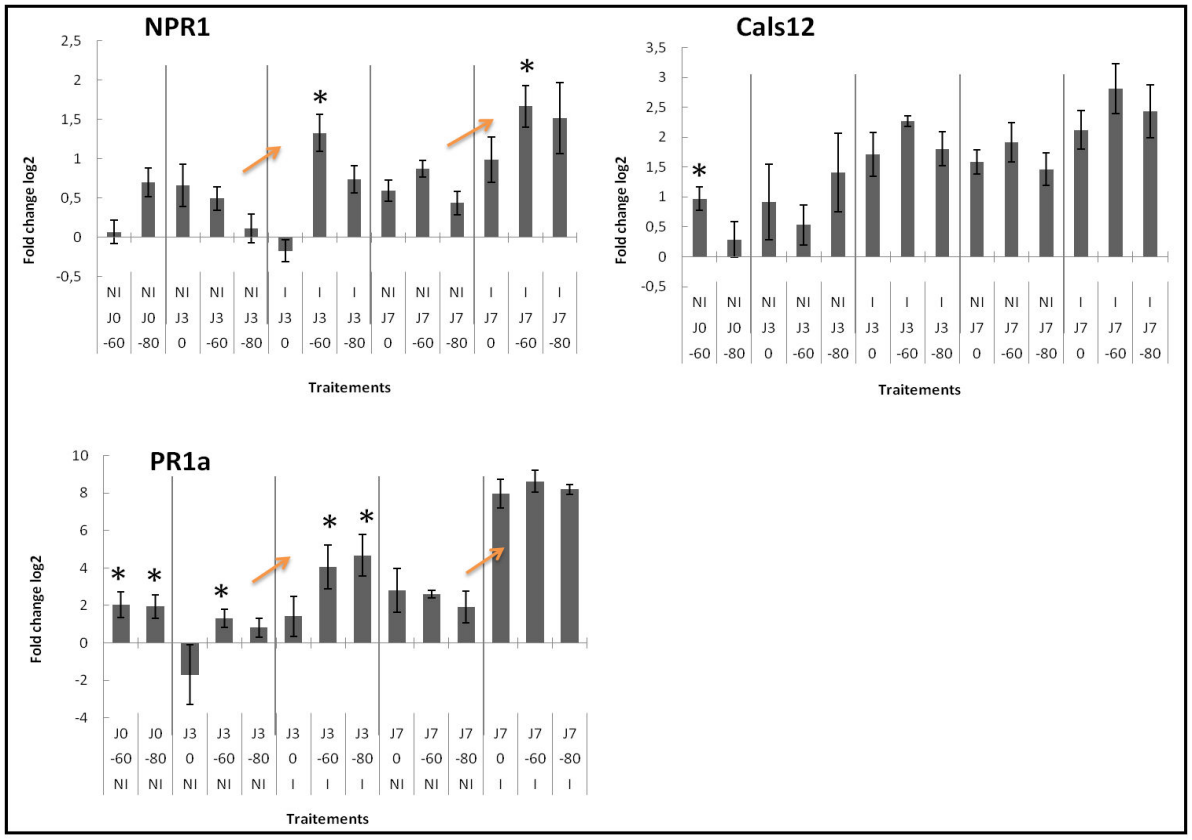

Figure 7: Expression relative des gènes de *NPR1*, *PR1a)* et *Cals12* par rapport aux plantes témoin à J0 sans infection et sans WD, contenus dans les tiges de Momor pour chaque traitement et dates de récolte. Les données sont exprimées par les moyennes ± SE (n = 3). Les symboles * indiquent les différences significatives à P < 0.05 (Tukey) entre le témoin et les déficits hydriques de chaque catégorie (séparation par des barres) et les flèches indiquent des différences significatives entre plantes non infectées et plantes infectées à J3 et J7 en conditions de déficits hydriques.

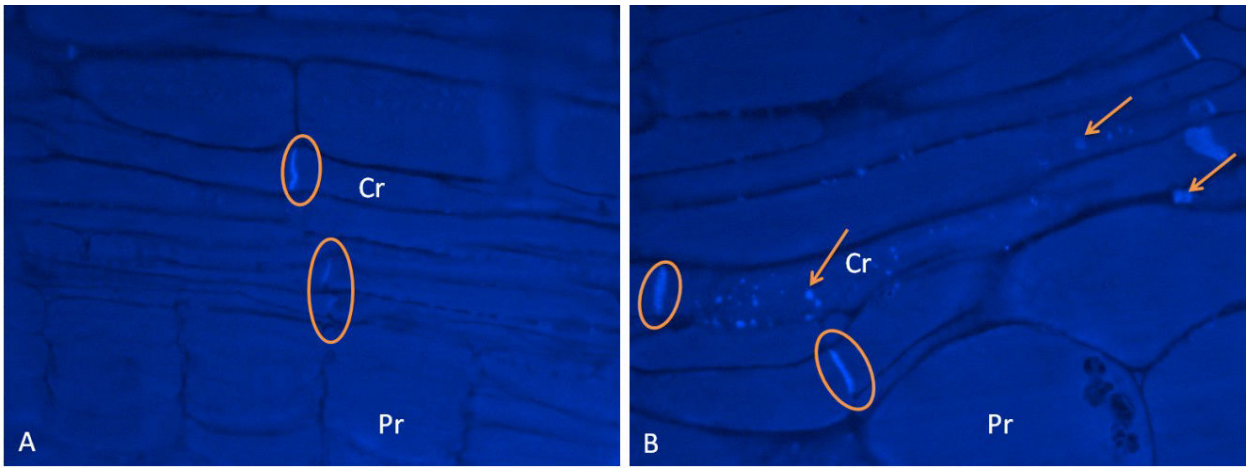

Figure 8: Observations en microscopie à fluorescence des dépôts de callose (zone fluorescente indiquée avec des flèches), après coloration au bleu d'aniline, au niveau des cribles (encerclés) des cellules criblées (Cr) et des cellules parenchymateuses (Pr), dans des tiges de tomate inoculées par *Botrytis cinerea*. La figure A représente une coupe transversale de tige en zone saine, en condition témoin non infecté. La figure B représente une coupe transversale dans la zone nécrosée de la tige, en condition témoin infecté (taille de la zone de coupe observée: 0.16 µm, zoom x400, 340-380 nm excitation et 425 nm émission).

IV- Discussion

La réponse des plantes en WD a été similaire aux réponses observées pour les plantes isohydriques (Tardieu et Simonneau, 1998) via l'ajustement de l'ouverture des stomates afin de maintenir leur contenu en eau. Cependant, la réduction de l'ouverture des stomates est souvent à l'origine d'une synthèse accrue de ROS (via l'induction d'un stress oxydatif) qui peuvent impacter les défenses et interagir avec l'infection (Atkinson et Urwin, 2012). Dans cette étude, les résultats suggèrent que les plantes en WD n'ont pas été impactées au niveau des photosystèmes, probablement par une bonne gestion du stress (i.e. amélioration des capacités photosynthétiques) déduite de l'augmentation des performances au niveau des PSII (PI) et des flux d'électrons entre les PSII et les accepteurs primaires des PSI (J_0^{RE1}/J^{ABS}). La croissance des plantes n'a également pas été impactée par ces WD, au vue de l'absence d'effet sur la surface foliaire, suggérant que ces WD ont été des stress modérés (Chaves *et al*., 2003). Globalement, le WD-60 a eu un impact plus faible sur la réponse physiologique des plantes en termes de potentiel hydrique au midi solaire et de conductance stomatique que le WD-80.

Il a été observé que les symptômes des infections sur tige des plantes en WD ont été plus importants que ceux des plantes témoins (+117 à +119 %), contrairement aux infections sur feuilles détachées. La plus faible intensité des symptômes sur feuilles détachées pour les plantes en WD est cohérente avec les résultats d'Achuo *et al*. (2006). Cependant, elle n'a été observée que pour les plantes WD préalablement infectées sur tige. Il apparait donc que l'infection d'organes distaux peut modifier l'effet du stress hydrique sur la sensibilité des feuilles de tomate à *B. cinerea*. Cependant, il n'a pas été observé de différences entre les niveaux d'infections sur feuilles des plantes non inoculées sur tige. L'interprétation de l'ensemble de ces résultats reste complexe. L'application de WD par réduction des apports d'eau sur une période de 21 jours, comme réalisé dans notre étude, ne stimulerait donc pas autant les défenses que lors d'alternance de WD (par arrêt des apports) et de périodes de récupération comme pratiqué par Achuo *et al.* (2006). La souche BC1 utilisée ici est connue pour être une des plus virulentes (Lecompte *et al*., 2010), ce qui peut masquer les différences de défenses des plantes lors des inoculations sur feuilles détachées, très favorables au pathogène. Les effets observés sur feuilles détachées pourraient être plus importants avec des souches moins virulentes.

L'antagonisme avec les infections sur tige nécessite des études complémentaires afin d'étudier un possible rôle de l'ABA (actuellement non dosé dans les tiges). En effet, Beyers *et*

al. (2014) ont observé que les mutants ABA étaient plus résistants aux infections sur tige que les plantes non mutées. Les teneurs foliaires en ABA mesurées dans notre étude ont montré une augmentation de cette hormone dans les plantes WD infectées et non infectées. Une contradiction, entre les observations des symptômes des infections sur feuilles détachées des mutants *sitiens* et *flacca* qui étaient moins forts et les résultats d'une stimulation de la résistance de la plante sous stress hydrique, ressort clairement (Asselbergh *et al*., 2007; Audenaert *et al.,* 2002; Achuo *et al*., 2006). L'ABA ne semble donc pas avoir un rôle prépondérant dans la réponse à l'infection lors d'un WD dans les feuilles comme suggéré par Achuo *et al*. (2006). Son implication dans les tiges reste à déterminer.

Les différences de pathogénicité entre organes sources et organes puits chez la tomate pourraient être reliées aux teneurs en métabolites primaires de ces organes. Les folioles utilisées pour les infections sur feuilles détachées étaient enfermées dans une boite hermétique fortement humidifiée, permettant une absence de fermeture des stomates. La baisse des symptômes sur feuilles détachées chez les plantes WD-80 pourrait donc être due à une amélioration des défenses, qui semble avoir été induite lorsque les plantes étaient infectées sur tige par un message systémique entre organes. En effet, les plantes en WD-80 infectées possédaient une forte quantité de sucres dans les feuilles sans que les ratios ne soient changés (Annexe 2 et Tab. 3) dont une forte quantité de saccharose (2.64 vs. 0.78 g 100 g^{-1} MS pour Momor et 4.17 vs. 0.88 g 100 g^{-1} MS pour Monalbo), comme cela a été observé sur laitues par Lecompte *et al*. (2013), probablement en lien avec son utilisation pour alimenter les voies de défenses.

En conditions témoins, les tiges ont des teneurs beaucoup plus élevées en sucres solubles que les feuilles (13.4 vs. 3.1 g 100 g^{-1} MS, moyenne pour les témoins non infectés) pour les deux génotypes. Les infections sur tige ont affecté négativement la teneur en amidon dans les feuilles et les tiges des plantes stressées non infectées et infectées. Cette baisse de l'amidon à la fois dans les feuilles et les tiges suggèrent son utilisation afin de fournir du saccharose pour alimenter les métabolismes de défense comme il a été observé grâce aux réseaux de corrélations partielles (Fig. 5). Les réseaux de corrélations partielles ont aussi mis en évidence un lien négatif entre la teneur relative en saccharose et les symptômes de la maladie, et ce en lien avec une augmentation de la teneur relative du glucose. En effet, les symptômes de la maladie étaient plus importants lorsque les tissus étaient plus riches en glucose. L'hypothèse de stimulation des défenses via la synthèse d'hexoses (en lien avec la stimulation des invertases), qui est normalement induite en réponse aux stress biotiques

(Berger *et al*., 2007) et par l'ABA (Kim *et al*., 2000) afin d'alimenter les voies de signalisation et d'activation des défenses, apparaît ici néfaste à la survie de la plante lors d'infection sur tige. Le glucose pourrait servir de substrat à la propagation de *B. cinerea*, qui est connu pour stimuler l'activité des invertases (Geissmann *et al*., 1991; Berger *et al*., 2004). D'un autre côté, les réseaux de corrélations partielles ont mis en évidence un lien entre les modifications engendrées par les WD sur la teneur relative en fructose dans les tiges et les symptômes des infections sur tige, expliquant le fait que les plantes témoins ayant une proportion de fructose supérieure dans les tiges ont des chancres plus petits. Une augmentation du fructose par rapport au glucose suppose l'intervention de l'enzyme sucrose synthase (SUSY) qui permet d'obtenir du fructose et de l'UDPG dans les organes puits (Fig. 9). Une augmentation de l'activité de la SUSY pourrait donc permettre à la plante de générer plus de fructose (comme pour les plantes témoins) afin d'alimenter les défenses des organes puits, et ce sans favoriser le développement du pathogène par une diminution du glucose. De plus, l'UDPG est un intermédiaire plus approprié pour la voie de synthèse de la callose dans des conditions pauvres en oxygène comme dans le phloème et pourrait donc être favorisé dans le cas d'une infection par un pathogène (Koch, 2004). Les résultats des dosages de l'expression de l'enzyme callose synthase Cals12 ont certes mis en évidence une augmentation de celle-ci lorsque les plantes étaient infectées mais aussi lorsqu'elles ne l'étaient pas, sans différence d'expression entre plantes WD et témoins. Le rôle de la callose n'apparaît donc pas majoritaire lors de l'interaction WD - *B. cinerea* dans les tiges, comme cela a été suggéré par Vicedo *et al*. (2009). Cependant, son rôle dans les infections sur feuilles détachées reste à vérifier lors de cette interaction.

Le ratio C/N est utilisé comme indicateur de l'état des défenses de la plante (Royer *et al*., 2013), de par l'implication du carbone et de l'azote dans la synthèse de nombreuses molécules de défenses. Ce ratio a été diminué dans les plantes WD et infectées sur tige. Huanosto Magaña *et al*. (2009) ont suggéré que les différentes sources d'azote et carbone pour la plante seraient les acides malique et citrique (pour le carbone) et les acides aminés (pour l'azote). Libik-Konieczny *et al*. (2012) ont également observé que dans les plantes de type C3, les infections par des spores de *B. cinerea* conduisaient à une augmentation de l'enzyme NADP-ME permettant l'utilisation de l'acide malique pour fournir de l'énergie pour alimenter les voies de défenses. Effectivement, une baisse des acides citrique et malique a été mise en évidence lors des infections sur tige et par les WD, suggérant leur utilisation pour alimenter les voies de défenses JA/ET (lors de l'infection) et la voie de l'ABA (lors des WD) (Fig. 9).

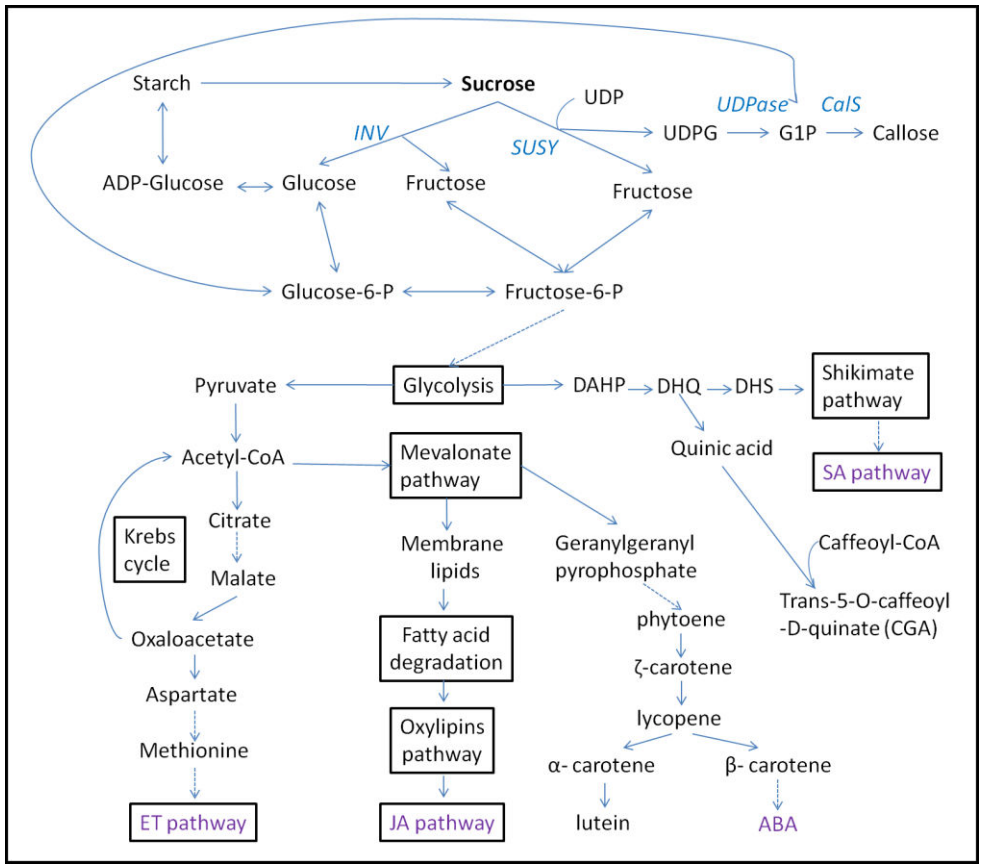

Figure 9: Représentation du métabolisme des sucres en relation avec la synthèse d'hormones induites lors de stress biotique et abiotique. Les flèches pleines symbolisent une relation directe tandis que les flèches en pointillés symbolisent un raccourci dans les voies de biosynthèse. Les encadrés noirs représentent des voies spécifiques non détaillées amenant aux composés d'intérêt étudiés. Les composés écrits en bleu et en italique représentent des enzymes catalysant les réactions décrites. Le saccharose (sucrose) peut être clivé en fructose et en uridine diphosphate glucose (UDPG) par la sucrose synthase (SUSY), enzyme cytoplasmique dans les tissus puits (Granot *et al*., 2013). L'UDPG via l'action de l'UDPase donnera du glucose-1-phosphate (G1P), qui part l'action de calloses synthases permettra la formation de callose (Koch, 2004). D'un autre côté, des invertases présentes dans les organes sources et puits, vont cliver le saccharose en glucose et fructose qui permettront la formation d'amidon via le glucose et qui alimenteront la glycolyse via le fructose-6-phosphate. La glycolyse va permettre de générer du pyruvate qui va alimenter le cycle de Krebs, dont un des composés l'oxaloacétate va permettre la synthèse d'aspartate, qui sera le point de départ de plusieurs réactions amenant à la voie l'éthylène (ET) (Blanco-Ulate *et al*., 2013). La voie principale de l'acide jasmonique (JA) provient de la voie du mévalonate alimentée par l'acétyl-coA du cycle de Krebs et de la dégradation des membranes lipidiques via la voie de l'oxylipine (Browse, 2009). De même, l'acide abscissique (ABA) qui dérive de la synthèse des caroténoïdes est lié à la voie du mévalonate (Taylor *et al*., 2000). Enfin la glycolyse permet aussi d'alimenter la voie de l'acide salycilique (SA) via la voie du shikimate (Vlot *et al*., 2009) et de l'acide quinique qui par combinaison avec le caffeoyl-CoA va permettre la formation de trans-5-O-caffeoyl-D-quinate qui induit une résistance aux pathogènes chez la pomme de terre (Dixon *et al*., 2002).

Cependant, cette baisse de ces acides issus du cycle de Krebs semblerait plutôt due à une favorisation de la synthèse d'acide quinique dans les plantes WD infectées (ratio de 0.33 à J3 vs. 0.37 à J7 en WD-80; Tab. 3 et 4 et Fig. 5). L'acide quinique a été décrit comme un accélérateur du transport d'électrons (Barba-Behrens *et al*., 1993), confortant les observations d'augmentation des flux entre photosystèmes observées lors des WD, et l'hypothèse de stimulation de cette voie par un WD modéré (Chapitre II). L'acide quinique est également lié aux voies de synthèse du shikimate et des phénylpropanoïdes qui ont un rôle dans la régulation de la croissance des plantes et dans la mise en place de mécanismes de résistance aux pathogènes décrits dans l'introduction. La voie du shikimate conduit également à la voie de SA (Fig. 9), qui a été stimulée via l'augmentation des expressions des gènes de NPR1 et PR1a) observée dans les tiges des plantes WD infectées. El Oirdi *et al*. (2011) ont observé une manipulation de la voie SA par des éliciteurs induits par *B. cinerea* chez la tomate. Ces éliciteurs favoriseraient la voie SA pour promouvoir la synthèse de NPR1, protéine antagoniste de la protéine PDF1.2 issue de la voie de JA permettant une meilleure défense contre les pathogènes nécrotrophes. La voie SA serait donc doublement stimulée via le pathogène et en conséquence de la stimulation de la voie de l'acide quinique par les WD, d'où la forte augmentation des expressions observée pour NPR1 et PR1a) à J7. Il a été observé que l'enzyme Cals12 serait NPR1-dépendante chez Arabidopsis (Dong *et al*., 2008) et cela semble aussi être le cas pour la tomate au vue de nos résultats. En effet, l'expression de Cals12 a été augmentée pour toutes les plantes infectées dont l'expression de NPR1 a aussi été augmentée, par rapport au témoin J0 (53 % de corrélation entre NPR1 et Cals12, et 68 % de corrélation entre PR1a) et Cals12, test de Pearson).

Ainsi, l'équilibre entre les régulations hormonales et l'utilisation des ressources primaires pour alimenter les voies de défenses en cas de stress est complexe. L'antagonisme entre la voie SA et les voies JA/ET aurait favorisé l'infection des tiges par *B. cinerea* lors des WD. L'interaction WD - *B. cinerea* est certes délétère pour les organes puits comme les tiges mais s'avère positive pour les organes sources comme les feuilles via un effet systémique déclenché par l'infection sur tige. Des souches moins virulentes seraient intéressantes à étudier afin de vérifier si les défenses de la plante sont augmentées, sans que la survie de la plante ne soit menacée. De même, il serait intéressant d'étudier des mutants sur-exprimant et/ou déficient au niveau de l'enzyme SUSY afin de vérifier son possible rôle dans l'augmentation de la teneur en fructose engendrée dans les tiges lors de l'infection et la baisse de l'intensité des symptômes observée.

V- Conclusion

Les WD appliqués par une réduction des apports en eau de -60 et -80 %, ont mis en évidence une augmentation de la sensibilité des tiges à *B. cinerea*, en lien avec une expression plus forte de la voie SA, antagoniste aux voies JA/ET nécessaires à la mise en place des défenses contre les pathogènes nécrotrophes, et en lien avec l'augmentation du contenu relatif en glucose. Il a été suggéré que la voie SA serait doublement stimulée par *B. cinerea* via des éliciteurs et par les WD via l'augmentation des teneurs en acide quinique. Egalement *B. cinerea* et les WD via l'ABA favorisent l'action des invertases générant une augmentation du glucose qui s'est révélée favorable au pathogène. Contrairement à la relation fructose et infection sur tige qui semble défavorable au pathogène. Il a donc été suggéré que l'enzyme SUSY pourrait jouer un rôle important dans la résistance à *B. cinerea*. Cependant cette interaction WD - *B. cinerea* peut aussi être positive. En effet, un effet systémique des infections sur tige pour les plantes en WD-80 a permis une meilleure défense des feuilles lors des infections sur feuilles détachées révélant un antagonisme entre organes puits et sources. Cette diminution des infections sur feuilles détachées semble liée à une teneur plus importante en saccharose dans les feuilles en WD-80 et suggère un faible rôle de l'ABA. Il serait intéressant de vérifier si cet effet systémique des infections peut être exploité sans que la survie de la plante ne soit menacée à l'aide de souches moins virulentes ou en concentration non délétère.

Remerciements

La réalisation de ces travaux a été soutenue par la Structure Fédérative de Recherche Tersys via la mise en place d'un projet de collaboration et le département EA de l'INRA (pari scientifique). Les auteurs remercient les équipes de l'Unité de Pathologie Végétale de l'INRA d'Avignon, le laboratoire d'analyses de l'Unité Plantes et Systèmes de culture Horticoles (INRA, Avignon) et le laboratoire d'analyses EA4279 Physiologie des Fruits et Légumes de l'Université d'Avignon pour leur implication dans la mise en place des cultures, les analyses de laboratoire et la mise en place de nouveaux protocoles de recherche.

Synthèse et discussion

L'objectif de la thèse était d'évaluer les effets bénéfiques et délétères du déficit hydrique sur les processus de croissance impliqués dans l'élaboration du rendement et de qualité des fruits et sur les mécanismes d'adaptation pouvant modifier la sensibilité de la plante vis à vis d'autres stress. Ainsi, pour atteindre ces objectifs, la thèse a été organisée autour de 5 questions de recherche :

- Peut-on identifier un seuil d'humidité du sol et des critères pertinents pour caractériser la réponse de la plante au cours du dessèchement du sol ?
- Quels sont les impacts d'un WD sur les stratégies adaptatives de la plante, la qualité (organoleptique et nutritive) des fruits et le rendement en fonction des stades de développement de la plante ou du fruit affectés ?
- Peut-on stimuler les défenses de la plante et l'adaptation au WD via des stress répétés ? Quels impacts ces stress répétés auraient-ils sur les métabolites primaires et secondaires et la qualité du fruit ?
- Des modifications du métabolisme primaire induites par le WD modifient-elles la sensibilité des plantes à *Botrytis cinerea* ?
- Quelle est la variabilité génétique des réponses observées?

L'une des finalités importantes de ces travaux était de fournir des connaissances pour une gestion plus durable des ressources en eau, tout en permettant d'améliorer la qualité des fruits sans perte excessive de rendement. Le travail présenté a effectivement apporté un certain nombre de réponses mais il a aussi généré de nouvelles interrogations liées à la complexité et à la variabilité des réponses physiologiques et métaboliques mises en jeu par les différents génotypes.

Mise en évidence d'un seuil d'humidité du substrat au-dessous duquel la physiologie de la plante est fortement affectée

La recherche de seuils d'humidité du sol lors des cinétiques de dessèchement a permis de mettre en évidence une relation entre les potentiels hydriques (base et minimum) et l'humidité des substrats testés. En effet, en dessous de 20 % d'humidité du substrat, seuil atteint après trois jours sans apport d'eau (Chapitre IV) et cinq jours avec de légers apports (Chapitres II et III) pour nos conditions de cultures, les plantes au stade reproducteur marquent réellement le WD par une baisse des potentiels hydriques de tige et feuille. La même tendance est observée pour certains indicateurs de fluorescence de la chlorophylle *a*

notamment l'indice de performance de Strasser *et al*. (2004) PI (Chapitre IV) qui indique une réponse variable en fonction de l'intensité du WD perçue par la plante. Une baisse de la conservation de l'énergie au niveau des PSII a été observée en fonction des génotypes, comme cela a été observé sur haricot (Goltsev *et al*., 2012). Toutefois des mesures de potentiels hydriques du substrat seraient nécessaires afin de mieux caractériser le seuil de 20 %.

Lors des expérimentations des chapitres II, III et V, la gamme de variations de l'humidité du substrat pour les plantes stressées variait entre 10 et 30 % avec des valeurs de potentiels hydriques de base et midi (Fig. 1) proches de celles retrouvées dans d'autres études sur tomate (par exemple entre -0.48 et -0.83 MPa pour Ealson and Richards, 2009) ou sur vigne (entre -0.66 et -0.94 MPa pour Coupel-Ledru *et al*., 2014). Ces valeurs de potentiels hydriques et les résultats observés en termes de régulation des pertes en eau (réduction de la conductance stomatique et de la transpiration) suggèrent une adaptation des plantes stressées aux différentes intensités (de -38 % à -80 % d'apport d'eau par rapport au témoin) et durées (entre 15 et 21 jours) de WD appliquées dans la thèse. La gestion du stress oxydatif induit par le WD a été mise en évidence, notamment via l'augmentation de J_0^{RE1}/J^{ABS} (chapitre II) et semble variable en fonction du stade de développement de la plante (Chapitre IV).

Les plantes végétatives et reproductives se distinguent par les mécanismes d'adaptation mis en jeu au cours du dessèchement

Les photosystèmes II et I sont au cœur de la réponse physiologique des plantes, aux contraintes environnementales. Les mesures de fluorescence chlorophyllienne permettent de caractériser les différentes pertes dans le flux de transport d'énergie qui pourraient survenir lors d'un stress, notamment à l'aide des paramètres OJIP (Strasser et Strasser, 1995), et des paramètres OJIPSMT (Stirbet *et al*., 2014). Ces mesures simples à mettre en œuvre ont l'avantage d'être non destructives et discriminantes des effets du WD en fonction du stade et des génotypes comme vu dans les chapitres II et IV.

D'après nos résultats, les rendements quantiques des étapes OJIP peuvent être classés en fonction de leur sensibilité au WD, avec du moins sensible au plus sensible:

- J_0^{TR}/J^{ABS} représentant le transfert d'électrons entre P680 et Q_A,
- J_0^{ET2}/J^{ABS} de Q_A vers PQ,
- J_0^{RE1}/J^{ABS} de PQ vers les accepteurs primaires du PSI.

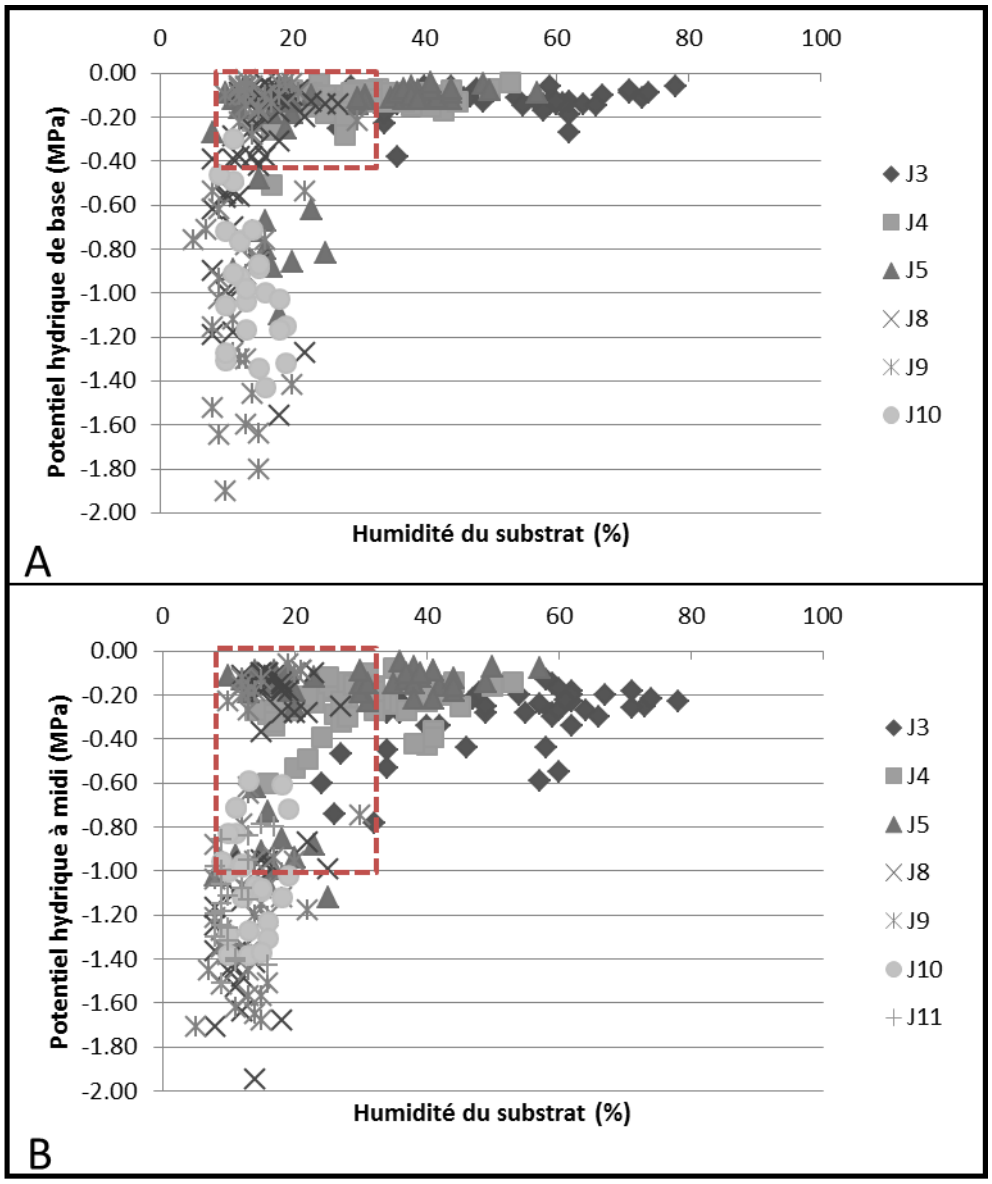

Figure 1: Potentiels hydriques de tige de base (A) et au midi solaire (B) en fonction de l'humidité du substrat mesurée lors des cinétiques de dessèchements du substrat (chapitre IV). Les symboles indiquent des jours (J) de mesures à partir de l'arrêt total des apports d'eau indiquées en légende. Les encadrés rouges en pointillés représentent les gammes de valeurs d'humidité du substrat et des potentiels hydriques de base et à midi observées lors des expérimentations avec des WD modérés dans les chapitres II, III et V, pour ces mêmes variables.

Cette classification retrouvée aussi dans le cas du haricot (Goltsev *et al*., 2012) est valable pour les deux stades de développement étudiés. Ces rendements quantiques montrent une sensibilité au WD plus forte pour les réactions photochimiques autour du PSI qu'autour du PSII. Le paramètre PI_{ABS}^{TOT} qui intègre ces 3 principaux rendements quantiques ressort comme un bon indicateur de l'état de stress lors d'un WD, au contraire de l'indicateur PI comme suggéré par Živčák *et al*. (2008).

Toutefois, la réponse des plants de tomate aux deux stades de développement étudiés a mis en évidence différentes stratégies en termes de régulation de l'excès d'énergie perçue lors d'un WD au niveau des photosystèmes. Ces stratégies sont schématisées sur la figure 2. En effet, les plantes végétatives ont évacué le trop plein d'énergie perçue au niveau des PSII en utilisant d'autres moyens que le piégeage d'électrons assimilables à des sorties sous forme de chaleur (à l'exception de Plovdiv). De plus, les plantes végétatives présentaient plus de signe de perte d'énergie au niveau de Q_A et du pool de PQ probablement en lien avec la chlororespiration (Feild *et al*., 1998 ; Rumeau *et al*., 2007), accentuant la baisse du transfert d'électrons vers les accepteurs du PSI. Les plantes reproductives ont plus fortement réduit l'ouverture de leurs stomates que les plantes végétatives et ont augmenté leur teneur relative en chlorophylles dans les feuilles. Les résultats observés sur les plantes reproductives témoignent d'un maintien de l'activité photosynthétique et donc de la production d'assimilats pour maintenir la croissance des organes puits. Ce type de réponse a été observé sur peuplier, maïs ou soja en condition de dessèchement (Boyer, 1970 ; Bogeat-Triboulot *et al*., 2007) La modulation des réponses entre plantes végétatives et reproductives pourrait être liée à la demande des organes puits qui serait plus importante pour les plantes reproductives en raison de la présence de fruits.

Ainsi, certains paramètres ont pu être sélectionnés pour caractériser l'impact du WD dont certains sont communs aux deux stades de développement de plante. Pour les plantules: F_0, Sm, N, J_0^{ET2} et J_0^{RE1}, PI_{ABS}^{TOT}; pour les plantes reproductives: F_M, PI, F_V/F_0, RC/ABS, $(1-V_J)/V_J$, J_0^{ET2}, J_0^{RE1} et PI_{ABS}^{TOT}.

<u>L'impact du WD sur la qualité des fruits dépendant de la phase de développement affectée par le WD</u>

La phase de division cellulaire du fruit semble la plus sensible au WD pour les deux génotypes testés (chapitre III) avec une amélioration de la qualité gustative (plus de sucres et

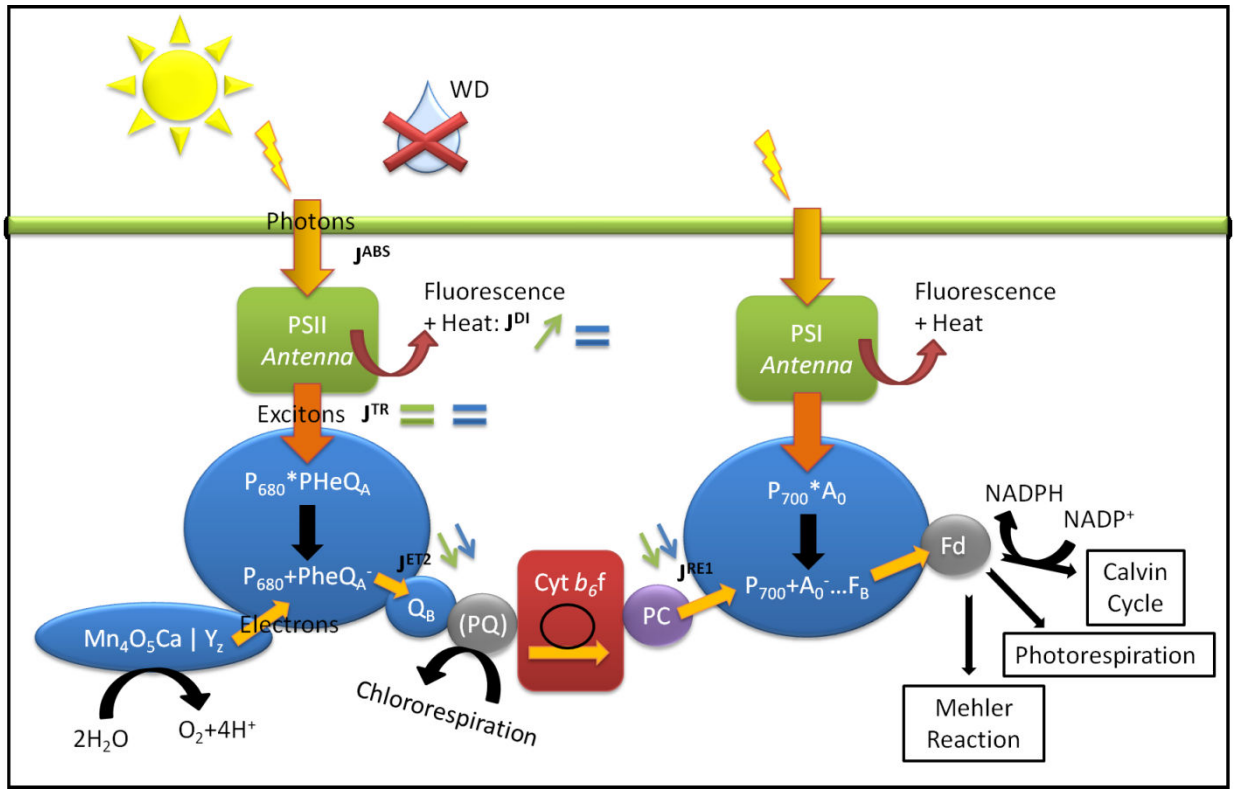

Figure 2: Représentation des effets d'un WD intense sur le fonctionnement de l'appareil photosynthétique, inspiré de Stirbet et Govindjee (2011). Les principaux paramètres de fluorescence établie par Strasser *et al.* (2004) sont représentés avec: J^{DI} qui représente la dissipation d'énergie au niveau des photosystèmes PSII sous forme de chaleur, J^{TR} représente le taux d'excitons piégés et le nombre d'événement de réduction de Q_A, J^{ET2} représente le transport d'électrons entre Q_A et Q_B, J^{RE1} représente le transport d'électron jusqu'aux accepteurs primaires des photosystèmes PSI. La sortie vers la chlororespiration représentée est basée sur l'hypothèse de Feild *et al.* (1998). Les symboles (↗ ↘ =) verts représentent les variations observées dans la thèse sur des plantes végétatives soumises au WD tandis que les symboles bleus représentent les variations observées pour les plantes reproductives. Toutes les variations observées ne sont pas représentées sur ce schéma notamment les baisses de Sm et N pour les plantes végétatives uniquement.

moins d'acides) et peu d'impact sur la croissance des fruits. Ces résultats sont en accord avec ceux observés par Nuruddin *et al*. (2003), où la période de floraison, qui correspond au début de la période de division cellulaire dans notre étude, serait favorable à l'application de WD en termes de maintien du rendement et d'amélioration de la qualité du fruit (évaluée en °Brix par ces auteurs). La croissance des fruits du génotype LA1420 a également été améliorée suite au WD appliqué en phase de division cellulaire comme vu pour la pêche (Li *et al*., 1989). Cette augmentation de la croissance des fruits est probablement liée à une augmentation des capacités photosynthétiques (chapitres III et IV) et à une meilleure plasticité lors de la récupération pendant la phase d'expansion cellulaire (chapitre III).

Cependant, pour les trois phases de développement, les effets observés étaient variables en fonction des génotypes. Cette variabilité pourrait dépendre des différences de composition initiale des génotypes. En effet, le génotype ayant la plus faible teneur en sucres en condition témoin était aussi celui pour lequel l'augmentation des sucres en réponse au WD a été la plus importante. De plus, quelle que soit la phase de développement du fruit affectée, le WD a eu un impact très contrasté au niveau des caroténoïdes et de l'acide ascorbique allant de négatif, à neutre ou positif, selon le composé étudié et le génotype. Ces résultats sont en accord avec la littérature (Ripoll *et al*., 2014). Ces réponses contrastées et variables pourraient être liées à la gestion du stress oxydatif en amont dans les organes sources, limitant l'impact des ROS et du statut redox sur la qualité des fruits.

Stimulation de l'effet mémoire des plantes au cours de cycles stress/récupération

L'alternance de périodes de WD et de périodes de récupération est un phénomène naturel notamment en saison estivale. Un premier stress de faible intensité tend à avoir un effet positif lors d'un second stress de plus forte intensité via la stimulation plus rapide des mécanismes d'adaptation (Bruce *et al*., 2007). Ainsi, des intensités croissantes de WD ont été appliquées afin de stimuler un effet « mémoire » des plantes et de mesurer les effets au cours d'un second stress (chapitre II).

Un léger effet de priming a été observé lors du premier cycle de DI1/RP1/DI2 mais lors du second cycle DI2/RP2/DI3 les plantes étaient globalement plus sensibles. Sur l'ensemble de la période de culture, les génotypes à gros fruits (plus de 50 g) ont été plus sensibles avec une réduction plus importante de la transpiration et de la conductance stomatique, ainsi que du rendement photochimique au niveau des PSII (PI). Cependant lors de

l'alternance WD/RP, la plupart des génotypes ont maximisé les performances au niveau du PSI via le flux cyclique d'électrons (augmentation de J_0^{RE1}/J^{ABS}), qui constitue une stratégie adaptative rarement observée pour les plantes en C3 permettant de limiter la formation des ROS en recyclant le trop plein d'énergie (Johnson, 2011 ; Rumeau *et al*., 2007). Les effets de l'alternance WD/RP sur ces plantes au stade reproductif était clairement contraire à ce qui a été observé lors de la cinétique de dessèchement (Chapitre III), auquel la plante n'a pu s'adapter.

L'alternance a affecté différentes phases de développement du fruit. En effet les phases de division et d'expansion cellulaire du fruit ont été les plus touchées. Les fruits récoltés à maturité ont été séparés par rapport aux périodes de WD/RP subis, avec un lot n°1 récolté suite à l'étape considérée comme un priming et un lot n°2 récolté en fin d'expérimentation sur des plantes ayant subis l'ensemble du traitement. Globalement, le premier cycle de stress a impacté positivement la qualité des fruits des génotypes à petits fruits (en termes de teneurs en matière sèche, de sucres solubles et d'amidon), suggérant une plus grande disponibilité en carbone (sans impacter la croissance) mais sans impact positif sur les teneurs en caroténoïdes totaux comme observé par Stoeva *et al*. (2010 ; 2012). Le second cycle de stress a eu des effets plus contrastés (nuls, négatifs ou positifs) sur la qualité des fruits pour tous les génotypes. Le second cycle a également eu un impact plutôt négatif sur la croissance des fruits.

Le WD module la sensibilité de la plante à *Botrytis cinerea* via des interactions entre métabolites primaires et régulations hormonales

L'étude de l'interaction WD - *Botrytis cinerea* avait pour objectif de tester l'hypothèse selon laquelle des manipulations des teneurs en sucres solubles et les régulations hormonales de la plante influenceraient la pathogénicité du Botrytis et donc l'interaction WD - *B. cinerea*. Deux intensités de WD modérés ont été appliquées sur deux génotypes de tomates commerciales dont la sensibilité aux maladies diffère (Momor moins sensible que Monalbo). Cependant, aucune différence de sensibilité n'a été observée entre les deux génotypes lors des infections sur tige, au contraire des infections foliaires sur feuilles détachées, probablement en raison du choix de la souche BC1 (très virulente) et de son injection à une concentration délétère.

La figure 3 résume les effets observés lors de l'interaction WD - *B. cinerea* à l'échelle des deux organes (feuilles et tiges) sur les métabolites primaires et les principales hormones étudiées. Le WD a modifié les équilibres en sucres solubles. L'augmentation des teneurs en saccharose et en glucose dans les tiges a favorisé la pathogénicité de *B. cinerea*. Cette augmentation du glucose est apparue corrélée à une baisse des acides du cycle de Krebs et à une augmentation de la voie de l'acide quinique conduisant à la voie SA (Fig. 9 Chapitre V). Les régulations hormonales mises en jeu semblent augmenter l'activité des invertases pour synthétiser des hexoses qui sont stimulées à la fois par l'ABA (Kim *et al*., 2000) et possiblement par des éliciteurs émis par *Botrytis* (Geissmann *et al*., 1991). De plus, la voie SA serait stimulée par *B. cinerea* (El Oirdi *et al*., 2011) afin de bloquer la voie JA/ET qui est la voie de défense la plus efficace contre les pathogènes nécrotrophes et possiblement par les WD modérés (Chapitre V). Un lien entre la voie SA, observé au niveau des gènes codant pour NPR1 et PR1a), et l'activité de la callose synthase Cals12 a également été mis en évidence dans la thèse. Ces résultats confirment sur tomate un lien déjà observé chez Arabidopsis (Dong *et al*., 2008). Bien que la callose constitue une des premières barrières limitante pour la propagation des pathogènes (Koch, 2004), la callose ne semble pas avoir un rôle prépondérant dans la résistance à *Botrytis* lors de l'interaction WD - *B. cinerea*.

D'un autre côté, une corrélation négative entre la teneur relative en fructose dans les tiges et le développement du pathogène sur tige a été observée. Nous avons émis l'hypothèse que l'enzyme SUSY qui permet de synthétiser du fructose et de l'UDPG à partir du saccharose dans les tiges, pourrait limiter les ressources disponibles (notamment le glucose) pour la croissance du pathogène (Nassr et Barakat, 2013) et probablement limiter la stimulation de la voie SA.

Bien que les infections sur tige aient été plus importantes pour les plantes en WD par rapport au témoin, un effet positif du WD a pu être observé au niveau des infections sur feuilles détachées, lorsque les plantes étaient infectées sur tige. Ce résultat laisse supposer un effet systémique, qui a été observé pour les deux génotypes. L'hypothèse d'une barrière physique mise en place au travers de la structure des feuilles ou de la conductance stomatique a été écartée (Chapitre V). D'autres hypothèses seraient une augmentation des dépôts de callose (non observés au niveau des feuilles) et la disponibilité en ressources pour alimenter les voies de défense (notamment le saccharose, Chapitre V). Le WD semble donc stimuler les mécanismes de défenses contre *B. cinerea* au niveau des feuilles via un signal transmis depuis le site d'infection sur tige.

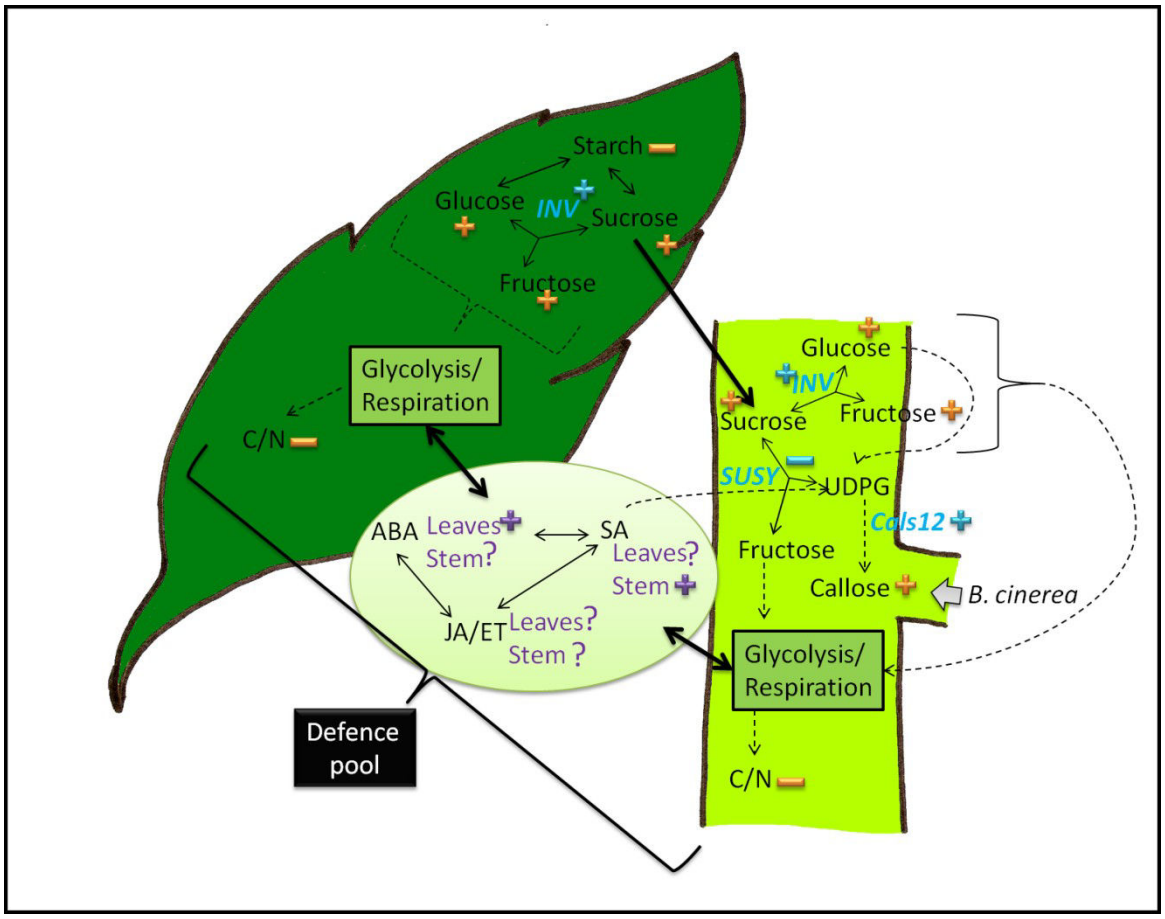

Figure 3: Schéma récapitulatif des effets de l'interaction entre un WD et *B. cinerea* (infection sur tige) sur les sucres solubles, le ratio C/N et les principales hormones mises en jeu à l'échelle de la feuille et de la tige. Les symboles + et – indiquent des effets positifs ou négatifs significatifs sur les métabolites primaires (orange), les enzymes (bleu) et les hormones (violet), à l'exception de la régulation de la voie JA/ET qui est hypothétique, de même pour les enzymes sucrose synthase (SUSY) et les invertases (INV). Les enzymes INV, SUSY et callose synthase 12 (Cals12) ont été représentées. Les régulations hormonales étudiées sont l'acide abscissique (ABA) et l'acide salicylique (SA); la voie de l'acide jasmonique (JA) et de l'éthylène (ET) n'ont pas pu être évaluées à ce jour.

Importance de la variabilité génétique dans la réponse au WD: étude des parents de la population MAGIC TOM

Les 8 parents de la population MAGIC sélectionnés parmi 360 accessions de tomates pour leur importante variabilité allélique (Ranc, 2010) constituent une bonne base pour caractériser la variabilité génétique des réponses au WD. Cette population est composée pour rappel de tomates d'origines diverses ayant des calibres contrastés, de tomates cerise à des tomates gros calibre.

La caractérisation de quatre de ces génotypes lors de cinétiques de dessèchement a permis d'établir une classification de leur sensibilité (en termes de gestion de l'état hydrique et du stress oxydatif engendré par le WD) en fonction du stade de développement de la plante avec du plus sensible au moins sensible:

- Plante végétative: Cervil > Plovdiv > Levovil > LA1420
- Plante reproductive: Levovil > Plovdiv > Cervil > LA1420

Il est néanmoins plus difficile d'établir une classification des génotypes suite au traitement d'alternance de phases de WD et de récupération. Globalement, la sensibilité au WD semble plus importante pour les génotypes à gros fruits (plus de 50 g). Au contraire chez les génotypes à petits fruits, nous avons observé une amélioration de la qualité gustative sans impact notable sur le rendement. Cette différence pourrait être due à une plus grande disponibilité en carbone et à une plus faible demande, favorables à la croissance et au métabolisme des fruits (Muller *et al*., 2011).

Parmi les 8 parents de la population MAGIC, le génotype LA1420, qui est le génotype le plus proche du parent sauvage *S. l. pimpinellifollium* connu pour sa meilleure résistance aux stress (Foolad *et al*., 2003), a été le moins sensible au WD (Chapitres II et III) en termes d'ajustement osmotique et de pertes de rendement. Les fruits de LA1420 sont de qualité médiocre en conditions témoin et les effets positifs du WD sur cette qualité ont été relativement importants. De fait, malgré une réduction de l'ouverture des stomates, la croissance des fruits a été augmentée, suggérant une amélioration des capacités photosynthétiques en condition de WD, comme cela a été suggéré par Muller *et al*. (2011). Ce génotype a donc un potentiel intéressant à exploiter pour la sélection de génotypes adaptés à la contrainte hydrique.

Conclusions et perspectives

Des réponses aux différents sous-objectifs ont pu être apportées à l'échelle de la plante et du fruit en termes de stratégie de réponses au WD et en termes de qualité des fruits, dont les principales variations sont résumées dans la figure 1. Toutefois de nouvelles pistes de recherche ont été soulevées.

Nous avons pu identifier des paramètres de fluorescence chlorophyllienne autour des photosystèmes II qui varient en fonction des génotypes, du stade de développement de la plante et du type de WD appliqué. Des compléments d'analyses pourraient permettre de caractériser l'impact de WD au sein des photosystèmes I (notamment au niveau des P700, figure 2, chapitre VI) via des indicateurs non abordés dans la thèse en utilisant un appareil spécifique, tel le Dual-PAM-100 (Heinz Walz, Effeltrich, Germany). De même, des paramètres supplémentaires OJIPSMT définie par Kalaji *et al*. (2014) pourraient être utilisés. Des analyses biochimiques de la composition des feuilles (échantillons prélevés mais non analysés) pourraient également permettre de compléter la compréhension des mécanismes de réponse mis en jeu aux deux stades de développement étudiés. Il serait également intéressant de coupler les approches de fluorescence chlorophyllienne à l'échelle des deux photosystèmes avec les mesures de photosynthèse en conditions de WD afin de compléter les connaissances déjà établies (Porcar-Castell *et al*., 2014).

Concernant les difficultés expérimentales rencontrées au cours de la thèse, un point critique a été l'application du WD pendant une phase de développement précise du fruit. En effet, sur des plantes à croissance indéterminée, plusieurs bouquets d'âge variables coexistent le long de la tige. L'évaluation de la variabilité génétique sur les huit parents de la population MAGIC TOM a également engendré des expérimentations lourdes en raison du nombre de plantes suivies. La caractérisation de sous lots de ces génotypes dans des conditions de stress bien contrôlées pourrait permettre de valider nos hypothèses.

Il serait également intéressant de tester d'autres modalités d'application du WD soit par des arrêts consécutifs des apports d'eau sur des périodes courtes soit par un stress continu mais faible sur une large sélection de variétés commerciales afin de trouver le meilleur compromis entre qualité et rendement. De plus, les graines issues des fruits stressés pourraient présenter un intérêt. Il serait intéressant de vérifier si l'amélioration de la qualité est préservée à la génération suivante et si une mémoire du WD existe permettant à la plante de s'adapter

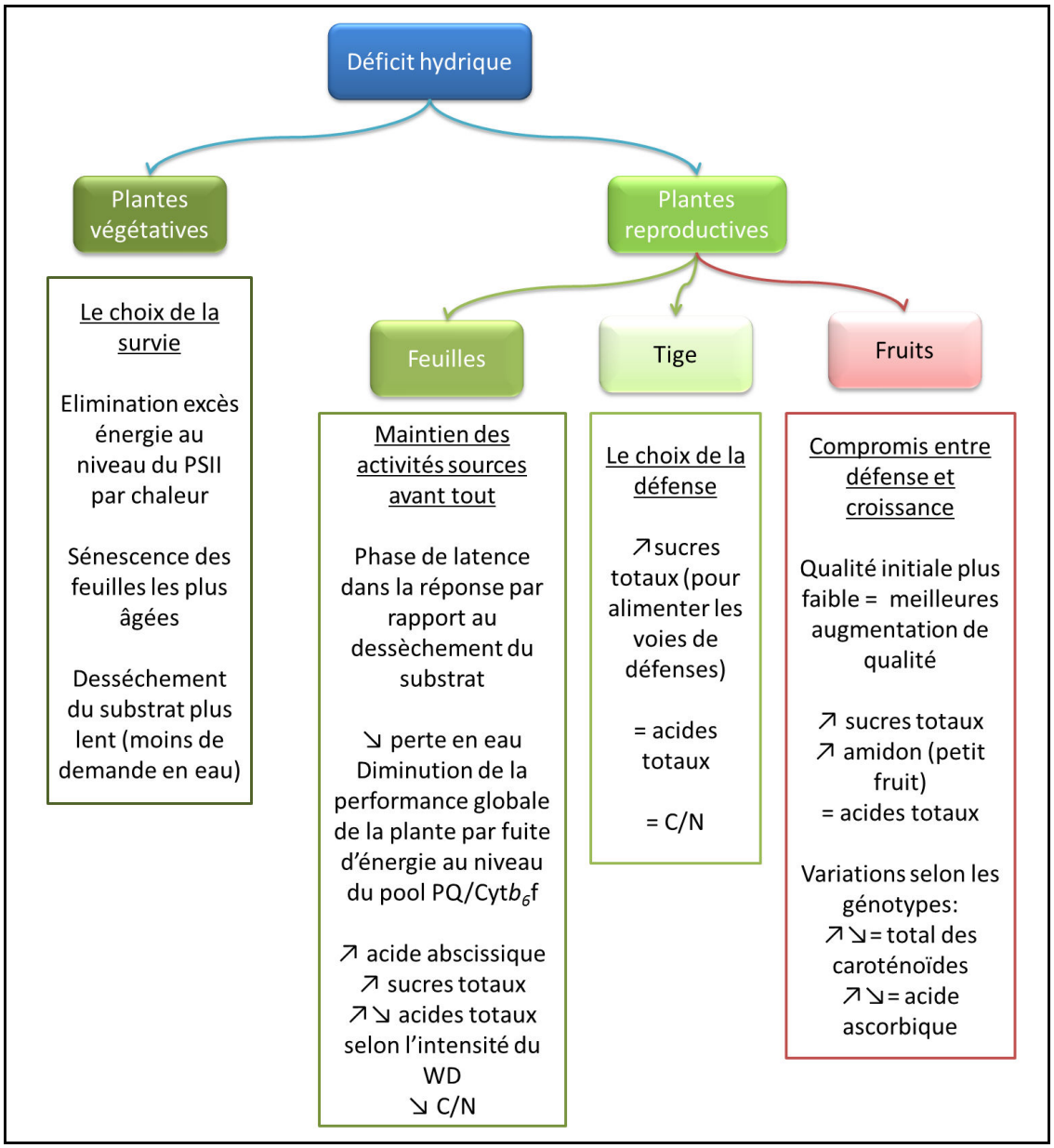

Figure 1: Récapitulatif des principaux impacts du WD d'intensité forte (plantes végétatives) à modéré (plantes reproductives) en fonction du stade de développement de la plante. Pour les plantes reproductives, les effets ont été étudiés à l'échelle des différents organes: source (feuilles) et puits (tige et fruits). Les symboles ↗ ↘ = indiquent les changements significatifs observés suite à un WD lors des travaux de thèse.

plus facilement (potentiellement par des effets épigénétiques).

Enfin, l'étude de l'interaction entre un WD et la sensibilité à *Botrytis cinerea* mérite d'être approfondie. Le choix du pathogène a été guidé par l'absence de moyen de lutte efficace contre *B. cinerea* et par des travaux de l'unité PSH montrant un rôle du métabolisme des sucres dans la pathogénicité. La question du rôle de l'enzyme SUSY lors de l'infection par *B. cinerea*, serait intéressante à approfondir, en utilisant des mutants spécifiques. De même, l'observation de l'effet systémique entre infection sur tige et infection sur feuilles ouvre de nouvelles perspectives de recherche afin de stimuler les défenses des plantes face aux pathogènes en injectant des pathogènes à des concentrations non délétères dans les plantes et/ou en utilisant des souches moins agressives.

Mes travaux suggèrent donc qu'il est possible de gérer le WD en conditions de productions, sans impacter la croissance des fruits et en améliorant la qualité gustative des fruits en fonction des génotypes, via l'alternance de cycles de WD et de RP ou via un WD appliqué lors de la division cellulaire des fruits (mais difficile à mettre en oeuvre sur plantes à croissance indéterminée). De même la stimulation des défenses de la plante par un WD serait plus efficace lorsque le WD est suivie d'une période de récupération. Les économies d'eau réalisées au cours des expérimentations de la thèse sont de l'ordre de 20 à 25 % sur l'ensemble des périodes de culture.

Références bibliographiques

A

Abbott JA. Quality measurement of fruits and vegetables. *Postharvest Biology and Technology*. 1999, **15**, 207-225.

AbuQamar S, Chai M-F, Luo H, Song F, Mengiste T. Tomato Protein Kinase 1b Mediates Signaling of Plant Responses to Necrotrophic Fungi and Insect Herbivory. *The Plant Cell Online*. 2008, **20**, 1964-1983.

Achuo E.A., Prinsen E., Höfte M. Influence of drought, salt stress and abscisic acid on the resistance of tomato to *Botrytis cinerea* and *Oidium neolycopersici*. *Plant Pathology*. 2006, **55**, 178-186.

Adato I, Gazit S. Water-deficit stress, ethylene production, and ripening in avocado fruits. *Plant Physiology*. 1974, **53**, 45-46.

Akinci S, Losel DM. The soluble sugars determination in Cucurbitaceae species under water stress and recovery periods. *Advances in Environmental Biology*. 2009, **3**, 175-183.

Albacete AA, Martínez-Andújar C, Pérez-Alfocea F. Hormonal and metabolic regulation of source–sink relations under salinity and drought: From plant survival to crop yield stability. *Biotechnology Advances*. 2014, **32**, 12-30.

Al Gehani I. Effet des stades d'application et de l'intensité de stress hydriques répétés sur l'état hydrique et la croissance des plantes et des fruits de tomate. Thèse, Aix-Marseille, Université d'Avignon et des pays du Vaucluse. 2005.

Allen JF. Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain. *Trends in Plant Science*. 2003, **8**, 15-19.

Anttonen MJ, Hoppula KI, Nestby R, Verheul MJ, Karjalainen RO. Influence of fertilization, mulch color, early forcing, fruit order, planting date, shading, growing environment, and genotype on the contents of selected phenolics in strawberry (Fragaria x ananassa Duch.) fruits. *Journal of agricultural and food chemistry*. 2006, **54**, 2614-2620.

Apelbaum A, Yang SF. Biosynthesis of stress ethylene induced by water deficit. *Plant Physiology*. 1981, **68**, 594-596.

Arasimowicz-Jelonek M, Kosmala A, Janus Ł, Abramowski D, Floryszak-Wieczorek J. The proteome response of potato leaves to priming agents and S-nitrosoglutathione. *Plant Science*. 2013, **198**, 83-90.

Araus JL, Cairns JE. Field high-throughput phenotyping: the new crop breeding frontier. *Trends in Plant Science*. 2014, **19**, 52-61.

Armond PA, Schreiber U, Bjorkman O. Photosynthetic Acclimation to Temperature in the Desert Shrub, Larrea divaricata: II. Light-harvesting Efficiency and Electron Transport. *Plant Physiol.* 1978, **61**, 411-415.

Armond PA, Bjorkman O, Staehelin LA. Dissociation of supramolecular complexes in chloroplast membranes. A manifestation of heat damage to the photosynthetic apparatus. *Biochim Biophys Acta*. 1980, **601**, 433-443.

Asselbergh B, De Vleesschauwer D, Höfte M. Global Switches and Fine-Tuning—ABA Modulates Plant Pathogen Defense. *Molecular Plant-Microbe Interactions.* 2008, **21**, 709-719.

Asselbergh B, Curvers K, Franca SC, Audenaert K, Vuylsteke M, Van Breusegem F, Hofte M. Resistance to Botrytis cinerea in sitiens, an abscisic acid-deficient tomato mutant, involves timely production of hydrogen peroxide and cell wall modifications in the epidermis. *Plant Physiology.* 2007, **144**, 1863-77.

Asselbergh B, De Vleesschauwer D, Höfte M. 2008. Global Switches and Fine-Tuning—ABA Modulates Plant Pathogen Defense. Molecular Plant-Microbe Interactions 21, 709-719. Atherton JG, Harris GP. Flowering. In: Rudish Aa, ed. *The tomato crop*. London: Chapman and Hall, 1986, 167-194.

Atherton JG, Harris GP. Flowering. In: Atherton J, Rudich J, eds. *The Tomato Crop*: Springer Netherlands. 1986, 167-200.

Atkinson KE. *An introduction to numerical analysis*: John Wiley & Sons. 2008.

Atkinson NJ, Dew TP, Orfila C, Urwin PE. Influence of Combined Biotic and Abiotic Stress on Nutritional Quality Parameters in Tomato (Solanum lycopersicum). *Journal of Agricultural and Food Chemistry.* 2011, **59**, 9673-9682.

Atkinson NJ, Urwin PE. The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany.* 2012, 1-21.

Aurand R, Faurobert M, Page D, Maingonnat JF, Brunel B, Causse M, Bertin N. Anatomical and biochemical trait network underlying genetic variations in tomato fruit texture. *Euphytica.* 2012, **187**, 99-116.

Audenaert K, De Meyer GB, Höfte MM. Abscisic Acid Determines Basal Susceptibility of Tomato to Botrytis cinerea and Suppresses Salicylic Acid-Dependent Signaling Mechanisms. Plant Physiology. 2002, **128**, 491-501.

B

Baba M, Shiraiwa Y. Biosynthesis of Lipids and Hydrocarbons in Algae. Photosynthesis, Prof. Zvy Dubinsky (Ed.), ISBN: 978-953-51-1161-0, InTech, 2013. DOI: 10.5772/56413.

Baker NR. Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. *Annual Review of Plant Biology.* 2008, **59**, 89-113.

Bajguz A, Hayat S. Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiology and Biochemistry.* 2009, **47**, 1-8.

Baldazzi V, Pinet A, Vercambre G, Benard C, Biais B, Génard M. In-silico analysis of water and carbon relations under stress conditions. A multi-scale perspective centered on fruit. *Frontiers in Plant Science.* 2013, 4, 495.

Baldet P, Hernould M, Laporte F, Mounet F, Just D, Mouras A, Chevalier C, Rothan C. The expression of cell proliferation-related genes in early developing flowers is affected by a fruit load reduction in tomato plants. *Journal of Experimental Botany.* 2006, **57**, 961-970.

Baldwin EA, Scott JW, Einstein MA, Malundo TMM, Carr BT, Shewfelt RL, Tandon KS. Relationship between sensory and instrumental analysis for tomato flavor. *Journal of the American Society for Horticultural Science.* 1998, **123**, 906-915.

Balibrea ME, Cuartero J, Bolarin MC, Perez-Alfocea F. Sucrolytic activities during fruit development of Lycopersicon genotypes differing in tolerance to salinity. *Physiol Plant.* 2003, **118**, 38-46.

Barba-Behrens N, Carrasco-Fuentes ME, Castillo-Blum SE, Mendoza JL, Salazar F, Tovar A, Lotina-Hennsen B, Contreras R, Flores-Parra A. Coordination compounds quinic acid as decouplers on photosynthesis. *Biophysical Chemistry.* 1993, **47**, 67-75.

Baron-Epel O, Hernandez D, Jiang LW, Meiners S, Schindler M. Dynamic continuity of cytoplasmic and membrane compartments between plant cells. *Journal of Cell Biology.* 1988, **106**, 715-721.

Barry CS, Giovannoni JJ. Ethylene and fruit ripening. *Journal of Plant Growth Regulation.* 2007, **26**, 143-159.

Beckles DM, Hong N, Stamova L, Luengwilai K. Biochemical factors contributing to tomato fruit sugar content: a review. *Fruits.* 2012, **67**, 49-64.

Beckers GJM, Jaskiewicz M, Liu YD, Underwood WR, He SY, Zhang SQ, Conrath U. Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in Arabidopsis thaliana. *Plant Cell.* 2009, **21**, 944-953.

Behboudian MH, Dixon J, Pothamshetty K. Plant and fruit responses of lysimeter-grown 'Braeburn' apple to deficit irrigation. *Journal of Horticultural Science and Biotechnology.* 1998, **73**, 781-785.

Benard C, Gautier H, Bourgaud F, Grasselly D, Navez B, Caris-Veyrat C, Weiss M, Genard M. Effects of Low Nitrogen Supply on Tomato (Solanum lycopersicum) Fruit Yield and Quality with Special Emphasis on Sugars, Acids, Ascorbate, Carotenoids, and Phenolic Compounds. *Journal of agricultural and food chemistry.* 2009, **57**, 4112-4123.

Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T. Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. *Physiologia Plantarum*. 2004, **122**, 419-428.

Berger S, Sinha AK, Roitsch T. Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany*. 2007, **58**, 4019-4026.

Bertin N, Martre P, Genard M, Quilot B, Salon C. Under what circumstances can process-based simulation models link genotype to phenotype for complex traits? Case-study of fruit and grain quality traits. *Journal of Experimental Botany.* 2010, **61**, 955-967.

Bertin, N.; Lecomte, A.; Brunel, B.; Fishman, S.; Genard, M. A model describing cell polyploidization in tissues of growing fruit as related to cessation of cell proliferation. *Journal of Experimental Botany*. **2007**, 58, 1903-1913.

Bertin N. Analysis of the tomato fruit growth response to temperature and plant fruit load in relation to cell division, cell expansion and DNA endoreduplication. *Annals of Botany.* 2005, **95**, 439-447.

Bertin N, Genard M, Fishman S. A model for an early stage of tomato fruit development: cell multiplication and cessation of the cell proliferative activity. *Annals of Botany.* 2003, **92**, 65-72.

Bertin, N.; Gautier, H.; Roche, C. Number of cells in tomato fruit depending on fruit position and source-sink balance during plant development. *Plant Growth Regulation*. 2002, **36**: 105-112.

Bertin N, Guichard S, Leonardi C, Longuenesse JJ, Langlois D, Navez B. Seasonal evolution of the quality of fresh glasshouse tomatoes under Mediterranean conditions, as affected by air vapour pressure deficit and plant fruit load. *Annals of Botany.* 2000, **85**, 741-750.

Bertin, N.; Gary, C.; Tchamitchian, M.; Vaissiere, B.E. Influence of cultivar, fruit position and seed content on tomato fruit weight during a crop cycle under low and high competition for assimilates. *Journal of Horticultural Science and Biotechnology*. 1998, **73**, 541-548.

Beyers T, Vos C, Aerts R, Heyens K, Vogels L, Seels B, Höfte M, Cammue BPA, De Coninck B. Resistance against Botrytis cinerea in smooth leaf pruning wounds of tomato does not depend on major disease signalling pathways. Plant Pathology. 2014, **63**, 165-173.

Bidel LPR, Coumans M, Baissac Y, Doumas P, Jay-Allemand C. *Biological activity of phenolics in plant cells*. Ames,: Wiley-Blackwell. 2010.

Bindon KA, Dry PR, Loveys BR. Influence of plant water status on the production of C13-norisoprenoid precursors in Vitis vinifera L. Cv. cabernet sauvignon grape berries. *Journal of Agricultural and Food Chemistry.* 2007, **55**, 4493-500.

Bishop N. The Possible Role of Plastoquinone (QA-) in the Electron Transport System of Photosynthesis, Ciba Foundation Symposium-Quinones in Electron Transport, Wiley Online Library. 1961. pp. 385-424.

Blanco-Ulate B, Vincenti E, Powell ALT, Cantu D. Tomato transcriptome and mutant analyses suggest a role for plant stress hormones in the interaction between fruit and Botrytis cinerea. *Frontiers in Plant Science.* 2013, **4**, 142.

Bleeker PM, Mirabella R, Diergaarde PJ, VanDoorn A, Tissier A, Kant MR, Prins M, Vos Md, Haring MA, Schuurink RC. Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. *Proceedings of the National Academy of Sciences of the United States of America.* 2012, **109**, 20124-20129.

Blum A. Drought resistance – is it really a complex trait? *Functional Plant Biology.* 2011, **38**, 753-757.

Blum, A. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regulation*. 1996, **20**, 135-148.

Bogeat-Triboulot M-B, Brosché M, Renaut J, Jouve L, Le Thiec D, Fayyaz P, Vinocur B, Witters E, Laukens K, Teichmann T, Altman A, Hausman J-F, Polle A, Kangasjärvi J, Dreyer E. Gradual Soil Water Depletion Results in Reversible Changes of Gene Expression, Protein Profiles, Ecophysiology, and Growth Performance in Populus euphratica, a Poplar Growing in Arid Regions. *Plant Physiology*. 2007, **143**, 876-892.

Bohner J, Bangerth F. Effects of fruit set sequence and defoliation on cell number, cell size and hormone levels of tomato fruits (Lycopersicon esculentum Mill.) within a truss. *Plant Growth Regulation.* 1988, **7**, 141-155.

Bolton MD. Primary Metabolism and Plant Defense-Fuel for the Fire. *Molecular Plant-Microbe Interactions*. 2009, **22**, 487-497.

Boyer JS. Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. *Plant Physiol.* 1970, **46**, 236-239.

Boyer JS. Cell enlargement and growth-induced water potentials. *Physiologia Plantarum.* 1988, **73**, 311-316.

Bradford KJ, Hsiao TC. Stomatal Behavior and Water Relations of Waterlogged Tomato Plants. *Plant Physiology.* 1982, **70**, 1508-1513.

Bräutigam K, Dietzel L, Kleine T, Stroher E, Wormuth D, Dietz KJ, Radke D, Wirtz M, Hell R, Dormann P, Nunes-Nesi A, Schauer N, Fernie AR, Oliver SN, Geigenberger P, Leister D, Pfannschmidt T. Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in Arabidopsis. *Plant Cell.* 2009, **21**, 2715-2732.

Bravdo B. Effect of cultural practices and environmental factors on fruit and wine quality. *Agriculturae Conspectus Scientificus.* 2001, **66**, 13-20.

Bray EA. Plant responses to water deficit. *Trends in Plant Science.* 1997, **2**, 48-54.

Browse J. Jasmonate passes muster: a receptor and targets for the defense hormone. *Annual Review of Plant Biology.* 2009, **60**, 183-205.

Bruce TJA, Matthes MC, Napier JA, Pickett JA. Stressful "memories" of plants: evidence and possible mechanisms. *Plant Science.* 2007, **173**, 603-608.

Buchanan BB, Holmgren A, Jacquot J-P, Scheibe R. Fifty years in the thioredoxin field and a bountiful harvest. *Biochimica et Biophysica Acta (BBA) - General Subjects.* 2012, **1820**, 1822-1829.

Buer CS, Muday GK, Djordjevic MA. Flavonoids are differentially taken up and transported long distances in Arabidopsis. *Plant Physiology.* 2007, **145**, 478-490.

Bünger-Kibler, S.; Bangerth, F. Relationship between cell number, cell size and fruit size of seeded fruits of tomato (Lycopersicon esculentum Mill.), and those induced parthenocarpically by the application of plant growth regulators. *Plant Growth Regulation*. 1983, **1**, 143-154.

Buttery RG, Ling LC, Light DM. Tomato leaf volatile aroma components. *Journal of Agricultural and Food Chemistry.* 1987, **35**, 1039-1042.

C

Capanoglu E. The potential of priming in food production. *Trends in Food Science & Technology.* 2010, **21**, 399-407.

Castellarin SD, Pfeiffer A, Sivilotti P, Degan M, Peterlunger E, Gaspero Gd. Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant, Cell and Environment.* 2007, **30**, 1381-1399.

Causse M, Buret M, Robini K, Verschave P. Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. *Journal of Food Science.* 2003, **68**, 2342-2350.

Causse M, Desplat N, Pascual L, Le Paslier M-C, Sauvage C, Bauchet G, Berard A, Bounon R, Tchoumakov M, Brunel D, Bouchet J-P. Whole genome resequencing in tomato reveals variation associated with introgression and breeding events. *BMC Genomics.* 2013, **14**, 791.

Cavanagh C., Morell M., Mackay I., Powell W., Biotechnology P., Leach E.J., Greenl A. From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Current Opinion in Plant Biology.* 2008, **11**, 215-221. DOI: 10.1016/j.pbi.2008.01.002.

Cazzonelli CI, Pogson BJ. Source to sink: regulation of carotenoid biosynthesis in plants. *Trends in Plant Science.* 2010, **15**, 266-274.

Centeno DC, Osorio S, Nunes-Nesi A, Bertolo ALF, Carneiro RT, Araujo WL, Steinhauser MC, Michalska J, Rohrmann J, Geigenberger P, Oliver SN, Stitt M, Carrari F, Rose JKC, Fernie AR. Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. *Plant Cell.* 2011, **23**, 162-184.

Cerovic ZG, Moise N, Agati G, Latouche G, Ben Ghozlen N, Meyer S. New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. *Journal of Food Composition and Analysis.* 2008, **21**, 650-654.

Chapman DM, Roby G, Ebeler SE, Guinard J-X, Matthews MA. Sensory attributes of Cabernet Sauvignon wines made from vines with different water status. *Australian Journal of Grape and Wine Research.* 2005, **11**, 339-347.

Chaves, M.M. Effects of Water Deficits on Carbon Assimilation. *Journal of Experimental Botany*. 1991, **42**, 1-16.

Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought - from genes to the whole plant. *Functional Plant Biology.* 2003, **30**, 239-264.

Chen P, McCarthy MJ, Kauten R. NMR for internal quality evaluation of fruits and vegetables. *Transactions of the ASAE.* 1989, **32**, 1747-1753.

Chen J, Kang S, Du T, Guo P, Qiu R, Chen R, Gu F. Modeling relations of tomato yield and fruit quality with water deficit at different growth stages under greenhouse condition. *Agricultural Water Management.* 2014, **146**, 131-148.

Christen D, Schönmann S, Jermini M, Strasser RJ, Défago G. Characterization and early detection of grapevine (Vitis vinifera) stress responses to esca disease by in situ chlorophyll fluorescence and comparison with drought stress. *Environmental and Experimental Botany.* 2007, **60**, 504-514.

Claeys H, Inze D. The agony of choice: how plants balance growth and survival under water-limiting conditions. *Plant Physiology.* 2013, **162**, 1768-1779.

Clement A, Dorais M, Vernon M. Multivariate approach to the measurement of tomato maturity and gustatory attributes and their rapid assessment by vis-NIR spectroscopy. *Journal of Agricultural and Food Chemistry.* 2008, **56**, 1538-1544.

Conrath U. Molecular aspects of defence priming. *Trends in Plant Science.* 2011, **16**, 524-531.

Copolovici L, Kannaste A, Pazouki L, Niinemets U. Emissions of green leaf volatiles and terpenoids from Solanum lycopersicum are quantitatively related to the severity of cold and heat shock treatments. *Journal of Plant Physiology.* 2012, **169**, 664-672.

Copolovici L, Niinemets U. Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. *Plant Cell Environ.* 2010, **33**, 1582-94.

Cosgrove DJ. Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *Plant Cell.* 1997, **9**, 1031-1041.

Costa, J.M.; Ortuno, M.F.; Chaves, M.M. Deficit irrigation as a strategy to save water: physiology and potential application to horticulture. *Journal of Integrative Plant Biology*. 2007, **49**, 1421-1434.

Coupel-Ledru A, Lebon É, Christophe A, Doligez A, Cabrera-Bosquet L, Péchier P, Hamard P, This P, Simonneau T. Genetic variation in a grapevine progeny (Vitis vinifera L. cvs Grenache×Syrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought. *Journal of Experimental Botany.* 2014, **65**, 6205-6218.

Craufurd PQ, Wheeler TR. Climate change and the flowering time of annual crops. *Journal of Experimental Botany.* 2009, **60**, 2529-2539.

Cristescu SM, De Martinis D, te Lintel Hekkert S, Parker DH, Harren FJM. Ethylene Production by Botrytis cinerea In Vitro and in Tomatoes. Applied and Environmental Microbiology. 2002, **68**, 5342-5350.

Csardi G., Nepusz T. 2006. The igraph software package for complex network research, *InterJournal Complex Systems.***1695**. http://igraph.org.

Cunningham FX. Regulation of carotenoid synthesis and accumulation in plants. *Pure and Applied Chemistry.* 2002, **74**, 1409-1417.

Curvers K, Seifi H, Mouille G, Rycke Rd, Asselbergh B, Hecke Av, Vanderschaeghe D, Hofte H, Callewaert N, Breusegem Fv, Hofte M. Abscisic acid deficiency causes changes in cuticle permeability and pectin composition that influence tomato resistance to Botrytis cinerea. *Plant Physiology.* 2010, **154**, 847-860.

D

Dai Z, Vivin P, Robert T, Milin S, Li S, Genard M. Model-based analysis of sugar accumulation in response to source-sink ratio and water supply in grape (*Vitis vinifera*) berries. *Functional Plant Biology.* 2009, **36**, 527-540.

Damour G, Vandame M, Urban L. Long-term drought results in a reversible decline in photosynthetic capacity in mango leaves, not just a decrease in stomatal conductance. *Tree Physiology.* 2009, **29**, 675-684.

Damour G, Simonneau T, Cochard H, Urban L. An overview of models of stomatal conductance at the leaf level. *Plant, Cell & Environment.* 2010, **33**, 1419-1438.

Danquah A, de Zelicourt A, Colcombet J, Hirt H. The role of ABA and MAPK signaling pathways in plant abiotic stress responses. *Biotechnology Advances.* 2014, **32**, 40-52.

Davies, W.J.; Bacon, M.A.; Stuart-Thompson, D.; Sobeih, W.; González-Rodríguez, L. Regulation of leaf and fruit growth in plants growing in drying soil: exploitation of the

plants' chemical signalling system and hydraulic architecture to increase the efficiency of water use in agriculture. *Journal of Experimental Botany*. 2000, **51**, 1617-1626.

Deborde C, Maucourt M, Baldet P, Bernillon S, Biais B, Talon G, Ferrand C, Jacob D, Ferry-Dumazet H, Daruvar A, Rolin D, Moing A. Proton NMR quantitative profiling for quality assessment of greenhouse-grown tomato fruit. *Metabolomics*. 2009, **5**, 183-198.

Deluc L, Quilici D, Decendit A, Grimplet J, Wheatley M, Schlauch K, Merillon J-M, Cushman J, Cramer G. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics*. 2009, **10**, 212.

De Oliveira GA, Bureau S, Renard CM-GC, Pereira-Netto AB, de Castilhos F. Comparison of NIRS approach for prediction of internal quality traits in three fruit species. *Food Chemistry*. 2014, **143**, 223-230.

De Pascale S, Maggio A, Fogliano V, Ambrosino P, Ritieni A. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. *Journal of Horticultural Science & Biotechnology*. 2001, **76**, 447-453.

De Pascale S, Martino A, Raimondi G, Maggio A. Comparative analysis of water and salt stress-induced modifications of quality parameters in cherry tomatoes. *Journal of Horticultural Science & Biotechnology*. 2007, **82**, 283-289.

Devoto A, Turner JG. Regulation of Jasmonate-mediated Plant Responses in Arabidopsis. *Annals of Botany*. 2003, **92**, 329-337.

Dicke M, Takabayashi J, Posthumus MA, Schutte C, Krips OE. Plant-phytoseiid interactions mediated by herbivore-induced plant volatiles: variation in production of cues and in responses of predatory mites. *Experimental & Applied Acarology*. 1998, **22**, 311-333.

Diretto G, Tavazza R, Welsch R, Pizzichini D, Mourgues F, Papacchioli V, Beyer P, Giuliano G. Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. *BMC Plant Biology*. 2006, **6**, 13.

Dixon RA, Achnine L, Kota P, Liu C-J, Reddy MSS, Wang L. The phenylpropanoid pathway and plant defence—a genomics perspective. *Molecular Plant Pathology*. 2002, **3**, 371-390.

Dong X, Hong Z, Chatterjee J, Kim S, Verma D. Expression of callose synthase genes and its connection with Npr1 signaling pathway during pathogen infection. *Planta*. 2008, **229**, 87-98.

Dulermo T, Rascle C, Chinnici G, Gout E, Bligny R, Cotton P. Dynamic carbon transfer during pathogenesis of sunflower by the necrotrophic fungus Botrytis cinerea: from plant hexoses to mannitol. *New Phytologist*. 2009, **183**, 1149-1162.

Dumas J.B.A. Procédés de l'analyse organique. *Annual Review of Physical Chemistry* (Paris). 1831, **2**, 198-213.**Dumville JC, Fry SC**. Solubilisation of tomato fruit pectins by ascorbate: a possible non-enzymic mechanism of fruit softening. *Planta.* 2003, **217**, 951-961.

Durán Zuazo VH, Rodriguez Pleguezuelo CR, Franco Tarifa D. Impact of sustained-deficit irrigation on tree growth, mineral nutrition, fruit yield and quality of mango in Spain. *Fruits (Paris)*. 2011, **66**, 257-268.

E

Easlon HM, Richards JH. Drought response in self-compatible species of tomato (Solanaceae). *American Journal of Botany.* 2009, **96**, 605-611.

Ebel RC, Mattheis JP, Buchanan DA. Drought stress of apple trees alters leaf emissions of volatile compounds. *Physiologia Plantarum.* 1995, **93**, 709-712.

El-Oirdi M, El-Rahman TA, Rigano L, El-Hadrami A, Rodriguez MC, Daayf F, Vojnov A, Bouarab K. Botrytis cinerea manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *Plant Cell*. 2011, **23**, 2405-2421.

El-Soda M, Boer MP, Bagheri H, Hanhart CJ, Koornneef M, Aarts MGM. Genotype–environment interactions affecting preflowering physiological and morphological traits of Brassica rapa grown in two watering regimes. *Journal of Experimental Botany.* 2014, **65**, 697-708.

Essmann J, Schmitz-Thom I, Schoen H, Sonnewald S, Weis E, Scharte J. RNA interference-mediated repression of cell wall invertase impairs defense in source leaves of tobacco. *Plant Physiology*. 2008, **147**, 1288-1299.

Esteban MA, Villanueva MJ, Lissarrague JR. Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (Vitis vinifera L) grape berries during ripening. *Journal of the Science of Food and Agriculture.* 2001, **81**, 409-420.

Etienne A, Genard M, Lobit P, Mbeguie-A-Mbeguie D, Bugaud C. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *Journal of Experimental Botany.* 2013, **64**, 1451-1469.

F

Fanciullino AL, Bidel LPR, Urban L. Carotenoid responses to environmental stimuli: integrating redox and carbon controls into a fruit model. *Plant, Cell & Environment*, 2014, **37**, 273-289.

Farag MA, Pare PW. C6-Green leaf volatiles trigger local and systemic VOC emissions in tomato. *Phytochemistry.* 2002, **61**, 545-54.

Favati F, Lovelli S, Galgano F, Miccolis V, Di Tommaso T, Candido V. Processing tomato quality as affected by irrigation scheduling. *scientia Horticulturae.* 2009, **122**, 562-571.

Fazio G, Stevens MR, Scott JW. Identification of RAPD markers linked to Fusarium crown and root rot resistance (Frl) in tomato. *Euphytica.* 1999, **105**, 205-210.

Feild TS, Nedbal L, Ort DR. 1998. Nonphotochemical reduction of the plastoquinone pool in sunflower leaves originates from chlororespiration. *Plant Physiol* **116**, 1209-1218.

Ferri M, Righetti L, Tassoni A. Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. *Journal of Plant Physiology.* 2011, **168**, 189-195.

Filippou P, Tanou G, Molassiotis A, Fotopoulos V. Plant Acclimation to Environmental Stress Using Priming Agents. In: N Tuteja and S Singh Gill, eds. *Plant Acclimation to Environmental Stress*: Springer New York, 2013, 1-27.

Fischer I, Camus-Kulandaivelu L, Allal F, Stephan W. Adaptation to drought in two wild tomato species: the evolution of the Asr gene family. *New Phytologist.* 2011, **190**, 1032-1044.

Floryszak-Wieczorek J, Arasimowicz-Jelonek M, Milczarek G, Janus L, Pawlak-Sprada S, Abramowski D, Deckert J, Billert H. Nitric Oxide–Mediated Stress Imprint in Potato as an Effect of Exposure to a Priming Agent. *Molecular Plant-Microbe Interactions.* 2012, **25**, 1469-1477.

Flors V, Ton J, Jakab G, Mauch-Mani B. Abscisic Acid and Callose: Team Players in Defence Against Pathogens? *Journal of Phytopathology.* 2005, **153**, 377-383.

Foolad M. Current Status Of Breeding Tomatoes For Salt And Drought Tolerance. In: M Jenks, et al., eds. *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops*: Springer Netherlands, 2007, 669-700.

Foolad MR, Zhang LP, Subbiah P. Genetics of drought tolerance during seed germination in tomato: inheritance and QTL mapping. *Genome.* 2003, **46**, 536-545.

Foolad MR, Lin GY. Relationships between cold- and salt-tolerance during seed germination in tomato: germplasm evaluation. *Plant Breeding.* 1999, **118**, 45-48.

Fox J, Weisberg S. An R Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Boots/Companion. 2011.

Fraser PD, Enfissi EMA, Halket JM, Truesdale MR, Yu DM, Gerrish C, Bramley PM. Manipulation of phytoene levels in tomato fruit: Effects on isoprenoids, plastids, and intermediary metabolism (vol 19, pg 3194, 2007). *Plant Cell.* 2007, **19**, 4131-4132.

Fray RG, Wallace A, Grierson D, Lycett GW. Nucleotide sequence and expression of a ripening and water stress-related cDNA from tomato with homology to the MIP class of membrane channel proteins. *Plant Molecular Biology.* 1994, **24**, 539-543.

Fritz C, Palacios-Rojas N, Feil R, Stitt M. Regulation of secondary metabolism by the carbon-nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. *Plant Journal*. 2006, **46**, 533-548.

Fry SC, Dumville JC, Miller JG. Fingerprinting of polysaccharides attacked by hydroxyl radicals in vitro and in the cell walls of ripening pear fruit. *Biochemical Journal.* 2001, **357**, 729-737.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology.* 2006, **9**, 436-442.

G

Gao X, Giorgi F. Increased aridity in the Mediterranean region under greenhouse gas forcing estimated from high resolution simulations with a regional climate model. *Global and Planetary Change.* 2008, **62**, 195-209.

Garchery C, Gest N, Do PT, Alhagdow M, Baldet P, Menard G, Rothan C, Massot C, Gautier H, Aarrouf J, Fernie AR, Stevens R. A diminution in ascorbate oxidase activity affects carbon allocation and improves yield in tomato under water deficit. *Plant, Cell & Environment.* 2013, **36**, 159-175.

García, A.L.; Marcelis, L. ; Garcia-Sanchez, F.; Nicolas, N.; Martínez, V. Moderate water stress affects tomato leaf water relations in dependence on the nitrogen supply. *Biologia Plantarum*. 2007, **51**, 707-712.

García-Tejero I, Romero-Vicente R, Jimenez-Bocanegra JA, Martínez-García G, Durán-Zuazo VH, Muriel-Fernández JL. Response of citrus trees to deficit irrigation during different phenological periods in relation to yield, fruit quality, and water productivity. *Agricultural Water Management.* 2010, **97**, 689-699.

Gautier H, Massot C, Stevens R, Serino S, Genard M. Regulation of tomato fruit ascorbate content is more highly dependent on fruit irradiance than leaf irradiance. *Annals of Botany.* 2009, **103**, 495-504.

Geissmann M, Frey T, Ruffner HP. Occurrence and properties of acid invertase in cultures of Botrytis cinerea. *Mycological Research*. 1991, **95**, 1321-1327.

Génard M, Bertin N, Borel C, Bussières P, Gautier H, Habib R, Léchaudel M, Lecomte A, Lescourret F, Lobit P, Quilot B. Towards a virtual fruit focusing on quality: modelling features and potential uses. *Journal of Experimental Botany.* 2007, **58**, 917-928.

Gibson SI. Control of plant development and gene expression by sugar signaling. *Current Opinion in Plant Biology.* 2005, **8**, 93-102.

Gillaspy, G.; Ben-David, H.; Gruissem, W. Fruits: a developmental perspectives. *The Plant Cell*. 1993, **5**, 1439-1451.

Gillet C, Voué M, Cambier P. Site-specific counterion binding and pectic chains conformational transitions in the Nitella cell wall. *Journal of Experimental Botany.* 1998, **49**, 797-805.

Giraud C., Huet S., Verzelem N. Graph selection with GGMselect. http://fr.arxiv.org/abs/0907.0619. 2009.

Giraudoux, P. Pgirmess: Data analysis in ecology. R package version 1.5.9. 2014.

Girona J, Marsal J, Mata M, Arbones A, Dejong TM. A comparison of the combined effect of water stress and crop load on fruit growth during different phenological stages in young peach trees. *Journal of Horticultural Science and Biotechnology.* 2004, **79**, 308-315.

Gladden LA, Wang Y, Hsieh C, Tsou I. Using deficit irrigation approach for evaluating the effects of water restriction on field grown tomato (Lycopersicon esculentum). *African Journal of Agricultural Research.* 2012, **7**, 2083-2095.

Gollotte A, Secco B, Mercy L, Lemoine MC, Durand P, Prost M, Gianinazzi S. Raspberry breeding and biotisation for increasing plant stress tolerance and antioxidant activity. In: E Kruger, et al., eds. *Acta Horticulturae*. Leuven, Belgium: International Society for Horticultural Science (ISHS), 2009, 145-150.

Goltsev V, Zaharieva I, Chernev P, Kouzmanova M, Kalaji HM, Yordanov I, Krasteva V, Alexandrov V, Stefanov D, Allakhverdiev SI, Strasser RJ. Drought-induced modifications of photosynthetic electron transport in intact leaves: Analysis and use of neural networks as a tool for a rapid non-invasive estimation. *Biochimica et Biophysica Acta (BBA) - Bioenergetics.* 2012, **1817**, 1490-1498.

Gomes MdMdA, Lagôa AMMA, Medina CL, Machado EC, Machado MA. Interactions between leaf water potential, stomatal conductance and abscisic acid content of orange

trees submitted to drought stress. *Brazilian Journal of Plant Physiology*. 2004, **16**, 155-161.

Gomez AH, He Y, Pereira AG. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis/NIR-spectroscopy techniques. *Journal of Food Engineering*. 2006, **77**, 313-319.

Gomez L, Bancel D, Rubio E, Vercambre G. The microplate reader: an efficient tool for the separate enzymatic analysis of sugars in plant tissues - validation of a micro-method. *Journal of the Science of Food and Agriculture*. 2007, **87**, 1893-1905.

Gomez, L.; Rubio, E.; Auge, M. A new procedure for extraction and measurement of soluble sugars in ligneous plants. *Journal of the Science and Food Agriculture*. 2002, **82,** 360-369.

Gong P, Zhang J, Li H, Yang C, Zhang C, Zhang X, Khurram Z, Zhang Y, Wang TY, Fei Z, Ye Z. Transcriptional profiles of drought-responsive genes in modulating transcription signal transduction, and biochemical pathways in tomato. *Journal of Experimental Botany*. 2010, **61**, 3563-3575.

Goodwin, T.W. Biosynthesis of carotenoids and plant triterpenes. *Biochemical Journal* . 1971, **123,** 293-329.

Gorovits R, Czosnek H. Biotic and Abiotic Stress Responses in Tomato Breeding Lines Resistant and Susceptible to Tomato Yellow Leaf Curl Virus. In: H Czosnek, ed. *Tomato Yellow Leaf Curl Virus Disease*: Springer Netherlands, 2007, 223-237.

Gouinguene SP, Turlings TCJ. The effects of abiotic factors on induced volatile emissions in maize plants. *Plant Physiology*. 2002, **129**, 1296-1307.

Granot D, David-Schwartz R, Kelly G. Hexose kinases and their role in sugar-sensing and plant development. *Frontiers in Plant Science*. 2013, **4**.

Grassmann J, Hippeli S, Elstner EF. Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiology and Biochemistry*. 2002, **40**, 471-478.

Graves S., Piepho H.-P., Selzer L. with the help from Dorai-Raj S. mulcompView: Visualizations of Paired Comparisons. R package version 0.1-5. http://CRAN.R-project.org/package=mulcompView. 2012.

Grinnan R, Carter TE, Jr., Johnson MTJ. Effects of drought, temperature, herbivory, and genotype on plant-insect interactions in soybean (Glycine max). *Arthropod - Plant Interactions*. 2013, **7**, 201-215.

Gucci R, Lodolini EM, Rapoport HF. Water deficit-induced changes in mesocarp cellular processes and the relationship between mesocarp and endocarp during olive fruit development. *Tree Physiology*. 2009, **29**, 1575-1585.

Guichard S, Bertin N, Leonardi C, Gary C. Tomato fruit quality in relation to water and carbon fluxes. *Agronomie*. 2001, 385-392.

Guichard S, Gary C, Leonardi C, Bertin N. Analysis of growth and water relations of tomato fruits in relation to air vapor pressure deficit and plant fruit load. *Journal of Plant Growth Regulation*. 2005, **24**, 201-213.

Gur A, Zamir D. Unused natural variation can lift yield barriers in plant breeding. *PLoS Biology*. 2004, **2**, e245.

Guri AJ, Hontecillas R, Si H, Liu D, Bassaganya-Riera J. Dietary abscisic acid ameliorates glucose tolerance and obesity-related inflammation in db/db mice fed high-fat diets. *Clinical Nutrition.* 2007, **26**, 107-116.

Guri AJ, Misyak SA, Hontecillas R, Hasty A, Liu D, Si H, Bassaganya-Riera J. Abscisic acid ameliorates atherosclerosis by suppressing macrophage and $CD4^+$ T cell recruitment into the aortic wall. *The Journal of nutritional biochemistry.* 2010, **21**, 1178-1185.

Gutbrodt B, Dorn S, Mody K. Drought stress affects constitutive but not induced herbivore resistance in apple plants. *Arthropod - Plant Interactions.* 2012, **6**, 171-179.

H

Hamon, W.R. *Computation of Direct Runoff Amounts from Storm Rainfall*: International Association of Scientific Hydrology Publication. 1963, **63,** 52-62.

Harker FR, Redgwell RJ, Hallett IC, Murray SH, Carter G. Texture of fresh fruit. *Horticultural Reviews.* 1997, **20**, 121-224.

Harrell F.E. Jr. with contributions from Dupont C. and many others. Hmisc: Harrell Miscellaneous. R package version 3.14-6. http://CRAN.R-project.org/package=Hmisc. 2014.

Harren FJM, Cristescu SM. Online, real-time detection of volatile emissions from plant tissue. *AoB Plants.* 2013, **5**, 1-18.

He JX, Wang J, Liang HG. Effects of water stress on photochemical function and protein metabolism of photosystem II in wheat leaves. *Physiologia Plantarum.* 1995, **93**, 771-777.

Herbers K, Meuwly P, Frommer WB, Metraux JP, Sonnewald U. Systemic acquired resistance mediated by the ectopic expression of invertase: Possible hexose sensing in the secretory pathway. *Plant Cell*. 1996, **8**, 793-803.

Herms DA, Mattson WJ. The dilemma of plants - To grow of defend. *Quarterly Review of Biology.* 1992, **67**, 283-335.

Hetherington AM, Woodward FI. The role of stomata in sensing and driving environmental change. Nature. 2003, **424**, 901-908.

Higashi K, Hosoya K, Ezura H. Histological analysis of fruit development between two melon (Cucumis melo L. reticulatus) genotypes setting a different size of fruit. *Journal of Experimental Botany.* 1999, **50**, 1593-1597.

Hnilickova H, Duffek J. The effect of water deficit and subsequent regeneration on selected physiological characteristics in tomatoes (Lycopersicum esculentum Mill.). *Scientia Agriculturae Bohemica.* 2004, **35**, 26-31.

Hnilickova H, Duffek J, Hnilicka F. Effects of low temperatures on photosynthesis and growth in selected tomato varieties (Lycopersicon esculentum Mill.). *Scientia Agriculturae Bohemica.* 2002, **33**, 101-105.

Ho, L.C. Metabolism and Compartmentation of Imported Sugars in Sink Organs in Relation to Sink Strength. *Annual Review of Plant Physiology and Plant Molecular Biology.* 1988**, 39**, 355-378.

Ho LC. The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. *Journal of Experimental Botany.* 1996a, **47**, 1239-1243.

Ho LC. *Tomato*: New York: Marcel Dekker Inc. 1996b.

Hockema BR, Etxeberria E. Metabolic contributors to drought-enhanced accumulation of sugars and acids in oranges. *Journal of the American Society for Horticultural Science.* 2001, **126**, 599-605.

Holopainen JK, Heijari J, Nerg AM, Vuorinen M, Kainulainen P. Potential for the use of exogenous chemical elicitors in disease and insect pest management of conifer seedling production. *Open Forest Science Journal.* 2009, **2**, 17-24.

Hooijdonk BMv, Dorji K, Behboudian MH. Fruit quality of 'Pacific Rose' TM apple grown under partial rootzone drying and deficit irrigation. *Journal of Food, Agriculture & Environment.* 2007, **5**, 173-178.

Horsfall G, Dimond AE. Interactions of tissue sugar, growth substances and disease susceptibility. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*. 1957, **64**, 415-421.

Howles PA, Sewalt V, Paiva NL, Elkind Y, Bate NJ, Lamb C, Dixon RA. Overexpression of L-Phenylalanine Ammonia-Lyase in Transgenic Tobacco Plants Reveals Control Points for Flux into Phenylpropanoid Biosynthesis. *Plant Physiology.* 1996, **112**, 1617-1624.

Huanosto Magaña R, Adamowicz S, Pagès L. Diel changes in nitrogen and carbon resource status and use for growth in young plants of tomato (Solanum lycopersicum). *Annals of Botany*. 2009, **103**, 1025-1037.

Hummel I, Pantin F, Sulpice R, Piques M, Rolland G, Dauzat M, Christophe A, Pervent M, Bouteille M, Stitt M, Gibon Y, Muller B. Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiology.* 2010, **154**, 357-372.

Husson F., Josse J., Le S. and Mazet J. FactoMineR : Multivariate Exploratory Data Analysis and Data Mining with R. R package version 1.26. http://CRAN.R-project.org/package=FactoMineR. 2014.

I

Iglesias DJ, Tadeo FR, Legaz F, Primo-Millo E, Talon M. In vivo sucrose stimulation of colour change in citrus fruit epicarps: Interactions between nutritional and hormonal signals. *Physiologia Plantarum*. 2001, **112**, 244-250.

J

Jackman RL, Gibson HJ, Stanley DW. Effects of chilling on tomato fruit texture. *Physiologia Plantarum.* 1992, **86**, 600-608.

James DG. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Journal of Chemical Ecology.* 2005, **31**, 481-495.

Jansen RMC, Hofstee JW, Wildt J, Vanthoor BHE, Verstappen FWA, Takayama K, Bouwmeester HJ, Henten EJv. Health monitoring of plants by their emitted volatiles: a model to predict the effect of Botrytis cinerea on the concentration of volatiles in a large-scale greenhouse. *Biosystems Engineering.* 2010, **106**, 37-47.

Jobic C, Boisson A-M, Gout E, Rascle C, Fevre M, Cotton P, Bligny R. Metabolic processes and carbon nutrient exchanges between host and pathogen sustain the disease development during sunflower infection by Sclerotinia sclerotiorum. *Planta*. 2007, **226**, 251-265.

Johnson G.N. Physiology of PSI cyclic electron transport in higher plants. Biochimica et Biophysica Acta (BBA) - *Bioenergetics.* 2011*,* **1807**, 384-389.

Johnson GN. Cyclic electron transport in C3 plants: fact or artefact? *Journal of Experimental Botany.* 2005*,* **56**, 407-416.

Jouquet P, Capowiez Y, Bottinelli N, Traoré S. Potential of Near Infrared Reflectance Spectroscopy (NIRS) for identifying termite species. *European Journal of Soil Biology.* 2014, **60**, 49-52.

K

Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, Brestic M, Bussotti F, Calatayud A, Dąbrowski P, Elsheery NI, Ferroni L, Guidi L, Hogewoning SW, Jajoo A, Misra AN, Nebauer SG, Pancaldi S, Penella C, Poli D, Pollastrini M, Romanowska-Duda ZB, Rutkowska B, Serôdio J, Suresh K, Szulc W, Tambussi E, Yanniccari M, Zivcak M. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research.* 2014, **122**, 121-158.

Kant MR, Ament K, Sabelis MW, Haring MA, Schuurink RC. Differential Timing of Spider Mite-Induced Direct and Indirect Defenses in Tomato Plants. *Plant Physiology.* 2004, **135**, 483-495.

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P. Systemic Signaling and Acclimation in Response to Excess Excitation Energy in *Arabidopsis*. *Science.* 1999, **284**, 654-657.

Keutgen AJ, Pawelzik E. Modifications of strawberry fruit antioxidant pools and fruit quality under NaCl stress. *Journal of agricultural and food chemistry.* 2007, **55**, 4066-4072.

Kim J-Y, Mahé A, Brangeon J, Prioul J-L. A Maize Vacuolar Invertase, IVR2, Is Induced by Water Stress. Organ/Tissue Specificity and Diurnal Modulation of Expression. *Plant Physiology*. 2000, **124**, 71-84.

Kim, S.H.; Jeong, J.C.; Park, S.; Bae, J.Y.; Ahn, M.J.; Lee, H.S.; Kwak, S.S. Down-regulation of sweetpotato lycopene beta-cyclase gene enhances tolerance to abiotic stress in transgenic calli. *Molecular Biology Reports.* 2014, **12,** 1-12.

Knee M. Properties of polygalacturonate and cell cohesion in apple fruit cortical tissue. *Phytochemistry.* 1978, **17**, 1257-1260.

Kobashi K, Sugaya S, Gemma H, Iwahori S. Effect of abscisic acid (ABA) on sugar accumulation in the flesh tissue of peach fruit at the start of the maturation stage. *Plant Growth Regulation.* 2001, **35**, 215-223.

Kocal N, Sonnewald U, Sonnewald S. Cell Wall-Bound Invertase Limits Sucrose Export and Is Involved in Symptom Development and Inhibition of Photosynthesis during Compatible Interaction between Tomato and Xanthomonas campestris pv vesicatoria. *Plant Physiology*. 2008, **148**, 1523-1536.

Koch K. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology.* 2004, **7**, 235-46.

Koricheva J. Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. *Oecologia.* 1999, **119**, 467-473.

Kornberg MD, Sen N, Hara MR, Juluri KR, Nguyen JVK, Snowman AM, Law L, Hester LD, Snyder SH. GAPDH mediates nitrosylation of nuclear proteins. *Nat Cell Biol.* 2010, **12**, 1094-1100.

Koundouras S, Hatzidimitriou E, Karamolegkou M, Dimopoulou E, Kallithraka S, Tsialtas JT, Zioziou E, Nikolaou N, Kotseridis Y. Irrigation and rootstock effects on the phenolic concentration and aroma potential of Vitis vinifera L. cv. cabernet sauvignon grapes. *J Agric Food Chem.* 2009, **57**, 7805-13.

Kranner I, Kastberger G, Hartbauer M, Pritchard HW. Noninvasive diagnosis of seed viability using infrared thermography. *Proceedings of the National Academy of Sciences of the United States of America.* 2010, **107**, 3912-3917.

Krause GH, Weis E. Chlorophyll Fluorescence and Photosynthesis: The Basics. *Annual Review of Plant Physiology and Plant Molecular Biology.* 1991, **42**, 313-349.

Krauss S, Schnitzler WH, Grassmann J, Woitke M. The influence of different electrical conductivity values in a simplified recirculating soilless system on inner and outer fruit quality characteristics of tomato. *Journal of agricultural and food chemistry.* 2006, **54**, 441-448.

Krumbein A, Schwarz D, Klaring HP. Effects of environmental factors on carotenoid content in tomato (Lycopersicon esculentam (L.) Mill.) grown in a greenhouse. *Journal of Applied Botany and Food Quality-Angewandte Botanik.* 2006, **80**, 160-164.

Kulkarni M, Deshpande U. In Vitro screening of tomato genotypes for drought resistance using polyethylene glycol. *African Journal of Biotechnology.* 2007, **6**, 691-696.

Kunert M, Biedermann A, Koch T, Boland W. Ultrafast sampling and analysis of plant volatiles by a hand-held miniaturised GC with pre-concentration unit: kinetic and quantitative aspects of plant volatile production. *Journal of Separation Science.* 2002, **25**, 677-684.

L

Labate JA, Grandillo S, Fulton T, Munos S, Caicedo AL, Peralta I, Ji YF, Chetelat RT, Scott JW, Gonzalo MJ, Francis D, Yang WC, Knaap Evd, Baldo AM, Smith-White B, Mueller LA, Prince JP, Blanchard NE, Storey DB, Stevens MR, Robbins MD, Wang JF, Liedl BE, O'Connell MA, Stommel JR, Aoki K. *Tomato*. Heidelberg, Germany: Springer-Verlag GmbH. 2007.

Laercio; J.S. Laercio: Duncan test, Tukey test and Scott-Knott test. R package version 1.0-1. 2010.

Laothawornkitkul J, Moore JP, Taylor JE, Possell M, Gibson TD, Hewitt CN, Paul ND. Discrimination of plant volatile signatures by an electronic nose: a potential technology for plant pest and disease monitoring. *Environmental Science & Technology*. 2008, **42**, 8433-8439.

Le Bot J, Benard C, Robin C, Bourgaud F, Adamowicz S. The 'trade-off' between synthesis of primary and secondary compounds in young tomato leaves is altered by nitrate nutrition: experimental evidence and model consistency. *Journal of Experimental Botany*. 2009, **60**, 4301-4314.

Lechaudel, M. Croissance et qualité organoleptique de la mangue (Mangifera indica): analyse expérimentale et modélisation de l'effet de la disponibilité hydrique et carbonée, Institut National Agronomique Paris Grignon, Centre de Coopération Internationale en Recherche Agronomique pour le Développement. 2004, 150 p.

Lechaudel M, Genard M, Lescourret F, Urban L, Jannoyer M. Modeling effects of weather and source-sink relationships on mango fruit growth. *Tree Physiology*. 2005, **25**, 583-597.

Lechaudel M, Urban L, Joas J. Chlorophyll fluorescence, a nondestructive method to assess maturity of mango fruits (cv. 'Cogshall') without growth conditions bias. *Journal of Agricultural and Food Chemistry*. 2010, **58**, 7532-7538.

Lecompte F, Abro MA, Nicot P. Contrasted responses of Botrytis cinerea strains developing on tomato plants grown under different nitrogen nutrition regimes. *Plant Pathology*. 2010, **59**, 891-899.

Lecompte F, Abro MA, Nicot PC. Can plant sugars mediate the effect of nitrogen fertilization on lettuce susceptibility to two necrotrophic pathogens: Botrytis cinerea and Sclerotinia sclerotiorum? *Plant and Soil*. 2013, **369**, 387-401

Lenth, R.V. Lsmeans: Least-Squares Means. R package version 2.0.5. 2014.

Li SH, Huguet JG, Schoch PG, Orlando P. Response of peach tree growth and cropping to soil water deficit at various phenological stages of fruit development. *Journal of Horticultural Science*. 1989, **64**, 541-552.

Li X, Zhang L. SA and PEG-Induced Priming for Water Stress Tolerance in Rice Seedling. In: E Zhu and S Sambath, eds. *Information Technology and Agricultural Engineering*, Vol. 134: Springer Berlin Heidelberg, 2012, 881-887.

Libik-Konieczny M, Surowka E, Nosek M, Goraj S, Miszalski Z. Pathogen-induced changes in malate content and NADP-dependent malic enzyme activity in C3 or CAM performing *Mesembryanthemum crystallinum* L. plants. *Acta Physiologiae Plantarum*. 2012, **34**, 1471-1477.

Lillo C, Lea US, Ruoff P. Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. *Plant, Cell & Environment*. 2008, **31**, 587-601.

Liu H, Genard M, Guichard S, Bertin N. Model-assisted analysis of tomato fruit growth in relation to carbon and water fluxes. *Journal of Experimental Botany*. 2007, **58**, 3567-3580.

Livingston AK, Cruz JA, Kohzuma K, Dhingra A, Kramer DM. An Arabidopsis Mutant with High Cyclic Electron Flow around Photosystem I (hcef) Involving the NADPH Dehydrogenase Complex. *The Plant Cell Online.* 2010, **22**, 221-233.

Lo Bianco R, Rieger M, Sung S-JS. Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. *Physiologia Plantarum.* 2000, **108**, 71-78.

Lockhart J. ed. *Cell extension*: Ed. J. Bomaer & J. E. Varner. Academic Press, New York9, 1965, pp. 827-849.

Loomis WE. Growth-differentiation balance vs. carbohydrate nitrogen ratio. *Proceedings of the American Society for Horticultural Science.* 1932, **29**, 240-245.

Lopez A, Zon K, Dussi MC, Reeb P, Giardina G, Leskovar M, Flores L. Economic evaluation between chemical thinning vs. hand thinning in 'Williams' pear. In: EE Sanchez, et al., eds. *Acta Horticulturae*. Leuven, Belgium: International Society for Horticultural Science (ISHS), 2011, 29-37.

Loreto F, Schnitzler JP. Abiotic stresses and induced BVOCs. *Trends in Plant Science.* 2010, **15**, 154-166.

Lovisolo C, Perrone I, Carra A, Ferrandino A, Flexas J, Medrano H, Schubert A. Drought-induced changes in development and function of grapevine (Vitis spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. *Functional Plant Biology*. 2010, **37**, 98-116.

Luis A, Sandalio LM, Corpas FJ, Palma JM, Barroso JB. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. *Plant Physiology.* 2006, **141**, 330-335.

M

Magwaza LS, Opara UL, Terry LA, Landahl S, Cronje PJR, Nieuwoudt HH, Hanssens A, Saeys W, Nicolaï BM. Evaluation of Fourier transform-NIR spectroscopy for integrated external and internal quality assessment of Valencia oranges. *Journal of Food Composition and Analysis.* 2013, **31**, 144-154.

Mahdid M., Kameli A., Ehlert C., Simonneau T. Rapid changes in leaf elongation, ABA and water status during the recovery phase following application of water stress in two durum wheat varieties differing in drought tolerance. *Plant Physiology and Biochemistry.* 2011, **49**, 1077-1083. DOI: 10.1016/j.plaphy.2011.08.002.

Mäkelä P, Kärkkäinen J, Somersalo S. Effect of Glycinebetaine on Chloroplast Ultrastructure, Chlorophyll and Protein Content, and RuBPCO Activities in Tomato Grown under Drought or Salinity. *Biologia Plantarum.* 2000*,* **43**, 471-475.

Manaa A, Ahmed HB, Valot B, Bouchet JP, Aschi-Smiti S, Causse M, Faurobert M. Salt and genotype impact on plant physiology and root proteome variations in tomato. *Journal of Experimental Botany.* 2011, **62**, 2797-2813.

Marin A, Rubio JS, Martinez V, Gil MI. Antioxidant compounds in green and red peppers as affected by irrigation frequency, salinity and nutrient solution composition. *Journal of the Science of Food and Agriculture.* 2009, **89**, 1352-1359.

Marjanovic, M.; Stikic, R.; Vucelic-Radovic, B.; Savic, S.; Jovanovic, Z.; Bertin, N.; Faurobert, M. Growth and proteomic analysis of tomato fruit under partial root-zone drying. *OMICS A Journal of Integrative Biology*. 2012, **16**, 343-356.

Marsal J, Rapoport HF, Manrique T, Girona J. Pear fruit growth under regulated deficit irrigation in container-grown trees. *Scientia Horticulturae.* 2000, **85**, 243-259.

Martre P, He J, Gouis Jl, Allard V, Gaju O, Orford S, Heumez H, Griffiths S, Snape JW, Foulkes MJ, Semenov M. A system approach to wheat phenology: simulation of environmental and genetic variations of leaf number and anthesis date. *Aspects of Applied Biology*. 2011, 9-18.

Mateos RM, Leon AM, Sandalio LM, Gomez M, del Rio LA, Palma JM. Peroxisomes from pepper fruits (Capsicum annuum L.): purification, characterisation and antioxidant activity. *J Plant Physiol.* 2003, **160**, 1507-16.

Matthews MA, Anderson MM. Fruit ripening in Vitis vinifera L.: Responses to seasonal water deficits. *American Journal of Enology and Viticulture.* 1988, **39**, 313-320.

Matthews MA, Anderson MM, Schultz HR. Phenological and growth responses to early and late season water deficits in Cabernet franc. *Vitis.* 1987, **26**, 147-160.

McCarthy M, Loveys B, Dry P, Stoll M. Regulated deficit irrigation and partial rootzone drying as irrigation management techniques for grapevines. FAO Water Reports, 2002, **22**, 79–87.

McCormick AC, Unsicker SB, Gershenzon J. The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends Plant Sci.* 2012, **17**, 303-10.

Mehinagic E, Royer G, Bertrand D, Symoneaux R, Laurens F, Jourjon F. Relationship between sensory analysis, penetrometry and visible– NIR spectroscopy of apples belonging to different cultivars. *Food Quality and Preference.* 2003, **14**, 473-484.

Mengiste T, Chen X, Salmeron J, Dietrich R. The BOTRYTIS SUSCEPTIBLE1 Gene Encodes an R2R3MYB Transcription Factor Protein That Is Required for Biotic and Abiotic Stress Responses in Arabidopsis. *The Plant Cell Online.* 2003, **15**, 2551-2565.

Mercier V, Bussi C, Lescourret F, Genard M. Effects of different irrigation regimes applied during the final stage of rapid growth on an early maturing peach cultivar. *Irrigation Science.* 2009, **27**, 297-306.

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling.* 2009, **2**, 45.

Miller SA, Smith GS, Boldingh HL, Johansson A. Effects of water stress on fruit quality attributes of kiwifruit. *Annals of Botany.* 1998, **81**, 73-81.

Mingo DM, Bacon MA, Davies WJ. Non-hydraulic regulation of fruit growth in tomato plants (Lycopersicon esculentum cv. Solairo) growing in drying soil. *Journal of Experimental Botany.* 2003, **54**, 1205-1212.

Mirás-Avalos JM, Alcobendas R, Alarcón JJ, Valsesia P, Génard M, Nicolás E. Assessment of the water stress effects on peach fruit quality and size using a fruit tree model, QualiTree. *Agricultural Water Management.* 2013, **128**, 1-12.

Mitchell JP, Shennan C, Grattan SR, May DM. Tomato fruit yields and quality under water deficit and salinity. *Journal of the American Society for Horticultural Science.* 1991, **116**, 215-221.

Mitchell, J.P.; Shennan, C.; Grattan, S.R. Developmental changes in tomato fruit composition in response to water deficit and salinity. *Physiologia Plantarum*. 1991, **83**, 177-185.

Mittelstrass K, Treutter D, Plessl M, Heller W, Elstner EF, Heiser I. Modification of primary and secondary metabolism of potato plants by nitrogen application differentially affects resistance to Phytophthora infestans and Alternaria solani. *Plant Biology*. 2006, **8**, 653-661.

Mittler R. Abiotic stress, the field environment and stress combination. *Trends in Plant Science.* 2006, **11**, 15-19.

Mittler R, Blumwald E. Genetic Engineering for Modern Agriculture: Challenges and Perspectives. *Annual Review of Plant Biology.* 2010, **61**, 443-462.

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Van Breusegem F. ROS signaling: the new wave? *Trends in plant science.* 2011, **16**, 300-309.

Modise D, Wright C, Atherton J. Changes in strawberry aroma in response to water stress. *Bost. J Agric Appl Sci.* 2006, 2.

Molinier J, Ries G, Zipfel C, Hohn B. Transgeneration memory of stress in plants. *Nature (London).* 2006, **442**, 1046-1049.

Moreno, J.C.; Pizarro, L.; Fuentes, P.; Handford, M.; Cifuentes, V.; Stange, C. Levels of Lycopene β-Cyclase 1 Modulate Carotenoid Gene Expression and Accumulation in *Daucus carota*. *PLoS ONE*. 2013, **8,** e58144.

Morigasaki S, Shimada K, Ikner A, Yanagida M, Shiozaki K. Glycolytic enzyme GAPDH promotes peroxide stress signaling through multistep phosphorelay to a MAPK cascade. *Mol Cell.* 2008, **30**, 108-13.

Mpelasoka B, Behboudian MH, Dixont J, Neal S, Caspari HW. Improvement of fruit quality and storage potential of 'Braeburn' apple through deficit irrigation. *Journal of Horticultural Science & Biotechnology.* 2000, **75**, 615-621.

Mpelasoka BS, Behboudian M.H. Production of aroma volatiles in response to deficit irrigation and to crop load in relation to fruit maturity for 'Braeburn' apple. *Postharvest Biology and Technology.* 2002, **24**, 1-11.

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M, Gibon Y. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany.* 2011, **62**, 1715-1729.

Münch E. Die Stoffbewegungen in der Pflanze. Verlag von Gustav Fischer, Jena. 1930, **234**.

Muñoz-Bertomeu J, Cascales-Miñana B, Irles-Segura A, Mateu I, Nunes-Nesi A, Fernie AR, Segura J, Ros R. The Plastidial Glyceraldehyde-3-Phosphate Dehydrogenase Is Critical for Viable Pollen Development in Arabidopsis. *Plant Physiology.* 2010, **152**, 1830-1841.

Mur LAJ, Prats E, Pierre S, Hall MA, Hebelstrup KH. Integrating nitric oxide into salicylic acid and jasmonic acid/ ethylene plant defence pathways. *Frontiers in Plant Science.* 2013, **4**(215), 1-7.

Murchie EH, Pinto M, Horton P. Agriculture and the new challenges for photosynthesis research. *New Phytologist.* 2009, **181**, 532-552.

Murshed R, Lopez-Lauri F, Sallanon H. Effect of water stress on antioxidant systems and oxidative parameters in fruits of tomato (Solanum lycopersicon L, cv. Micro-Tom). *Physiology and Molecular Biology of Plants.* 2013, **19**, 363-378.

N

Nardozza S, Gamble J, Axten LG, Wohlers MW, Clearwater MJ, Feng J, Harker FR. Dry matter content and fruit size affect flavour and texture of novel Actinidia deliciosa genotypes. *Journal of the Science of Food and Agriculture.* 2011, **91**, 742-748.

Nassr, S. and Barakat, R. Effect of factors on conidium germination of Botrytis cinerea in vitro. *International Journal of Plant and Soil Science*. 2013, **2**, 41–54

Navarro JM, Flores P, Garrido C, Martinez V. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry*. 2006, **96**, 66-73.

Nedbal L, Soukupova J, Whitmarsh J, Trtilek M. Postharvest imaging of chlorophyll fluorescence from lemons can be used to predict fruit quality. *Photosynthetica.* 2000, **38**, 571-579.

Niinemets Ü. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management.* 2010, **260**, 1623-1639.

Niinemets U, Flexas J, Penuelas J. Evergreens favored by higher responsiveness to increased CO 2. *Trends in Ecology & Evolution.* 2011, **26**, 136-142.

Niinemets U, Kannaste A, Copolovici L. Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. *Frontiers in Plant Science.* 2013, **4**, 262.

Niu Y, Wang Y, Li P, Zhang F, Liu H, Zheng G. Drought stress induces oxidative stress and the antioxidant defense system in ascorbate-deficient vtc1 mutants of Arabidopsis thaliana. *Acta Physiologiae Plantarum.* 2013, **35**, 1189-1200.

Nora L, Dalmazo GO, Nora FR, Rombaldi CV. Controlled Water Stress to Improve Fruit and Vegetable Postharvest Quality. Water Stress, Prof. Ismail Md. Mofizur Rahman (Ed.). 2012.

Nowak M, Kleinwachter M, Manderscheid R, Weigel HJ, Selmar D. Drought stress increases the accumulation of monoterpenes in sage (Salvia officinalis), an effect that is compensated by elevated carbon dioxide concentration. *Journal of Applied Botany and Food Quality.* 2010, **83**, 133-136.

Nuruddin MM, Madramootoo CA, Dodds GT. Effects of water stress at different growth stages on greenhouse tomato yield and quality. *HortScience.* 2003, **38**, 1389-1393.

O

Oh M-M, Carey EE, Rajashekar CB. Regulated Water Deficits Improve Phytochemical Concentration in Lettuce. *Journal of the American Society for Horticultural Science.* 2010, **135**, 223-229.

Oikawa PY, Giebel BM, Sternberg Lda S, Li L, Timko MP, Swart PK, Riemer DD, Mak JE, Lerdau MT. Leaf and root pectin methylesterase activity and 13C/12C stable isotopic ratio measurements of methanol emissions give insight into methanol production in Lycopersicon esculentum. *New Phytol.* 2011, **191**, 1031-40.

Ojeda H, Deloire A, Carbonneau A. Influence of water deficits on grape berry growth. *Vitis.* 2001, **40**, 141-145.

Oukarroum A, Madidi SE, Schansker G, Strasser RJ. Probing the responses of barley cultivars (Hordeum vulgare L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering. *Environmental and Experimental Botany.* 2007, **60**, 438-446.

P

Panthee DR, Cao CX, Debenport SJ, Rodriguez GR, Labate JA, Robertson LD, Breksa AP, III, Knaap Evd, Gardener BBM. Magnitude of genotype * environment interactions affecting tomato fruit quality. *HortScience.* 2012, **47**, 721-726.

Parra M, Albacete A, Martínez-Andújar C, Pérez-Alfocea F. Increasing plant vigour and tomato fruit yield under salinity by inducing plant adaptation at the earliest seedling stage. *Environmental and Experimental Botany.* 2007, **60**, 77-85.

Pastore D, Di Pede S, Passarella S. Isolated durum wheat and potato cell mitochondria oxidize externally added NADH mostly via the malate/oxaloacetate shuttle with a rate that depends on the carrier-mediated transport. *Plant Physiol.* 2003, **133**, 2029-2039.

Pastore D, Trono D, Laus MN, Di Fonzo N, Flagella Z. Possible plant mitochondria involvement in cell adaptation to drought stress. A case study: durum wheat mitochondria. *J Exp Bot.* 2007, **58**, 195-210.

Patanè C, Cosentino SL. Effects of soil water deficit on yield and quality of processing tomato under a Mediterranean climate. *Agricultural Water Management.* 2010, **97**, 131-138.

Pelayo-Zaldívar C. Environmental Effects on Flavor Changes. *Handbook of Fruit and Vegetable Flavors*: John Wiley & Sons, Inc., 2010, 73-91.

Peleman J, Boerjan W, Engler G, Seurinck J, Botterman J, Alliotte T, Van Montagu M, Inze D. Strong cellular preference in the expression of a housekeeping gene of Arabidopsis thaliana encoding S-adenosylmethionine synthetase. *Plant Cell.* 1989, **1**, 81-93.

Penman H.L. Natural evaporation from open water, bare soil and grass. Proc. Roy. Soc. London A(194), 1948, S.: 120-145.

Penuelas J, Lluisa J, Gimeno BS. Effects of ozone concentrations on biogenic volatile organic compounds emission in the Mediterranean region. *Environmental Pollution.* 1999, **105**, 17-23.

Penuelas J, Staudt M. BVOCs and global change. *Trends in Plant Science.* 2010, **15**, 133-144.

Perez-Alfocea F, Ghanem ME, Gomez-Cadenas A, Dodd IC. Omics of root-to-shoot signaling under salt stress and water deficit. *Omics.* 2011, **15**, 893-901.

Perez-Martin A, Flexas J, Ribas-Carbó M, Bota J, Tomás M, Infante JM, Diaz-Espejo A. Interactive effects of soil water deficit and air vapour pressure deficit on mesophyll

conductance to CO2 in Vitis vinifera and Olea europaea. *Journal of Experimental Botany.* 2009, **60**, 2391-2405.

Perez-Pastor A, Ruiz-Sanchez MC, Martinez JA, Nortes PA, Artes F, Domingo R. Effect of deficit irrigation on apricot fruit quality at harvest and during storage. *Journal of the Science of Food and Agriculture.* 2007, **87**, 2409-2415.

Peter KV, Rai B. Stability parameters and genotype X environment interactions in tomato. *Indian Journal of Agricultural Sciences.* 1976, **46**, 395-398.

Petrikova K, Burg P, Pokluda R. Evaluation of selected tomato varieties for the resistance against Phytophthora infestans (Mont.) de Bary and comparison of their earliness and yield. *Acta Horticulturae et Regiotecturae.* 2003, **6**, 40-43.

Pfaffl MW. Quantification strategies in real-time PCR. *AZ of quantitative PCR.* 2004, **1**, 89-113.

Pilati S, Perazzolli M, Malossini A, Cestaro A, Dematte L, Fontana P, Dal Ri A, Viola R, Velasco R, Moser C. Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at veraison. *BMC Genomics.* 2007, **8**, 428.

Plaut, Z.; Grava, A.; Yehezkel, C.; Matan, E. How do salinity and water stress affect transport of water, assimilates and ions to tomato fruits? *Physiologia Plantarum*. 2004, **122**, 429-442.

Poiroux-Gonord F, Bidel LPR, Fanciullino A-L, Gautier H, Lauri-Lopez F, Urban L. Health benefits of vitamins and secondary metabolites of fruits and vegetables and prospects to increase their concentrations by agronomic approaches. *Journal of agricultural and food chemistry.* 2010, **58**, 12065-12082.

Poiroux-Gonord F, Fanciullino A-L, Bert L, Urban L. Effect of fruit load on maturity and carotenoid content of clementine (Citrus clementina Hort. ex Tan.) fruits. *Journal of the Science of Food and Agriculture.* 2012, **92**, 2076-2083.

Poiroux-Gonord F, Fanciullino AL, Poggi I, Urban L. Carbohydrate control over carotenoid build-up is conditional on fruit ontogeny in clementine fruits. *Physiologia Plantarum.* 2013a, **147**, 417-431.

Poiroux-Gonord F, Santini J, Fanciullino AL, Lopez-Lauri F, Giannettini J, Sallanon H, Berti L, Urban L. Metabolism in orange fruits is driven by photooxidative stress in the leaves. *Physiologia Plantarum.* 2013b, **149**, 175-187.

Poovaiah BW, Glenn GM, Reddy ASN. Calcium and Fruit Softening: Physiology and Biochemistry. *Horticultural Reviews*: John Wiley & Sons, Inc., 1988, 107-152.

Porcar-Castell A, Tyystjarvi E, Atherton J, van der Tol C, Flexas J, Pfundel EE, Moreno J, Frankenberg C, Berry JA. Linking chlorophyll a fluorescence to photosynthesis for remote sensing applications: mechanisms and challenges. *J Exp Bot.* 2014, **65**, 4065-4095.

Potters G, Horemans N, Jansen MAK. The cellular redox state in plant stress biology ‚Äì A charging concept. *Plant Physiology and Biochemistry.* 2010, **48**, 292-300.

Prudent M, Bertin N, Genard M, Munos S, Rolland S, Garcia V, Petit J, Baldet P, Rothan C, Causse M. Genotype-dependent response to carbon availability in growing tomato fruit. *Plant, Cell and Environment.* 2010, **33**, 1186-1204.

Q

Quiros-Gonzalez M. Phytophagous mite populations on Tahiti lime, Citrus latifolia, under induced drought conditions. *Experimental & Applied Acarology.* 2000, **24**, 897-904.

R

R Core Team. R : A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria. 2014.

Rahman S.M.L., Nawata E., Sakuratani T. Effect of water stress on growth, yield and eco-physiological responses of four tomato (*Lycopersicon esculentum* Mill.) cultivars. *Journal of the Japanese Society for Horticultural Science.* 1999, **68**, 499-504.

Ran N, Knop DR, Draths KM, Frost JW. Benzene-free synthesis of hydroquinone. *J Am Chem Soc.* 2001, **123**, 10927-10934.

Ranc N. Analyse du polymorphisme moléculaire de gènes de composantes de la qualité des fruits dans les ressources génétiques sauvages et cultivées de tomate ; recherche d'associations gènes/QTL, Académie de Montpellier, Ecole Nationale Supérieure Agronomique de Montpellier. 2010.

Ranc N, Muños S, Xu J, Le Paslier M-C, Chauveau A, Bounon R, Rolland S, Bouchet J-P, Brunel D, Causse M. Genome-Wide Association Mapping in Tomato (Solanum lycopersicum) Is Possible Using Genome Admixture of Solanum lycopersicum var. cerasiforme. *G3: Genes|Genomes|Genetics.* 2012, **2**, 853-864.

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylides C, Havaux M. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proceedings of the National Academy of Sciences of the United States of America.* 2012, **109**, 5535-5540.

Rapoport HF, Hammami SBM, Martins P, Pérez-Priego O, Orgaz F. Influence of water deficits at different times during olive tree inflorescence and flower development. *Environmental and Experimental Botany.* 2012, **77**, 227-233.

Reid JB, Brash DW, Sorensen IB, Bycroft B. Improvement in kiwifruit storage life caused by withholding early-season irrigation. *New Zealand Journal of Crop and Horticultural Science.* 1996, **24**, 21-28.

Reynolds AG, Lowrey WD, Tomek L, Hakimi J, de Savigny C. Influence of Irrigation on Vine Performance, Fruit Composition, and Wine Quality of Chardonnay in a Cool, Humid Climate. *American Journal of Enology and Viticulture.* 2007, **58**, 217-228.

Riggi E, Patane C, Ruberto G. Content of carotenoids at different ripening stages in processing tomato in relation to soil water availability. *Australian Journal of Agricultural Research.* 2008, **59**, 348-353.

Ripoll, J.; Urban, L.; Staudt, M.; Lopez-Lauri, F.; Bidel, L.P.R.; Bertin, N. Water shortage and quality of fleshy fruits—making the most of the unavoidable. *Journal of Experimental Botany*. 2014, 1-21.

Roitsch T, Gonzalez MC. Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science.* 2004, **9**, 606-613.

Rolland F, Moore B, Sheen J. Sugar sensing and signaling in plants. *Plant Cell.* 2002, **14**, S185-S205.

Rosales MA, Cervilla LM, Rios JJ, Blasco B, Sanchez-Rodriguez E, Romero L, Ruiz JM. Environmental conditions affect pectin solubilization in cherry tomato fruits grown in two experimental Mediterranean greenhouses. *Environmental and Experimental Botany.* 2009, **67**, 320-327.

Royer M., Larbat R., Le Bot J., Adamowicz S., Robin C. Is the C:N ratio a reliable indicator of C allocation to primary and defence-related metabolisms in tomato? *Phytochemistry*. 2013, **88**, 25-33.

Royston P. Remark AS R94: A Remark on Algorithm AS 181: The W-test for Normality. *Journal of the Royal Statistical Society. Series C (Applied Statistics).* 1995, **44**, 547-551.

Ruan Y, Jin Y, Yang Y, Li G, Boyer JS. Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Molecular Plant.* 2010, **3**, 942-955.

Rumeau D, Peltier G, Cournac L. Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. *Plant, Cell & Environment.* 2007, **30**, 1041-1051.

Rustioni L, Basilico R, Fiori S, Leoni A, Maghradze D, Failla O. Grape Colour Phenotyping: Development of a Method Based on the Reflectance Spectrum. *Phytochemical Analysis.* 2013, **24**, 453-459.

Ruuskanen TM, Müller M, Schnitzhofer R, Karl T, Graus M, Bamberger I, Hörtnagl L, Brilli F, Wohlfahrt G, Hansel A. Eddy covariance VOC emission and deposition fluxes above grassland using PTR-TOF. *Atmos. Chem. Phys.* 2011, **11**, 611-625.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD. Systemic acquired resistance. *Plant Cell.* 1996, **8**, 1809-1819.

S

Saha P, Das N, Deb P, Suresh CP. Effect of NAA and GA 3 on yield and quality of tomato (Lycopersicon esculentum Mill.). *Environment and Ecology.* 2009, **27**, 1048-1050.

Saladie M, Matas AJ, Isaacson T, Jenks MA, Goodwin SM, Niklas KJ, Ren XL, Labavitch JM, Shackel KA, Fernie AR, Lytovchenko A, O'Neill MA, Watkins CB, Rose JKC. A reevaluation of the key factors that influence tomato fruit softening and integrity. *Plant Physiology.* 2007, **144**, 1012-1028.

Salzman RA, Tikhonova I, Bordelon BP, Hasegawa PM, Bressan RA. Coordinate Accumulation of Antifungal Proteins and Hexoses Constitutes a Developmentally Controlled Defense Response during Fruit Ripening in Grape. *Plant Physiology*. 1998, **117**, 465-472.

Sams CE. Preharvest factors affecting postharvest texture. *Postharvest Biology and Technology.* 1999, **15**, 249-254.

Sanchez-Rodriguez E, Moreno DA, Ferreres F, Rubio-Wilhelmi MdM, Ruiz JM. Differential responses of five cherry tomato varieties to water stress: changes on phenolic metabolites and related enzymes. *Phytochemistry*. 2011, **72**, 723-729.

Sánchez-Rodríguez E, Rubio-Wilhelmi M, Cervilla LM, Blasco B, Rios JJ, Rosales MA, Romero L, Ruiz JM. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. *Plant Science.* 2010, **178**, 30-40.

Schaefer J., Opgen-Rhein R., Strimmer K. GeneNet: Modeling and Inferring Gene Networks. R package version 1.2.11. http://CRAN.R-project.org/package=GeneNet**.** 2014.

Scharte J, Schoen H, Tjaden Z, Weis E, von Schaewen A. Isoenzyme replacement of glucose-6-phosphate dehydrogenase in the cytosol improves stress tolerance in plants. *Proceedings of the National Academy of Sciences of the United States of America.* 2009, 106.

Schopfer P. Hydroxyl radical-induced cell-wall loosening in vitro and in vivo: implications for the control of elongation growth. *Plant J.* 2001, **28**, 679-88.

Schwartzenberg Kv, Schultze W, Kassner H. The moss Physcomitrella patens releases a tetracyclic diterpene. *Plant Cell Reports.* 2004, **22**, 780-786.

Serino S., Gomez L., Costagliola G., Gautier H. HPLC assay of tomato carotenoids: validation of a rapid microextraction technique. *Journal of Agricultural and Food Chemistry*. 2009, **57**, 8753-8760.

Seymour GB, Manning K, Eriksson EM, Popovich AH, King GJ. Genetic identification and genomic organization of factors affecting fruit texture. *Journal of Experimental Botany.* 2002, **53**, 2065-2071.

Shackel KA, Polito VS, Ahmadi H. Maintenance of turgor by rapid sealing of puncture wounds in leaf epidermal cells. *Plant Physiology.* 1991, **97**, 907-912.

Shakeel SN, Wang X, Binder BM, Schaller GE. Mechanisms of signal transduction by ethylene: overlapping and non-overlapping signalling roles in a receptor family. *AoB Plants.* 2013, **5**, 1-16.

Shao HB, Chu LY, Jaleel CA, Zhao CX. Water-deficit stress-induced anatomical changes in higher plants. *C R Biol.* 2008, **331**, 215-25.

Shavit N, Avron M. The effect of ultraviolet light on photophosphorylation and the Hill reaction. *Biochim Biophys Acta.* 1963, **66**, 187-195.

Shen J, Tieman D, Jones JB, Taylor MG, Schmelz E, Huffaker A, Bies D, Chen K, Klee HJ. A 13-lipoxygenase, TomloxC, is essential for synthesis of C5 flavour volatiles in tomato. *Journal of Experimental Botany.* 2014, **65**, 419-428.

Shewfelt RL. What is quality? *Postharvest Biology and Technology.* 1999, **15**, 197-200.

Shock C.C.B., J.Michael and Seddigh, Majid. Calibration of Watermark Soil Moisture Sensors for Irrigation Management. Proceedings of the International Irrigation Show, San Diego, CA. Irrigation Association. 1998, 139-146.

Silva ECd, Albuquerque MBd, Neto ADdA, Junior CDdS. *Drought and Its Consequences to Plants – From Individual to Ecosystem*. 2013.

Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B. Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. *Plant Physiology.* 2012, **158**, 835-843.

Slaymaker DH, Navarre DA, Clark D, Pozo Od, Martin GB, Klessig DF. The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which

exhibits antioxidant activity and plays a role in the hypersensitive defense response. *Proceedings of the National Academy of Sciences of the United States of America.* 2002, **99**, 11640-11645.

Solfanelli C, Poggi A, Loreti E, Alpi A, Perata P. Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. *Plant Physiology*. 2006, **140**, 637-646.

Song J, Shellie KC, Wang H, Qian MC. Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (Vitis vinifera L.). *Food Chem.* 2012, **134**, 841-50.

Srivastava A., Strasser R. Greening of peas: parallel measurements of 77 K emission spectra, OJIP chlorophyll a fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. *Photosynthetica*. 1999, **37**, 365-392.

Staudt M, Bertin N. Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (Quercus ilex L.) leaves. *Plant, Cell & Environment.* 1998, **21**, 385-395.

Steinhauser MC, Steinhauser D, Gibon Y, Bolger M, Arrivault S, Usadel B, Zamir D, Fernie AR, Stitt M. Identification of enzyme activity quantitative trait loci in a Solanum lycopersicum * Solanum pennellii introgression line population. *Plant Physiology.* 2011, **157**, 998-1014.

Stevens R, Page D, Gouble B, Garchery C, Zamir D, Causse M. Tomato fruit ascorbic acid content is linked with monodehydroascorbate reductase activity and tolerance to chilling stress. *Plant, Cell and Environment.* 2008, **31**, 1086-1096.

Stevens, R.; Buret, M.; Garchery, C.; Carretero, Y.; Causse, M. Technique for Rapid, Small-Scale Analysis of Vitamin C Levels in Fruit and Application to a Tomato Mutant Collection. *Journal of Agricultural and Food Chemistry*. 2006, **54**, 6159-6165.

Stewart P, Globig S. *Photosynthesis: Genetic, Environmental and Evolutionary Aspects*. Research Progress in Botany. CRC Press, 2011, 338 p.

Stikic, R.; Jovanovic, Z.; Prokic, L. Mitigation of plant drought stress in a changing climate. *Botanica Serbica*. 2014, **38**, 35-42.

Stikic R, Popovic S, Srdic M, Savic D, Jovanovic Z, Prokic L, Zdravkovic J. Partial root drying (PRD): a new technique for growing plants that saves water and improves the quality of fruit. *Bulgarian Journal of Plant Physiology*. 2003, **29**, 164-171.

Stirbet A, Govindjee. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: basics and applications of the OJIP fluorescence transient. *Journal of Photochemistry and Photobiology B:Biology.* 2011, **104**, 236-257.

Stirbet A, Riznichenko GY, Rubin AB, Govindjee. Modeling chlorophyll a fluorescence transient: relation to photosynthesis. *Biochemistry.* 2014, **79**, 291-323.

Stoeva N, Berova M, Vassilev A, Zlatev Z, Kaymakanova M, Ganeva D, Petkova V. Study on some enzyme activity in tomato plants during drought and recovery periods. *Agrarni Nauki.* 2012, **4**, 61-64.

Stoeva N, Berova M, Zlatev Z, Kaymakanova M, Koleva L, Ganeva D. Physiological test for evaluation of genotypes tolerance of tomato (Solanum lycopersicum) to water stress. *Agrarni Nauki.* 2010, **2**, 81-84.

Strasser R.J., Srivastava A. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria*. *Photochemistry and Photobiology*. 1995, **61**, 32-42.

Strasser BJ, Strasser RJ. Measuring fast fluorescence transients to address environmental questions: the JIP-test. Dordrecht, Netherlands, Kluwer Academic Publishers. 1995.

Strasser B. Donor side capacity of Photosystem II probed by chlorophyll a fluorescence transients. *Photosynthesis Research.* 1997, **52**, 147-155.

Strasser RJ, Srivastava A, Tsimilli-Michael M. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P, eds. *Probing Photosynthesis: Mechanism, Regulation and Adaptation*. London: Taylor and Francis. 2000, 445-483.

Strasser R, Tsimilli-Michael M, Srivastava A. Analysis of the Chlorophyll a Fluorescence Transient. In: G Papageorgiou and Govindjee, eds. *Chlorophyll a Fluorescence*, Vol. 19: Springer Netherlands. 2004, 321-362.

Struik PC, Yin XY, de Visser P. Complex quality traits: now time to model. *Trends in Plant Science.* 2005, **10**, 513-516.

Suarez L, Zarco-Tejada PJ, Gonzalez-Dugo V, Berni JAJ, Fereres E. The Photochemical Reflectance Index (PRI) as a water stress indicator in peach orchards from remote sensing imagery. In: J Girona and J Marsal, eds. *Acta Horticulturae.* 2012, 363-370.

Subramanian KS, Santhanakrishnan P, Balasubramanian P. Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. *Scientia Horticulturae.* 2006, **107**, 245-253.

Subrtova D, Hubacek J, Jankovsky M, Fialova D. Volatile substances of tomatoes (Lycopersicon esculentum). *Rostlinna Vyroba.* 1985, **31**, 871-880.

Sujata B, Kshitija S. Drought stress adaptation: metabolic adjustment and regulation of gene expression. *Plant Breeding.* 2013, **132**, 21-32.

Sundby C, Melis A, Mäenpää P, Andersson B. Temperature-dependent changes in the antenna size of Photosystem II. Reversible conversion of Photosystem IIα to Photosystem IIβ. *Biochimica et Biophysica Acta (BBA) – Bioenergetics.* 1986, **851**, 475-483.

T

Takabayashi J, Dicke M, Posthumus MA. Volatile herbivore-induced terpenoids in plant-mite interactions: variation caused by biotic and abiotic factors. *Journal of Chemical Ecology.* 1994, **20**, 1329-1354.

Tanksley SD, McCouch SR. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science (Washington).* 1997, **277**, 1063-1066.

Tardieu F, Cruiziat P, Durand J-L, Triboï E, Zivy M. 1.1.2. Perception de la sécheresse par la plante. Conséquences sur la productivité et sur la qualité des produits récoltés. *ESCo "Sécheresse et Agriculture"* **Chapitre 1-1**, 2006, 49-67.

Tardieu F, Granier C, Muller B. Water deficit and growth. Co-ordinating processes without an orchestrator? *Current Opinion in Plant Biology.* 2011, **14**, 283-289.

Tardieu F, Simonneau T. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany*. 1998, **49**, 419-432.

Tardieu, F.; Tuberosa, R. Dissection and modelling of abiotic stress tolerance in plants. *Current Opinion in Plant Biology*. 2010, **13**, 206-212.

Tauzin AS, Giardina T. Sucrose and invertases, a part of the plant defense response to the biotic stresses. *Frontiers in Plant Science.* 2014, **5**.

Taylor IB, Burbidge A, Thompson AJ. Control of abscisic acid synthesis. *Journal of Experimental Botany.* 2000, **51**, 1563-1574.

Telef N, Stammitti-Bert L, Mortain-Bertrand A, Maucourt M, Carde JP, Rolin D, Gallusci P. Sucrose deficiency delays lycopene accumulation in tomato fruit pericarp discs. *Plant Molecular Biology.* 2006, **62**, 453-469.

Terry LA, Chope GA, Bordonaba JG. Effect of water deficit irrigation and inoculation with Botrytis cinerea on strawberry (Fragaria * ananassa) fruit quality. *Journal of Agricultural and Food Chemistry.* 2007, **55**, 10812-10819.

Thompson DS. Extensiometric determination of the rheological properties of the epidermis of growing tomato fruit. *Journal of Experimental Botany.* 2001, **52**, 1291-1301.

Toivonen PMA, Brummell DA. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology.* 2008, **48**, 1-14.

Ton J, Flors V, Mauch-Mani B. The multifaceted role of ABA in disease resistance. *Trends in Plant Science.* 2009, **14**, 310-317.

Tuccio L, Remorini D, Pinelli P, Fierini E, Tonutti P, Scalabrelli G, Agati G. Rapid and non-destructive method to assess in the vineyard grape berry anthocyanins under different seasonal and water conditions. *Australian Journal of Grape and Wine Research.* 2011, **17**, 181-189.

U

Ulissi V, Antonucci F, Benincasa P, Farneselli M, Tosti G, Guiducci M, Tei F, Costa C, Pallottino F, Pari L, Menesatti P. Nitrogen concentration estimation in tomato leaves by VIS-NIR non-destructive spectroscopy. *Sensors.* 2011, **11**, 6411-6424.

Urban L, Le Roux X, Sinoquet H, Jaffuel S, Jannoyer M. A biochemical model of photosynthesis for mango leaves: evidence for the effect of fruit on photosynthetic capacity of nearby leaves. *Tree Physiology.* 2003, **23**, 289-300.

Urban L, Léchaudel M. Effect of leaf-to-fruit ratio on leaf nitrogen content and net photosynthesis in girdled branches of Mangifera indica L. *Trees.* 2005, **19**, 564-571.

Urban L, Jegouzo L, Damour G, Vandame M, François C. Interpreting the decrease in leaf photosynthesis during flowering in mango. *Tree Physiology.* 2008, **28**, 1025-1036.

Urban L, Alphonsout L. Girdling decreases photosynthetic electron fluxes and induces sustained photoprotection in mango leaves. *Tree Physiology.* 2007, **27**, 345-352.

V

Vallverdu X, Girona J, Echeverria G, Marsal J, Behboudian MH, Lopez G. Sensory quality and consumer acceptance of 'Tardibelle' peach are improved by deficit irrigation applied during stage II of fruit development. *HortScience.* 2012, **47**, 656-659.

Varga, A.; Bruinsma, J. Tomato. In: S M, ed. CRC *Handbook of Fruit Set and Development*. Boca Raton, FL:CRC Press. 1986, 461-491.

Varga A, Bruinsma J. Roles of Seeds and Auxins in Tomato Fruit Growth. *Zeitschrift für Pflanzenphysiologie*. 1976, **80**, 95-104.

Veit-Köhler U, Krumbein A, Kosegarten H. Effect of different water supply on plant growth and fruit quality of Lycopersicon esculentum. *Journal of Plant Nutrition and Soil Science.* 1999, **162**, 583-588.

Vercammen J, Pham-Tuan H, Sandra P. Automated dynamic sampling system for the on-line monitoring of biogenic emissions from living organisms. *Journal of Chromatography, A*. 2001, **930**, 39-51.

Vicedo B, Flors V, de la O Leyva M, Finiti I, Kravchuk Z, Real MD, García-Agustín P, González-Bosch C. Hexanoic Acid-Induced Resistance Against Botrytis cinerea in Tomato Plants. *Molecular Plant-Microbe Interactions.* 2009, **22**, 1455-1465.

Vlot AC, Dempsey DMA, Klessig DF. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology.* 2009, **47**, 177-206.

Vredenberg W, Kasalicky V, Durchan M, Prasil O. The chlorophyll a fluorescence induction pattern in chloroplasts upon repetitive single turnover excitations: Accumulation and function of QB-nonreducing centers. *Biochimica et Biophysica Acta (BBA) – Bioenergetics.* 2006, **1757**, 173-181.

W

Walker BJ, Strand DD, Kramer DM, Cousins AB. The Response of Cyclic Electron Flow around Photosystem I to Changes in Photorespiration and Nitrate Assimilation. *Plant Physiology.* 2014, 165, 453-462.

Wang D, Gartung J. Infrared canopy temperature of early-ripening peach trees under postharvest deficit irrigation. *Agricultural Water Management.* 2010, **97**, 1787-1794.

Wang Y, Frei M. Stressed food - the impact of abiotic environmental stresses on crop quality. *Agriculture, Ecosystems & Environment.* 2011, **141**, 271-286.

Wang, F.; Kang, S.; Du, T.; Li, F.; Qiu, R. Determination of comprehensive quality index for tomato and its response to different irrigation treatments. *Agricultural Water Management*. 2011, **98**, 1228-1238.

Wang L, Wang Y, Meng X, Meng Q. Overexpression of tomato GDP-L-galactose phosphorylase gene enhanced tolerance of transgenic tobacco to methyl viologen-mediated oxidative stress. *Plant Physiology Communications.* 2012, **48**, 689-698.

Ward EWB, Cahill DM, Bhattacharyya MK. Abscisic acid suppression of phenylalanine ammonia-lyase activity and mRNA, and resistance of soybeans to Phytophthora megasperma f.sp. glycinea. *Plant Physiology.* 1989, **91**, 23-27.

Warnes G.R., Bolker B., Bonebakker L., Gentleman R., Liaw W.H.A., Lumley T., Maechler M., Magnusson A., Moeller S., Schwartz M., Venables B. gplots: Various R programming tools for plotting data. R package version 2.14.2. 2014.

West G, Inzé D, Beemster GTS. Cell Cycle Modulation in the Response of the Primary Root of Arabidopsis to Salt Stress. *Plant Physiology.* 2004, **135**, 1050-1058.

Wheeler GL, Jones MA, Smirnoff N. The biosynthetic pathway of vitamin C in higher plants. *Nature.* 1998, **393**, 365-9.

Whitt SR, Wilson LM, Tenaillon MI, Gaut BS, Buckler ES. Genetic diversity and selection in the maize starch pathway. *Proceedings of the National Academy of Sciences.* 2002, **99**, 12959-12962.

Wiese J, Kranz T, Schubert S. Induction of pathogen resistance in barley by abiotic stress. *Plant Biol.* 2004, **6**, 529-36.

Wilkens RT, Spoerke JM, Stamp NE. Differential responses of growth and two soluble phenolics of tomato to resource availability. *Ecology.* 1996, **77**, 247-258.

Wilkinson S, Davies WJ. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell and Environment.* 2002, **25**, 195-210.

Wingate VPM, Lawton MA, Lamb CJ. Glutathione causes a massive and selective indution of plant defense genes *Plant Physiology.* 1988, **87**, 206-210.

Wu M, Buck JS, Kubota C. Effects of nutrient solution EC, plant microclimate and cultivars on fruit quality and yield of hydroponic tomatoes (*Lycopersicon esculentum*). In: DJ Cantliffe, et al., eds. *7th International Symposium on Protected Cultivation in Mild Winter Climates - Production, Pest Management and Global Competition*. Kissimmee, FL, 2004, 541-547.

X

Xoconostle-Cazares, B.; Ramirez-Ortega, F.A.; Flores-Elenes, L.; Ruiz-Medrano, R. Drought tolerance in crop plants. *American Journal of Plant Physiology.* 2010, **5**, 241-256.

Xu Z., Zhou G., Shimizu H. Plant responses to drought and rewatering. *Plant Signaling & Behavior.* 2010, **5**: 649-654.

Y

Yakushiji H, Nonami H, Fukuyama T, Ono S, Takagi N, Hashimoto Y. Sugar Accumulation Enhanced by Osmoregulation in Satsuma Mandarin Fruit. *Journal of the American Society for Horticultural Science.* 1996, **121**, 466-472.

Yang J, Zhang J, Wang Z, Zhu Q, Liu L. Activities of enzymes involved in sucrose-to-starch metabolism in rice grains subjected to water stress during filling. *Field Crops Research.* 2003, **81**, 69-81.

Yordanov I, Goltsev V, Stefanov D, Chernev P, Zaharieva I, Kirova M, Gecheva V, Strasser RJ. Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. *Journal of Plant Physiology.* 2008, **165**, 1954-1963.

Yoshioka T, Inokuchi T, Fujioka S, Kimura Y. Phenolic compounds and flavonoids as plant growth regulators from fruit and leaf of Vitex rotundifolia. *Z Naturforsch C.* 2004, **59**, 509-514.

Z

Zanor MI, Osorio S, Nunes-Nesi A, Carrari F, Lohse M, Usadel B, Kühn C, Bleiss W, Giavalisco P, Willmitzer L, Sulpice R, Zhou Y-H, Fernie AR. RNA Interference of LIN5 in Tomato Confirms Its Role in Controlling Brix Content, Uncovers the Influence of Sugars on the Levels of Fruit Hormones, and Demonstrates the Importance of Sucrose Cleavage for Normal Fruit Development and Fertility. *Plant Physiology*. 2009, **150**, 1204-1218.

Zhang Q, Li Q, Zhang G. Rapid Determination of Leaf Water Content Using VIS/NIR Spectroscopy Analysis with Wavelength Selection. *Spectroscopy: An International Journal*. 2012, **27**, 93-105.

Zhang S, Zhang Z, Qiao Y, Wu J, Tao S. Effects of fruit bagging at different stages on pear quality and sclereid development and the activities of their related enzymes in the pear variety kousui. *Acta Botanica Boreali-Occidentalia Sinica.* 2006, **26**, 1369-1377.

Zhang Y, Li H, Shu W, Zhang C, Zhang W, Ye Z. Suppressed Expression of Ascorbate Oxidase Gene Promotes Ascorbic Acid Accumulation in Tomato Fruit. *Plant Molecular Biology Reporter*. 2011, **29**, 638-645.

Živčák M, Brestič M, Olšovská K, Slamka P. Performance index as a sensitive indicator of water stress in Triticum aestivum L. *L. Plant Soil Environ.* 2008, **54**, 133-139.

Zushi K, Matsuzoe N. Effect of soil water deficit on vitamin C, sugar, organic acid, amino acid and carotene contents of large-fruited tomatoes. *Journal of the Japanese Society for Horticultural Science.* 1998, **67**, 927-933.

Webographie

[1] Food and Agriculture Organization of the United Nations (FAO) website, Aquastat©, Agricultural water withdrawal, from 2003 to 2007, selection for Mediterranean countries. http://www.fao.org/nr/water/aquastat/data/query/results.html. (*E.g.* 06 Sept. 2013)

[2] Food and Agriculture Organization of the United Nations (FAO) website, The State of Food Insecurity in the World 2012, Book. http://www.fao.org/publications/sofi/en (e.g. 16 sept 2013)

Annexes du Chapitre II

Genotype	Treatment	Predawn water potential	Midday stem water potential	Stomatal conductance	Net photosynthesis rate	Respiration	Transpiration rate	Length*number of leaves	Chlorophyll content	Fv'/Fm'	Fv/Fm	Cyclic fluxes	PI_{ABS}
CERVIL	Control	-7.87 ± 0.30	-14.54 ± 1.02	8.32 ± 0.46	317.98 ± 16.41	-246.98 ± 12.79	157.89 ± 6.06	100996.65 ± 2956.03	2494.95 ± 60.54	52.87 ± 0.25	58666.62 ± 2419.44	11.81 ± 0.39	215.25 ± 7.65
	Stress	-10.33 ± 0.81 *	-16.39 ± 1.31	8.71 ± 1.16	358.64 ± 26.21	-197.45 ± 16.27 *	136.05 ± 14.92	108093.90 ± 1704.76	2538.07 ± 8.51	51.96 ± 0.45 *	59367.85 ± 5730.39 *	12.88 ± 0.58	188.04 ± 19.83
CRIOLLO	Control	-5.47 ± 0.35	-9.18 ± 0.26	4.20 ± 0.49	149.84 ± 16.15	-236.98 ± 3.69	96.41 ± 8.90	103484.10 ± 1808.39	2056.86 ± 44.15	52.63 ± 0.19	60096.12 ± 582.31	8.61 ± 0.19	184.23 ± 14.34
	Stress	-6.51 ± 0.28	-11.78 ± 0.47 *	7.72 ± 0.69	188.19 ± 11.60	-209.57 ± 2.85	113.33 ± 7.11	93096.00 ± 2663.36 *	2196.73 ± 44.52 *	52.08 ± 0.08	60376.00 ± 4478.85	10.77 ± 0.34 *	153.00 ± 7.35
LA1420	Control	-4.94 ± 0.17	-8.19 ± 0.61	7.25 ± 0.56	346.11 ± 28.29	-229.31 ± 9.14	138.39 ± 8.33	91653.16 ± 3060.10	2160.80 ± 25.52	53.73 ± 0.05	63390.50 ± 2024.27	11.39 ± 0.43	236.78 ± 8.77
	Stress	-6.33 ± 0.63 *	-8.76 ± 0.66	6.41 ± 0.32	353.94 ± 20.87	-221.98 ± 16.51 *	107.16 ± 5.69	88908.40 ± 1332.13	2396.69 ± 36.51 *	52.52 ± 0.16	58072.03 ± 570.95 *	13.39 ± 0.38 *	189.90 ± 7.89 *
PLOVDIV	Control	-5.75 ± 0.34	-9.11 ± 0.69	13.48 ± 0.75	489.57 ± 10.79	-286.19 ± 17.47	222.00 ± 7.41	105410.25 ± 2029.38	2338.52 ± 29.16	53.96 ± 0.12	71113.80 ± 1750.22	13.06 ± 0.35	247.07 ± 7.56
	Stress	-7.67 ± 0.54 *	-10.42 ± 1.49	8.59 ± 0.70 *	373.52 ± 24.83 *	-213.17 ± 10.16 *	114.07 ± 6.45 *	88176.00 ± 4071.28 *	2402.17 ± 29.54 *	52.53 ± 0.26	59984.53 ± 1047.46 *	14.09 ± 0.55	199.09 ± 12.61 *
STUPICKE	Control	-5.29 ± 0.25	-9.94 ± 0.92	13.05 ± 1.36	429.98 ± 23.13	-240.17 ± 13.16	220.66 ± 13.04	89145.55 ± 3697.83	2411.63 ± 33.40	53.45 ± 0.11	65633.20 ± 2832.72	12.94 ± 0.49	225.96 ± 13.95
	Stress	-7.04 ± 0.52 *	-11.30 ± 0.85	10.34 ± 1.20	388.83 ± 16.27	-193.64 ± 6.15 *	134.32 ± 8.71 *	84549.40 ± 3040.26	2799.09 ± 33.90 *	51.84 ± 0.70	63197.05 ± 1590.46 *	16.13 ± 0.98 *	200.89 ± 12.14
FERUM	Control	-6.32 ± 0.34	-8.34 ± 0.84	13.94 ± 0.71	480.11 ± 16.82	-235.13 ± 9.13	199.97 ± 12.09	78904.85 ± 1296.92	2481.52 ± 40.83	53.96 ± 0.02	73362.73 ± 1643.42	15.38 ± 0.24	253.16 ± 5.74
	Stress	-8.21 ± 0.42 *	-12.92 ± 0.68 *	11.40 ± 2.04	450.38 ± 37.16	-238.51 ± 7.31	161.67 ± 21.22 *	64888.15 ± 1488.09 *	2592.70 ± 25.51	52.47 ± 0.29	61664.15 ± 2455.45 *	15.30 ± 0.80	175.06 ± 4.10 *
LA0147	Control	-7.50 ± 0.20	-10.31 ± 0.89	15.49 ± 0.96	477.42 ± 26.03	-241.35 ± 7.52	224.89 ± 10.46	99000.35 ± 4428.97	2635.71 ± 46.37	53.50 ± 0.16	73955.88 ± 2970.79	13.43 ± 0.83	197.53 ± 12.83
	Stress	-8.69 ± 0.63	-12.45 ± 0.82	11.84 ± 0.58 *	458.97 ± 15.06	-190.24 ± 8.60	154.69 ± 3.93 *	90489.75 ± 1204.72 *	2683.87 ± 46.74	52.08 ± 0.27	70382.05 ± 1926.80 *	16.52 ± 0.86 *	179.27 ± 6.76
LEVOVIL	Control	-6.48 ± 0.51	-13.17 ± 0.56	9.47 ± 1.69	430.81 ± 42.91	-246.30 ± 10.32	168.70 ± 18.01	94511.60 ± 2345.67	2329.68 ± 71.79	53.38 ± 0.27	66209.93 ± 2462.75	11.81 ± 0.17	203.77 ± 16.86
	Stress	-6.82 ± 0.34	-12.03 ± 0.82	7.69 ± 0.33	412.63 ± 20.88	-230.73 ± 3.44	114.13 ± 2.71 *	80060.30 ± 2472.75 *	2726.17 ± 57.49	51.92 ± 0.15	64833.35 ± 1162.45 *	13.12 ± 0.49	138.57 ± 5.98 *

Supplementary data 1: Total area under the curve (AUC) value with standard error (n = 5) for physiological parameters as water potentials, net photosynthesis rate, transpiration, stomatal conductance, respiration, the length*number of leaves, the chlorophyll contents in SPAD units, the maximum efficiency of PSII photochemistry when all the PSII centres were 'open' (F_V'/F_M'), Maximum quantum efficiency of PSII Photochemistry on dark adapted leaves (F_V/F_M), the performance index (PI) of Strasser *et al.* (2004) and cyclic electron fluxes (J_0^{RE1}/J^{ABS}) of Stirbet and Govindjee (2011) measured for control and stressed plants (alternative treatment). Symbols * indicated significant differences between treatments for each genotype for $P < 0.05$ (Tukey test).

Genotype	Treatment	Dry matter content	Glucose	Fructose	Saccharose	Total sugars	Starch	Citric acid	Malic acid	Quinic acid	Total acids
CERVIL	Control	834.49 ± 18.96	100.06 ± 7.25	316.48 ± 13.16	120.23 ± 2.51	520.91 ± 25.17	521.36 ± 43.43	236.57 ± 9.99	470.25 ± 13.70	121.67 ± 8.06	789.74 ± 42.50
	Stress	888.40 ± 6.95	114.14 ± 4.33 *	353.00 ± 12.20	114.16 ± 2.01	581.23 ± 17.05	495.96 ± 49.01	193.87 ± 1.76 *	436.06 ± 8.86	147.86 ± 5.04 *	777.82 ± 8.45
CRIOLLO	Control	674.30 ± 15.83	61.67 ± 6.81	126.37 ± 17.69	63.25 ± 3.57	251.27 ± 27.35	176.74 ± 28.86	193.41 ± 12.39	374.16 ± 10.74	69.34 ± 3.61	636.93 ± 9.61
	Stress	799.68 ± 18.60 *	71.79 ± 10.11 *	160.65 ± 17.15	71.06 ± 3.40	303.52 ± 29.25 *	316.70 ± 40.71 *	192.04 ± 6.24	409.19 ± 6.44	97.87 ± 5.02 *	699.06 ± 7.45
LA1420	Control	630.89 ± 27.06	75.48 ± 4.80	208.75 ± 9.83	83.47 ± 4.43	367.70 ± 18.72	170.84 ± 13.53	186.09 ± 7.17	244.95 ± 22.33	76.42 ± 3.19	507.28 ± 21.87
	Stress	711.31 ± 8.10 *	102.24 ± 0.80	231.99 ± 5.56 *	87.12 ± 3.55	421.46 ± 8.96 *	192.77 ± 15.06	173.25 ± 2.86	338.17 ± 8.62 *	95.26 ± 6.31 *	606.67 ± 11.91
PLOVDIV	Control	719.71 ± 13.90	113.50 ± 8.97	201.70 ± 10.63	59.44 ± 2.64	374.56 ± 20.46	82.31 ± 10.78	110.42 ± 4.15	335.61 ± 21.58	112.89 ± 9.16	558.82 ± 27.38
	Stress	820.02 ± 13.78 *	155.04 ± 8.11 *	263.34 ± 7.13 *	65.14 ± 1.27	483.41 ± 13.42 *	281.47 ± 29.97 *	114.54 ± 2.14	415.76 ± 7.74 *	151.37 ± 6.47 *	681.69 ± 10.32
STUPICKE	Control	709.67 ± 5.26	25.83 ± 4.02	92.83 ± 4.96	52.49 ± 1.87	171.10 ± 9.48	90.98 ± 15.75	191.78 ± 10.42	289.60 ± 16.30	98.84 ± 6.78	580.16 ± 17.80
	Stress	749.40 ± 40.09	62.80 ± 4.87*	159.48 ± 12.66 *	57.00 ± 5.12	279.40 ± 21.70 *	183.30 ± 50.77	189.86 ± 8.35	378.27 ± 14.81 *	123.82 ± 6.19 *	691.97 ± 24.26 *
FERUM	Control	853.26 ± 11.24	63.49 ± 7.03	179.84 ± 10.57	67.45 ± 1.19	310.79 ± 16.64	149.19 ± 10.07	177.25 ± 8.64	262.88 ± 18.23	94.40 ± 2.22	534.44 ± 25.06
	Stress	959.88 ± 42.29 *	69.75 ± 3.64	190.20 ± 6.97	71.27 ± 1.92	330.10 ± 14.13	440.87 ± 37.94 *	189.61 ± 12.79	329.80 ± 34.20 *	120.75 ± 9.31 *	643.17 ± 71.91 *
LA0147	Control	706.51 ± 22.39	42.33 ± 3.73	101.88 ± 5.03	52.84 ± 2.08	197.11 ± 9.28	56.67 ± 8.11	189.30 ± 4.83	303.02 ± 15.05	68.82 ± 6.55	561.10 ± 21.37
	Stress	802.01 ± 18.51 *	67.35 ± 5.09 *	150.38 ± 5.04 *	61.98 ± 2.08 *	279.73 ± 10.82 *	114.07 ± 10.18	175.24 ± 8.38	318.66 ± 21.39	114.63 ± 4.16 *	608.56 ± 26.09 *
LEVOVIL	Control	693.38 ± 24.48	33.26 ± 2.48	96.00 ± 8.01	50.85 ± 1.79	180.17 ± 10.77	138.94 ± 30.22	186.83 ± 4.61	206.40 ± 19.95	72.46 ± 3.43	465.72 ± 23.08
	Stress	803.09 ± 14.30 *	76.48 ± 3.81 *	165.24 ± 8.33 *	56.19 ± 1.00	287.94 ± 7.05 *	434.02 ± 59.00 *	164.25 ± 3.41 *	292.21 ± 12.01 *	119.50 ± 4.23 *	562.50 ± 15.57 *

Supplementary data 2: Total area under the curve (AUC) value with standard error (n = 5) for leaf composition i.e. dry matter content, soluble sugars, organic acids and starch, measured for control and stressed plants (alternative treatment). Symbols * indicated significant differences between treatments for each genotype for $P < 0.05$ (Tukey test).

ID	CERVIL	CRIOLLO	LA1420	PLOVDIV	STUPICKE	FERUM	LA0147	LEVOVIL
Dry matter content in %	***8.9***	5.4	-0.3	-1.3	12.7	5.1	2.2	4.3
Fresh weight (g)	7.2	-5.7	-18.9	-3.3	-10.7	-11.0	-5.2	-12.7
Glucose (g/100g)FW	***30.6***	13.7	6.7	-2.4	9.6	12.4	-0.7	10.7
Fructose (g/100g)FW	***31.6***	11.7	12.5	-4.8	10.9	10.5	0.7	8.0
Sucrose (g/100g)FW	42.0	27.8	-19.8	10.8	23.0	44.4	11.3	-20.8
Total sugar (g/100g)FW	***31.3***	12.9	9.0	-3.2	10.5	11.9	0.1	9.1
Citric acid (g/100g)FW	19.7	-6.0	-19.7	-11.2	7.9	-0.3	-18.5	3.4
Malic acid (g/100g)FW	37.9	-2.0	-3.6	-7.1	-8.2	8.1	-29.6	5.1
Quinic acid (g/100g)FW	***36.3***	9.4	15.6	0.5	***35.6***	13.8	-8.7	5.7
Total acid (g/100g)FW	***29.3***	0.1	-7.6	-5.0	17.2	7.1	-16.6	4.7
Luteine (mg/kg FW)	4.4	-1.9	43.7	-32.5	15.6	-11.0	-1.5	-10.0
Lycopene (mg/kg FW)	-15.9	26.0	33.2	13.1	-0.3	-7.1	19.8	-8.1
Bêta-carotene (mg/kg FW)	-1.9	4.5	23.5	-21.3	22.9	-2.0	4.3	-16.9
Phytoene (mg/kg FW)	-12.5	37.0	46.4	25.7	-11.1	-3.7	33.7	-8.1
Total carotenoids (mg/kg FW)	-14.3	25.8	34.2	12.9	0.2	-6.4	20.5	-8.7
Reduced C vit (mg/100g FW)	-1.0	1.9	24.6	-17.1	4.1	-2.5	2.4	-14.6
Total C Vit (mg/100g FW)	2.0	8.1	21.4	-18.3	7.3	3.5	0.8	-8.7
reduced/total C vit	-2.7	-6.0	2.3	1.5	-2.6	-5.8	1.7	-4.9
Starch (g/100g)FW	70.1							

Supplementary data 3: Relative differences in fruit metabolite content between control and alternation treatment. Soluble sugars, organic acids, ascorbic acid (C vit) and carotenoids were measured on a fresh weight basis on the 8 MAGIC TOM parents. Relative differences were calculated on the means of control values vs. the means of stressed values. Data are means of pools of 5 fruits ± SE ($n \geq 5$). Fruits (lot S1) were harvested in RP2 after the alternation of a first stress period during cell division (DI1) and a second stress period during cell expansion (DI2). Significant differences are indicated in red using bold, italic and underlined fonts for $P < 0.05$ (Two-way ANOVA test or Kruskal-Wallis test).

ID	CERVIL	CRIOLLO	LA1420	PLOVDIV	STUPICKE	FERUM	LA0147	LEVOVIL
Dry matter content in %	***21.0***	14.0	***22.3***	9.0	8.7	-9.5	3.6	***33.7***
Fresh weight (g)	-0.2	-21.0	-20.2	-23.7	-16.1	-0.4	-18.6	-42.8
Glucose (g/100g)FW	***48.6***	9.5	29.5	13.0	26.9	-9.1	1.8	***47.6***
Fructose (g/100g)FW	***46.3***	11.5	23.0	12.9	22.3	-4.6	2.3	***44.6***
Sucrose (g/100g)FW	***77.3***	143.2	78.8	3.8	77.2	-23.9	22.8	119.6
Total sugar (g/100g)FW	***48.5***	12.3	27.0	12.6	25.6	-7.2	2.6	***47.0***
Citric acid (g/100g)FW	16.2	15.0	-11.1	3.9	1.8	-5.0	5.5	5.0
Malic acid (g/100g)FW	16.7	10.4	30.9	13.2	4.7	-21.0	4.5	-10.6
Quinic acid (g/100g)FW	***46.9***	10.0	13.6	14.4	8.6	-0.2	3.6	***44.0***
Total acid (g/100g)FW	***29.9***	12.7	-0.1	10.1	5.0	-4.6	4.6	18.5
Luteine (mg/kg FW)	18.3	23.8	-2.0	41.1	20.4	16.8	28.0	23.6
Lycopene (mg/kg FW)	-14.6	4.4	15.2	10.0	-4.1	28.1	8.0	20.5
Bêta-carotene (mg/kg FW)	2.3	19.2	-10.0	6.7	0.4	-2.4	4.6	1.4
Phytoene (mg/kg FW)	0.5	5.8	21.1	-1.2	5.1	30.9	6.1	33.1
Total carotenoids (mg/kg FW)	-11.5	5.0	14.7	8.3	-2.9	27.6	8.0	20.5
Reduced C vit (mg/100g FW)	***23.5***	5.4	***31.2***	***30.3***	-1.9	-1.8	5.1	12.4
Total C Vit (mg/100g FW)	***29.8***	2.0	***32.4***	43.6	1.1	-0.1	6.7	21.5
reduced/total C vit	4.8	-3.6	-0.1	11.0	3.3	1.4	0.9	8.0
Starch (g/100g)FW	***124.6***							

Supplementary data 4: Relative differences in fruit metabolite content between control and alternation treatment. Soluble sugars, organic acids, ascorbic acid (C vit) and carotenoids were measured on a fresh weight basis on the 8 MAGIC TOM parents. Relative differences were calculated on the means of control values vs. the means of stressed values. Data are means of pools of 5 fruits ± SE ($n \geq 5$). Fruits (lot S2) were harvested in DI3 (Cervil, Criollo, Plovdiv and Stupicke) and RP3 (Levovil, LA1420, LA0147 and Ferum) after the alternation of a first stress period during cell division (DI2) and a second stress period during cell expansion and ripening (DI3). Significant differences are indicated in red using bold, italic and underlined fonts for $P < 0.05$ (Two-way ANOVA test or Kruskal-Wallis test).

Genotype	Cervil		Criollo		LA1420		Plovdiv	
Treatment	Control	Alternation	Control	Alternation	Control	Alternation	Control	Alternation
Fruit size (mm)	21.72 ± 0.31	22.23 ± 0.24	40.02 ± 0.88	38.41 ± 0.89	54.80 ± 3.20	51.08 ± 1.41	42.17 ± 1.17	43.94 ± 0.65
Fresh weight (g)	5.94 ± 0.30	6.36 ± 0.20	26.59 ± 1.51	25.07 ± 1.07	64.81 ± 7.78	52.54 ± 3.18	53.76 ± 1.23	51.99 ± 2.13
Dry matter content (g 100g FW^{-1})	11.48 ± 0.12	12.51 ± 0.15 *	6.51 ± 0.08	6.87 ± 0.11	5.71 ± 0.17	5.69 ± 0.07	8.19 ± 0.04	8.09 ± 0.05
Glucose (g 100 g DM^{-1})	18.11 ± 2.35	21.66 ± 0.63 *	19.09 ± 0.69	20.59 ± 0.49	17.40 ± 0.63	18.64 ± 0.54	23.34 ± 3.11	23.05 ± 0.43
Fructose (g 100 g DM^{-1})	18.87 ± 2.59	22.74 ± 0.68 *	20.83 ± 0.78	22.1 ± 0.55	18.04 ± 0.58	20.37 ± 0.37	22.23 ± 3.09	21.42 ± 0.42
Sucrose (g 100 g DM^{-1})	0.78 ± 0.25	1.01 ± 0.15	0.58 ± 0.06	0.71 ± 0.08	0.80 ± 0.03	0.64 ± 0.05	1.16 ± 0.42	1.30 ± 0.10
Total sugar (g 100 g DM^{-1})	37.76 ± 5.06	45.4 ± 1.43 *	40.50 ± 1.47	43.40 ± 1.02	36.24 ± 1.22	39.66 ± 0.94	46.73 ± 6.62	45.78 ± 0.83
Citric acid (g 100 g DM^{-1})	3.09 ± 0.47	3.38 ± 0.08	6.33 ± 0.41	5.65 ± 0.25	6.87 ± 0.44	5.51 ± 0.19 *	3.24 ± 0.52	2.92 ± 0.11
Malic acid (g 100 g DM^{-1})	0.51 ± 0.08	0.64 ± 0.04	0.52 ± 0.06	0.48 ± 0.06	0.55 ± 0.05	0.53 ± 0.03	1.51 ± 0.16	1.42 ± 0.07
Quinic acid (g 100 g DM^{-1})	3.62 ± 0.48	4.514 ± 0.10	4.2 ± 0.35	4.37 ± 0.19	3.45 ± 0.21	4.00 ± 0.07	4.18 ± 0.62	4.26 ± 0.06
Total acid (g 100 g DM^{-1})	7.22 ± 1.03	8.54 ± 0.18	11.04 ± 0.79	10.51 ± 0.45	10.87 ± 0.39	10.04 ± 0.26	8.93 ± 1.31	8.59 ± 0.19
Sugar / Acid ratio	5.28 ± 0.10	5.31 ± 0.08	3.70 ± 0.12	4.15 ± 0.14	3.35 ± 0.19	3.96 ± 0.10	5.23 ± 0.02	5.34 ± 0.17
Luteine (mg kg DM^{-1})	6.71 ± 0.37	6.42 ± 0.27	11.91 ± 0.65	11.12 ± 0.67	6.88 ± 0.87	10.01 ± 0.37	9.90 ± 0.46	6.76 ± 0.50
Lycopene (mg kg DM^{-1})	652.53 ± 35.09	503.94 ± 15.38	1170.66 ± 86.31	1405.73 ± 140.71	749.58 ± 54.62	1006.38 ± 53.56	674.47 ± 25.52	772.74 ± 65.64
Beta-carotene (mg kg DM^{-1})	45.58 ± 1.75	41.10 ± 0.84	63.52 ± 5.77	63.42 ± 7.48	72.17 ± 7.36	89.83 ± 5.41	46.62 ± 0.98	37.20 ± 2.79
Phytoene (mg kg DM^{-1})	172.56 ± 9.79	138.54 ± 4.89	133.12 ± 15.93	173.80 ± 14.72	113.19 ± 13.00	168.21 ± 16.27	154.40 ± 11.45	196.51 ± 14.81
Total carotenoid (mg kg DM^{-1})	877.38 ± 43.82	690 ± 20.51	1379.21 ± 103.78	1654.06 ± 159.25	941.82 ± 71.93	1274.43 ± 70.14	885.39 ± 38.41	1013.20 ± 78.96
Total AsA (mg 100 g DM^{-1})	341.02 ± 11.36	319.72 ± 5.86	303.36 ± 12.69	312.05 ± 13.12	230.75 ± 12.38	283.37 ± 23.99	211.70 ± 8.03	175.14 ± 8.40
Reduced AsA (mg 100 g DM^{-1})	335.88 ± 11.10	305.73 ± 6.33	301.30 ± 11.25	292.23 ± 15.69	221.62 ± 12.51	279.34 ± 25.76	195.39 ± 2.14	164.05 ± 7.06
Red / Tot AsA	0.98 ± 0.01	0.96 ± 0.03	0.99 ± 0.02	0.94 ± 0.02	0.96 ± 0.01	0.98 ± 0.01	0.92 ± 0.02	0.94 ± 0.01

Supplementary data 5: Fruit size, fresh weight, dry matter content and composition (sugars, acids, carotenoids and ascorbic acid) on a dry weight basis measured on the S1 lot for the 4 genotypes *S.l. var. cerasiforme* (Cervil, Criollo, LA1420 and PlovdivXXIVa). Data are means ± SE ($n \geq 5$). Symbols * indicated significant differences between treatments for each genotype for $P < 0.05$ (Tukey test).

Genotype	Stupicke		Ferum		LA0147		Levovil	
Treatment	Control	Alternation	Control	Alternation	Control	Alternation	Control	Alternation
Fruit size (mm)	53.38 ± 0.26	51.48 ± 0.98	66.65 ± 0.88	64.21 ± 0.54	67.16 ± 1.91	65.90 ± 1.87	78.64 ± 2.92	74.27 ± 0.71
Fresh weight (g)	81.69 ± 1.14	72.93 ± 2.99	146.63 ± 4.47	130.44 ± 3.80	141.68 ± 11.68	134.25 ± 10.68	219.98 ± 24.93	192.08 ± 3.96
Dry matter content (g 100g FW^{-1})	6.44 ± 0.11	7.26 ± 0.09 *	6.94 ± 0.14	7.29 ± 0.23	6.00 ± 0.09	6.13 ± 0.10	5.11 ± 0.14	5.33 ± 0.19
Glucose (g 100 g DM^{-1})	21.58 ± 0.62	20.95 ± 0.37	19.37 ± 0.48	20.68 ± 0.57	20.49 ± 0.18	19.88 ± 0.59	20.56 ± 0.34	21.77 ± 1.00
Fructose (g 100 g DM^{-1})	24.75 ± 0.74	24.31 ± 0.48	19.02 ± 0.47	19.97 ± 0.59	20.11 ± 0.18	19.78 ± 0.84	22.49 ± 0.32	23.22 ± 0.91
Sucrose (g 100 g DM^{-1})	0.92 ± 0.10	1.00 ± 0.04	0.49 ± 0.09	0.67 ± 0.09	0.55 ± 0.04	0.60 ± 0.03	0.35 ± 0.04	0.27 ± 0.02
Total sugar (g 100 g DM^{-1})	47.25 ± 1.40	46.26 ± 0.80	38.87 ± 0.97	41.33 ± 1.21	41.14 ± 0.38	40.27 ± 1.46	43.40 ± 0.65	45.26 ± 1.90
Citric acid (g 100 g DM^{-1})	5.41 ± 0.25	5.16 ± 0.43	3.54 ± 0.13	3.36 ± 0.09	5.15 ± 0.51	4.13 ± 0.36	3.50 ± 0.23	3.44 ± 0.16
Malic acid (g 100 g DM^{-1})	1.37 ± 0.08	1.11 ± 0.07	1.34 ± 0.07	1.36 ± 0.11	1.84 ± 0.23	1.28 ± 0.12 *	1.95 ± 0.09	1.97 ± 0.08
Quinic acid (g 100 g DM^{-1})	4.63 ± 0.13	5.54 ± 0.65	3.77 ± 0.08	4.07 ± 0.10	4.27 ± 0.36	3.82 ± 0.17	4.12 ± 0.11	4.17 ± 0.18
Total acid (g 100 g DM^{-1})	11.41 ± 0.44	11.81 ± 1.15	8.65 ± 0.16	8.79 ± 0.22	11.26 ± 1.07	9.23 ± 0.28	9.57 ± 0.16	9.58 ± 0.37
Sugar / Acid ratio	4.15 ± 0.05	4.04 ± 0.32	4.50 ± 0.08	4.70 ± 0.06	3.77 ± 0.30	4.38 ± 0.28	4.53 ± 0.08	4.72 ± 0.02
Luteine (mg kg DM^{-1})	20.39 ± 2.12	20.78 ± 1.73	12.22 ± 0.85	10.36 ± 0.58	15.88 ± 0.61	15.31 ± 0.48	20.61 ± 1.76	17.70 ± 0.71
Lycopene (mg kg DM^{-1})	1097.81 ± 84.61	970.13 ± 116.06	1101.26 ± 48.81	989.87 ± 132.62	1230.93 ± 138.13	1434.40 ± 248.94	1192.35 ± 133.02	1045.79 ± 48.30
Beta-carotene (mg kg DM^{-1})	72.50 ± 3.37	78.86 ± 6.20	46.84 ± 2.08	43.62 ± 3.21	48.31 ± 3.95	49.25 ± 1.18	97.9 ± 5.46	77.94 ± 5.70 *
Phytoene (mg kg DM^{-1})	126.36 ± 13.23	99.53 ± 14.61	243.70 ± 15.24	228.66 ± 39.68	156.30 ± 25.94	202.25 ± 54.29	182.36 ± 23.14	159.88 ± 14.03
Total carotenoid (mg kg DM^{-1})	1317.06 ± 99.32	1169.30 ± 136.01	1404.01 ± 61.76	1272.51 ± 173.57	1451.41 ± 166.55	1701.21 ± 302.07	1493.22 ± 157.96	1301.31 ± 56.61
Total AsA (mg 100 g DM^{-1})	300.43 ± 13.50	285.05 ± 9.29	220.30 ± 4.36	217.48 ± 7.19	260.59 ± 21.57	255.42 ± 22.03	264.32 ± 10.37	232.29 ± 13.72
Reduced AsA (mg 100 g DM^{-1})	301.11 ± 13.34	277.67 ± 9.22	223.84 ± 4.93	208.20 ± 10.54	263.47 ± 25.27	262.35 ± 26.56	222.73 ± 14.59	184.46 ± 15.98
Red / Tot AsA	1.00 ± 0.02	0.98 ± 0.03	1.02 ± 0.01	0.96 ± 0.03	1.01 ± 0.01	1.02 ± 0.02	0.84 ± 0.03	0.80 ± 0.07

Supplementary data 6: Fruit size, fresh weight, dry matter content and composition (sugars, acids, carotenoids and ascorbic acid) on a dry weight basis measured on the S1 lot for the 4 genotypes *S.l. var. esculentum* (Stupicke Polni Rane, Ferum, LA0147 and Levovil). Data are means ± SE ($n \geq 5$). Symbols * indicated significant differences between treatments for each genotype for $P < 0.05$ (Tukey test).

Genotype	Cervil		Criollo		LA1420		Plovdiv	
Treatment	Control	Alternation	Control	Alternation	Control	Alternation	Control	Alternation
Fruit size (mm)	21.00 ± 0.52	20.69 ± 0.53	32.44 ± 3.73	29.28 ± 3.96	45.55 ± 1.70	42.17 ± 2.73	42.07 ± 0.65	38.25 ± 0.98
Fresh weight (g)	5.1 ± 0.34	5.09 ± 0.31	15.85 ± 5.00	12.52 ± 4.60	40.83 ± 4.78	32.59 ± 5.57	46.42 ± 2.12	35.42 ± 2.96
Dry matter content (g 100g FW^{-1})	10.39 ± 0.41	12.56 ± 0.21 *	6.86 ± 0.32	7.82 ± 0.73	6.16 ± 0.18	7.54 ± 0.64 *	8.46 ± 0.14	9.22 ± 0.21
Glucose (g 100 g DM^{-1})	16.27 ± 0.88	20.15 ± 0.40	18.02 ± 1.34	17.54 ± 1.03	18.17 ± 0.61	19.15 ± 0.84	23.61 ± 0.40	24.49 ± 0.42
Fructose (g 100 g DM^{-1})	16.81 ± 0.64	20.44 ± 0.42	20.88 ± 0.50	20.56 ± 1.06	18.89 ± 0.60	19.07 ± 0.76	21.28 ± 0.28	22.02 ± 0.64
Sucrose (g 100 g DM^{-1})	1.24 ± 0.12	1.84 ± 0.03 *	0.49 ± 0.17	1.00 ± 0.30	0.60 ± 0.09	0.85 ± 0.24	1.62 ± 0.14	1.54 ± 0.17
Total sugar (g 100 g DM^{-1})	34.33 ± 1.59	42.44 ± 0.80	39.39 ± 1.76	39.1 ± 1.86	37.66 ± 1.25	39.07 ± 1.65	46.50 ± 0.66	48.04 ± 0.94
Citric acid (g 100 g DM^{-1})	3.51 ± 0.12	3.37 ± 0.11	5.89 ± 0.60	5.97 ± 0.16	7.34 ± 0.61	5.36 ± 0.30 *	3.43 ± 0.18	3.27 ± 0.09
Malic acid (g 100 g DM^{-1})	0.59 ± 0.10	0.56 ± 0.09	0.68 ± 0.11	0.65 ± 0.13	0.78 ± 0.12	0.90 ± 0.17	1.05 ± 0.08	1.09 ± 0.08
Quinic acid (g 100 g DM^{-1})	3.24 ± 0.14	3.96 ± 0.08 *	4.5 ± 0.10	4.36 ± 0.25	4.10 ± 0.27	3.83 ± 0.18	4.21 ± 0.14	4.42 ± 0.15
Total acid (g 100 g DM^{-1})	7.34 ± 0.21	7.88 ± 0.24	11.07 ± 0.59	10.98 ± 0.31	12.23 ± 0.91	10.08 ± 0.45	8.69 ± 0.30	8.78 ± 0.20
Sugar / Acid ratio	4.70 ± 0.24	5.4 ± 0.15	3.60 ± 0.37	3.56 ± 0.12	3.12 ± 0.15	3.89 ± 0.15	5.37 ± 0.18	5.47 ± 0.05
Luteine (mg kg DM^{-1})	6.61 ± 0.42	6.40 ± 0.36	18.18 ± 2.99	19.79 ± 2.03	11.63 ± 1.53	9.19 ± 0.41	7.27 ± 0.47	9.37 ± 1.05
Lycopene (mg kg DM^{-1})	845.54 ± 52.20	591.85 ± 33.06	2435.89 ± 134.71	2148.65 ± 454.56	1349.32 ± 81.68	1293.46 ± 60.92	1174.22 ± 100.60	1188.26 ± 49.76
Beta-carotene (mg kg DM^{-1})	45.65 ± 1.86	38.42 ± 2.13	70.64 ± 4.01	73.29 ± 7.57	62.15 ± 5.00	45.91 ± 1.72	40.91 ± 1.73	39.90 ± 2.28
Phytoene (mg kg DM^{-1})	150.48 ± 15.97	122.88 ± 7.77	219.52 ± 30.91	194.33 ± 48.65	152.58 ± 27.94	154.27 ± 10.24	227.96 ± 21.76	207.03 ± 15.26
Total carotenoid (mg kg DM^{-1})	1048.29 ± 68.96	759.54 ± 29.41	2744.24 ± 170.44	2436.07 ± 493.51	1575.68 ± 102.57	1502.84 ± 71.24	1450.36 ± 119.24	1444.56 ± 66.33
Total AsA (mg 100 g DM^{-1})	267.98 ± 13.35	274.89 ± 10.29	370.07 ± 23.79	342.68 ± 26.60	359.39 ± 30.97	396.85 ± 38.27	219.26 ± 17.31	262.70 ± 13.87
Reduced AsA (mg 100 g DM^{-1})	252.05 ± 10.09	271.41 ± 9.62	387.61 ± 28.47	345.36 ± 26.70	350.89 ± 26.28	391.80 ± 44.12	198.17 ± 27.38	261.14 ± 13.69
Red / Tot AsA	0.94 ± 0.02	0.99 ± 0.01	1.04 ± 0.01	1.01 ± 0.02	0.98 ± 0.03	0.98 ± 0.02	0.90 ± 0.09	0.99 ± 0.02

Supplementary data 7: Fruit size, fresh weight, dry matter content and composition (sugars, acids, carotenoids and ascorbic acid) on a dry weight basis measured on the S2 lot for the 4 genotypes *S.l. var. cerasiforme* (Cervil, Criollo, LA1420 and PlovdivXXIVa). Data are means ± SE ($n \geq 5$). Symbols * indicated significant differences between treatments for each genotype for $P < 0.05$ (Tukey test).

Genotype	Stupicke		Ferum		LA0147		Levovil	
Treatment	Control	Alternation	Control	Alternation	Control	Alternation	Control	Alternation
Fruit size (mm)	52.95 ± 1.44	49.38 ± 1.00	60.96 ± 1.32	60.38 ± 1.44	62.70 ± 0.67	58.32 ± 2.09	62.65 ± 4.11	53.42 ± 3.03
Fresh weight (g)	76.29 ± 5.41	64.02 ± 2.90	115.18 ± 5.02	114.71 ± 8.16	116.18 ± 3.59	94.62 ± 8.38	140.09 ± 18.15	80.08 ± 14.01
Dry matter content (g 100g FW^{-1})	7.21 ± 0.11	7.84 ± 0.08	8.82 ± 0.19	7.98 ± 0.20	6.99 ± 0.16	7.25 ± 0.63	5.90 ± 0.12	7.89 ± 0.13 *
Glucose (g 100 g DM^{-1})	21.2 ± 0.09	24.69 ± 3.44	22.15 ± 0.60	22.32 ± 0.73	21.28 ± 1.10	20.9 ± 0.40	22.35 ± 0.66	24.66 ± 1.03
Fructose (g 100 g DM^{-1})	23.51 ± 0.40	26.39 ± 3.25	20.50 ± 0.57	21.70 ± 0.90	19.33 ± 1.05	19.02 ± 0.35	22.46 ± 0.49	24.26 ± 1.08
Sucrose (g 100 g DM^{-1})	0.95 ± 0.09	1.54 ± 0.21 *	0.61 ± 0.13	0.52 ± 0.03	1.07 ± 0.08	1.23 ± 0.24	0.59 ± 0.10	0.97 ± 0.16
Total sugar (g 100 g DM^{-1})	45.66 ± 0.44	52.62 ± 6.86	43.25 ± 1.26	44.54 ± 1.63	41.67 ± 2.11	41.15 ± 0.62	45.41 ± 1.15	49.88 ± 2.26
Citric acid (g 100 g DM^{-1})	4.69 ± 0.10	4.39 ± 0.12	3.47 ± 0.09	3.65 ± 0.25	4.36 ± 0.24	4.45 ± 0.08	4.36 ± 0.35	3.42 ± 0.16
Malic acid (g 100 g DM^{-1})	0.98 ± 0.05	0.94 ± 0.04	0.95 ± 0.09	0.83 ± 0.06	1.51 ± 0.17	1.52 ± 0.11	1.84 ± 0.14	1.23 ± 0.10 *
Quinic acid (g 100 g DM^{-1})	4.23 ± 0.08	4.22 ± 0.11	3.81 ± 0.10	4.21 ± 0.19	3.79 ± 0.17	3.78 ± 0.06	4.42 ± 0.10	4.75 ± 0.25
Total acid (g 100 g DM^{-1})	9.90 ± 0.20	9.55 ± 0.21	8.22 ± 0.20	8.69 ± 0.42	9.65 ± 0.29	9.75 ± 0.13	10.62 ± 0.41	9.40 ± 0.47
Sugar / Acid ratio	4.62 ± 0.06	5.58 ± 0.89	5.27 ± 0.15	5.15 ± 0.17	4.34 ± 0.30	4.23 ± 0.11	4.31 ± 0.23	5.31 ± 0.10
Luteine (mg kg DM^{-1})	19.58 ± 1.02	21.70 ± 0.98	10.48 ± 0.51	13.56 ± 0.74	15.89 ± 0.82	21.12 ± 4.18	16.57 ± 1.53	15.26 ± 0.91
Lycopene (mg kg DM^{-1})	1493.37 ± 42.76	1317.75 ± 75.71	1157.64 ± 86.05	1636.25 ± 22.82	1203.81 ± 112.40	1309.67 ± 199.81	1553.84 ± 131.63	1400.64 ± 80.62
Beta-carotene (mg kg DM^{-1})	66.93 ± 1.87	61.77 ± 2.75	33.69 ± 1.63	36.60 ± 2.22	47.05 ± 3.51	50.07 ± 8.31	84.73 ± 7.84	63.93 ± 3.35 *
Phytoene (mg kg DM^{-1})	129.71 ± 9.22	125.75 ± 6.65	189.92 ± 21.81	273.92 ± 10.88	117.48 ± 17.33	121.89 ± 12.32	128.67 ± 13.48	128.34 ± 9.76
Total carotenoid (mg kg DM^{-1})	1709.59 ± 50.49	1526.96 ± 80.94	1391.73 ± 108.47	1960.32 ± 34.15	1384.23 ± 129.97	1502.75 ± 220.47	1783.81 ± 143.11	1608.17 ± 87.28
Total AsA (mg 100 g DM^{-1})	327.15 ± 15.28	295.01 ± 20.01	237.56 ± 3.89	261.05 ± 16.47	301.32 ± 17.81	314.39 ± 32.69	314.3 ± 12.50	264.68 ± 15.42
Reduced AsA (mg 100 g DM^{-1})	316.12 ± 17.96	293.32 ± 18.42	233.37 ± 3.23	260.82 ± 19.62	303.89 ± 18.56	321.56 ± 36.90	289.95 ± 10.25	263.64 ± 12.81
Red / Tot AsA	0.96 ± 0.02	1.00 ± 0.02	0.98 ± 0.02	1.00 ± 0.03	1.01 ± 0.02	1.02 ± 0.02	0.93 ± 0.03	1.00 ± 0.02

Supplementary data 8: Fruit size, fresh weight, dry matter content and composition (sugars, acids, carotenoids and ascorbic acid) on a dry weight basis measured on the S2 lot for the 4 genotypes *S.l. var. esculentum* (Stupicke Polni Rane, Ferum, LA0147 and Levovil). Data are means ± SE ($n \geq 5$). Symbols * indicated significant differences between treatments for each genotype for $P < 0.05$ (Tukey test).

Annexes du Chapitre III

Fruit quality traits	LA1420						Plovdiv XXIVa					
	Control	CD	Control	CE	Control	MT	Control	CD	Control	CE	Control	MT
Total sugar (g 100 g DM^{-1})	27.66 ± 0.90 **ab**	33.40 ± 0.73 **d**	26.25 ± 0.21 **bc**	30.37 ± 0.64 **b**	23.88 ± 0.92 **c**	28.66 ± 0.94 **ab**	36.83 ± 0.48 **a**	38.35 ± 1.15 **a**	39.56 ± 0.53 **ab**	38.15 ± 0.21 **a**	41.65 ± 1.14 **b**	37.51 ± 0.43 **a**
Glucose (g 100 g DM-1)	11.78 ± 0.55 **a**	15.01 ± 0.36 **d**	11.24 ± 0.09 **ab**	13.54 ± 0.37 **c**	9.92 ± 0.46 **b**	12.32 ± 0.43 **ac**	18.52 ± 0.23 **ab**	19.28 ± 0.53 **ab**	19.36 ± 0.16 **ab**	18.56 ± 0.13 **ab**	19.77 ± 0.59 **b**	17.93 ± 0.16 **a**
Fructose (g 100 g DM-1)	14.87 ± 0.32 **abc**	17.21 ± 0.36 **d**	14.12 ± 0.16 **ab**	16.00 ±0.34 **cd**	13.38 ± 0.45 **a**	15.59 ± 0.55 **bc**	17.36 ± 0.32 **a**	18.00 ± 0.63 **ab**	19.22 ± 0.33 **b**	18.59 ± 0.24 **ab**	20.83 ± 0.54 **c**	18.28 ± 0.37 **ab**
Sucrose (g 100 g DM-1)	1.01 ± 0.06 **ab**	1.18 ± 0.02 **b**	0.89 ± 0.01 **ab**	0.83 ± 0.04 **ac**	0.57 ± 0.03 **c**	0.75 ± 0.06 **ac**	0.95 ± 0.07 **a**	1.07 ± 0.14 **ab**	0.98 ± 0.08 **a**	1.00 ± 0.07 **a**	1.05 ± 0.11 **ab**	1.30 ± 0.11 **b**
Total acid (g 100 g DM-1)	12.56 ± 0.14 **a**	11.09 ± 0.39 **b**	12.65 ± 0.37 **a**	11.06 ± 0.20 **b**	12.86 ± 0.32 **a**	12.06 ± 0.21 **ab**	8.62 ± 0.30 **ab**	8.07 ± 0.05 **a**	9.10 ± 0.47 **abc**	8.91 ± 0.19 **ab**	10.15 ± 0.22 **c**	9.39 ± 0.27 **bc**
Citric acid (g 100 g DM-1)	8.97 ± 0.12 **a**	7.34 ± 0.34 **b**	9.09 ± 0.34 **a**	7.45 ± 0.19 **b**	9.20 ± 0.47 **a**	8.00 ± 0.40 **b**	3.42 ± 0.10 **a**	3.23 ± 0.04 **a**	3.52 ± 0.17 **a**	3.54 ± 0.11 **a**	4.13 ± 0.08 **a**	3.49 ± 0.07 **a**
Malic acid (g 100 g DM-1)	0.60 ± 0.04 **a**	0.40 ± 0.04 **a**	0.68 ± 0.03 **a**	0.42 ± 0.02 **a**	0.93 ± 0.19 **a**	0.83 ± 0.10 **a**	2.03 ± 0.16 **abc**	1.49 ± 0.08 **a**	2.06 ± 0.29 **bc**	1.98 ± 0.13 **ab**	2.17 ± 0.14 **bc**	2.56 ± 0.24 **c**
Quinic acid (g 100 g DM-1)	2.99 ± 0.06 **abc**	3.35 ± 0.06 **d**	2.89 ± 0.04 **ab**	3.18 ± 0.08 **bcd**	2.72 ± 0.06 **a**	3.22 ± 0.15 **cd**	3.17 ± 0.09 **a**	3.35 ± 0.10 **ab**	3.52 ± 0.07 **b**	3.38 ± 0.03 **ab**	3.85 ± 0.09 **c**	3.34 ± 0.06 **ab**
Total carotenoid (mg kg DM^{-1})	1163.44 ± 155.62 **ab**	859.61 ± 139.63 **a**	1318.04 ± 130.03 **ab**	1261.07 ± 147.87 **ab**	1569.41 ± 242.03 **b**	1574.80 ± 166.61 **b**	1106.49 ± 188.96 **a**	1043.02 ± 141.43 **a**	1354.35 ± 149.73 **a**	1260.00 ± 259.57 **a**	1431.37 ± 171.34 **a**	941.19 ± 175.21 **a**
Luteine (mg kg DM-1)	6.35 ± 0.68 **a**	5.18 ± 0.30 **a**	8.74 ± 0.38 **b**	6.20 ± 0.38 **a**	8.59 ± 0.65 **b**	4.84 ± 0.34 **a**	6.73 ± 0.34 **a**	5.92 ± 0.48 **ab**	5.22 ± 0.25 **ab**	6.19 ± 0.54 **a**	5.71 ± 0.31 **ab**	4.33 ± 0.25 **b**
Lycopene (mg kg DM-1)	690.59 ± 41.70 **ab**	499.85 ± 70.14 **a**	774.26 ± 68.52 **bc**	764.43 ± 49.61 **bc**	942.12 ± 98.00 **c**	958.99 ± 77.63 **c**	713.85 ± 52.48 **ab**	656.70 ± 44.49 **ab**	877.67 ± 60.59 **b**	811.98 ± 67.22 **ab**	839.74 ± 85.43 **ab**	597.87 ± 52.21 **a**
Beta-carotene (mg kg DM-1)	76.49 ± 3.87 **a**	82.60 ± 3.83 **a**	87.28 ± 6.49 **a**	82.78 ± 2.19 **a**	88.43 ± 6.27 **a**	63.76 ± 3.59 **b**	37.45 ± 0.73 **a**	40.76 ± 1.09 **a**	33.32 ± 1.12 **a**	34.89 ± 2.86 **a**	34.86 ± 3.87 **a**	30.81 ± 0.41 **a**
Phytoene (mg kg DM-1)	226.09 ± 11.85 **ab**	139.58 ± 13.44 **a**	258.63 ± 25.43 **bc**	226.54 ± 18.22 **ab**	301.46 ± 33.20 **bc**	325.38 ± 40.56 **c**	186.14 ± 16.11 **a**	189.77 ± 16.81 **a**	242.51 ± 23.61 **a**	222.21 ± 19.35 **a**	357.08 ± 42.54 **b**	171.00 ± 18.24 **a**
Total AsA (mg 100 g DM^{-1})	224.69 ± 10.94 **a**	234.49 ± 20.69 **a**	267.39 ± 35.87 **a**	235.58 ± 9.64 **a**	259.68 ± 41.44 **a**	226.60 ± 27.93 **a**	139.15 ± 1.43 **a**	154.97 ± 7.35 **a**	140.28 ± 9.79 **a**	138.87 ± 23.06 **a**	154.01 ± 4.26 **a**	104.38 ± 9.75 **a**

xSupplementary data 1: Effect of deficit irrigation (DI) treatments on sugars, organic acids, carotenoids and ascorbic acid (AsA) of LA1420 and PlovdivXXIVa fruits. CD treatments corresponds to the DI treatment applied during the cell division period, CE to the DI treatment applied during the cell expansion period, and MT to the DI treatment applied during the fruit maturation period. The composition in metabolites is expressed on a dry weight basis. Data are means ± SE (n ≥ 5). Each sample was made of 2 to 5 fruits pooled together. Different letters for a given parameter correspond to significant differences at the P = 0.05 threshold.

Fruit quality traits	LA1420						Plovdiv XXIVa					
	Control	CD	Control	CE	Control	MT	Control	CD	Control	CE	Control	MT
Total sugar (g 100 g FW^{-1})	1.94 ± 0.11 **ab**	2.43 ± 0.06 **c**	1.73 ± 0.02 **a**	1.99 ± 0.03 **b**	1.45 ± 0.06 **d**	1.90 ± 0.09 **ab**	2.96 ± 0.04 **a**	3.11 ± 0.07 **ab**	3.10 ± 0.04 **ab**	2.97 ± 0.07 **a**	3.26 ± 0.08 **b**	3.03 ± 0.04 **ab**
Glucose (g 100 g FW^{-1})	0.87 ± 0.04 **a**	1.11 ± 0.03 **d**	0.83 ± 0.01 **ab**	1.00 ± 0.03 **c**	0.73 ± 0.03 **b**	0.91 ± 0.03 **ac**	1.36 ± 0.02 **ab**	1.42 ± 0.04 **ab**	1.43 ± 0.01 **ab**	1.37 ± 0.01 **ab**	1.46 ± 0.04 **b**	1.32 ± 0.01 **a**
Fructose (g 100 g FW^{-1})	1.00 ± 0.06 **a**	1.24 ± 0.04 **c**	0.85 ± 0.02 **ab**	0.94 ±0.01 **a**	0.69 ± 0.03 **b**	0.95 ± 0.06 **a**	1.52 ± 0.02 **a**	1.60 ± 0.04 **ab**	1.60 ± 0.03 **ab**	1.52 ± 0.07 **a**	1.71 ± 0.04 **b**	1.60 ± 0.03 **ab**
Sucrose (g 100 g FW^{-1})	0.069 ± 0.007 **ac**	0.085 ± 0.003 **c**	0.054 ± 0.000 **ab**	0.049 ± 0.003 **ab**	0.030 ± 0.002 **b**	0.046 ± 0.005 **ab**	0.083 ± 0.006 **a**	0.095 ± 0.012 **ab**	0.081 ± 0.006 **a**	0.082 ± 0.007 **a**	0.087 ± 0.009 **a**	0.114 ± 0.010 **b**
Total acid (g 100 g FW^{-1})	0.84 ± 0.04 **a**	0.80 ± 0.03 **ab**	0.76 ± 0.03 **abc**	0.65 ± 0.02 **c**	0.66 ± 0.02 **c**	0.73 ± 0.03 **bc**	0.75 ± 0.01 **ab**	0.72 ± 0.02 **a**	0.75 ± 0.03 **ab**	0.73 ± 0.04 **a**	0.84 ± 0.03 **b**	0.82 ± 0.03 **ab**
Citric acid (g 100 g FW^{-1})	0.60 ± 0.03 **a**	0.53 ± 0.03 **b**	0.54 ± 0.03 **ab**	0.44 ± 0.02 **c**	0.48 ± 0.03 **bc**	0.49 ± 0.03 **bc**	0.30 ± 0.01 **a**	0.29 ± 0.01 **a**	0.29 ± 0.01 **a**	0.29 ± 0.02 **a**	0.34 ± 0.01 **a**	0.31 ± 0.01 **a**
Malic acid (g 100 g FW^{-1})	0.040 ± 0.003 **a**	0.029 ± 0.002 **a**	0.041 ± 0.002 **a**	0.025 ± 0.001 **a**	0.048 ± 0.009 **a**	0.050 ± 0.005 **a**	0.176 ± 0.010 **a**	0.134 ± 0.010 **a**	0.170 ± 0.022 **a**	0.163 ± 0.016 **a**	0.180 ± 0.013 **ab**	0.223 ± 0.022 **b**
Quinic acid (g 100 g FW^{-1})	0.201 ± 0.012 **a**	0.242 ± 0.006 **b**	0.173 ± 0.002 **ac**	0.188 ± 0.002 **a**	0.141 ± 0.003 **c**	0.196 ± 0.014 **a**	0.277 ± 0.004 **a**	0.297 ± 0.007 **ab**	0.293 ± 0.007 **ab**	0.276 ± 0.011 **a**	0.317 ± 0.012 **b**	0.291 ± 0.004 **ab**
Total carotenoid (mg kg FW^{-1})	66.71 ± 2.54 **ab**	52.32 ± 5.62 **a**	69.18 ± 5.95 **ab**	63.67 ± 3.78 **ab**	69.21 ± 6.19 **ab**	81.90 ± 7.28 **b**	82.42 ± 5.95 **ab**	79.14 ± 4.52 **ab**	96.56 ± 8.04 **b**	86.83 ± 4.44 **ab**	101.74 ± 9.78 **b**	70.08 ± 5.68 **a**
Luteine (mg kg FW^{-1})	0.42 ± 0.04 **ab**	0.37 ± 0.02 **ac**	0.52 ± 0.03 **b**	0.37 ± 0.03 **ac**	0.45 ± 0.04 **ab**	0.29 ± 0.02 **c**	0.59 ± 0.04 **a**	0.53 ± 0.05 **ab**	0.43 ± 0.02 **bc**	0.50 ± 0.04 **abc**	0.47 ± 0.02 **abc**	0.38 ± 0.02 **c**
Lycopene (mg kg FW^{-1})	46.00 ± 1.76 **ab**	35.94 ± 4.74 **a**	47.63 ± 4.44 **ab**	45.07 ± 2.82 **ab**	48.60 ± 4.76 **ab**	58.01 ± 4.81 **b**	62.33 ± 4.61 **ab**	58.21 ± 3.44 **ab**	73.14 ± 5.99 **b**	65.55 ± 3.33 **ab**	69.02 ± 6.69 **b**	52.10 ± 4.31 **a**
Beta-carotene (mg kg FW^{-1})	5.11 ± 0.20 **a**	5.97 ± 0.33 **b**	4.88 ± 0.20 **a**	4.89 ± 0.16 **a**	4.58 ± 0.35 **ac**	3.88 ± 0.29 **c**	3.27 ± 0.08 **ab**	3.63 ± 0.15 **b**	2.77 ± 0.11 **a**	2.81 ± 0.13 **ab**	2.87 ± 0.32 **ab**	2.69 ± 0.04 **a**
Phytoene (mg kg FW^{-1})	15.18 ± 0.99 **ab**	10.04 ± 0.87 **a**	16.16 ± 1.54 **ab**	13.34 ± 0.99 **ab**	15.58 ± 1.69 **ab**	19.71 ± 2.63 **b**	16.23 ± 1.36 **a**	16.77 ± 1.18 **a**	20.22 ± 2.18 **a**	17.96 ± 1.20 **a**	29.38 ± 3.45 **b**	14.91 ± 1.57 **a**
Total AsA (mg 100 g FW^{-1})	15.0 ± 0.47 **a**	16.9 ± 1.41 **a**	16.0 ± 2.00 **a**	13.9 ± 0.40 **a**	13.4 ± 2.00 **a**	13.8 ± 1.87 **a**	12.2 ± 0.35 **a**	13.8 ± 0.53 **a**	11.7 ± 0.96 **a**	11.1 ± 1.27 **a**	12.7 ± 0.28 **a**	9.1 ± 0.85 **a**

Supplementary data 2: Effect of deficit irrigation (DI) treatments on sugars, organic acids, carotenoids and ascorbic acid (AsA) of LA1420 and Plovdiv XXIVa fruits CD treatment corresponds to the DI treatment applied during the cell division period, CE to the DI treatment applied during the cell expansion period, and MT to the DI treatment applied during the fruit maturation period. The composition in metabolites is expressed on a fresh weight basis. Data are means ± SE (n ≥ 5). Each sample was made of 2 to 5 fruits pooled together. Different letters for a given parameter correspond to significant differences at the P = 0.05 threshold.

Tissue	Parameters	Effect of WD treatment	Genotype effect	Interactions effect	Seasonal effect on control plants
Leaflets	$\Psi_{S\ predawn}$	***	.		NA
	$\Psi_{S\ midday}$	*	.		NA
	$\psi\pi_L$	***			NA
	Stomatal conductance	***			.
	Transpiration rate	***	***	***	*
	SLA	***	*	*	NA
	DM	***	***		
Fruits	Size	***	***	***	.
	FW	***	***	***	.
	DM	***	***	*	
	Ψ_F	**	***		NA
	$\psi\pi_F$	***	***	**	NA
	Total sugar	***	***	***	***
	Total acid	***	***	*	
	Total carotenoid	.			
	Total AsA		***		***
Seed	Number	**	***	*	NA
	Weight	**	***	.	NA

Supplementary data 3: Effects of WD treatment (mean for all WD compared with mean of controls), genotype (all treatments averaged), their interactions (mean for all WD compared with mean of controls) and seasonal effect for control plants only (comparison of controls plants grown in spring 2012 with controls plants grown in autumn 2012 in Avignon) for the two genotypes LA1420 and PlovdivXXIVa.

Measured were: stem water potential at predawn and midday ($\Psi_{Spredawn}$ and $\Psi_{Smidday}$), stomatal conductance and transpiration rates, SLA: specific leaf surface area, DM: dry matter content in leaflets and fruits, fruit size and fresh mass (FW), Ψ_F: fruit water potential, $\Psi\pi_F$: fruit osmotic potential, fruit total sugar, acid, carotenoid and ascorbic acid (AsA) contents, and seed number and weight. Significant differences are indicated using (.) symbols for $P < 0.10$, * symbols for $P < 0.05$, ** symbols for $P < 0.01$, *** symbols for $P < 0.001$ and NA correspond to the absence of measures.

Annexes du Chapitre IV

Genotype	Treatment	Substrate humidity (%)	Stomatal conductance (mmol m^{-2} s^{-1})	SPAD units (UA)	Dry matter content (g 100g FW^{-1})	Dry mass (g)	Leaf surface area (cm^2)	SLA (cm^2 g^{-1})	Mass per surface (g cm^{-2})
Cervil	Initial value	66.2 ± 0.9	200.60 ± 22.80	33.37 ± 1.16	11.90 ± 0.31	0.01 ± 0.00	7.63 ± 0.50	536.55 ± 24.85	0.002 ± 0.000
Cervil	Stress	20.8 ± 1.2 *	93.25 ± 17.35 *	37.92 ± 1.07 *	18.57 ± 1.26 *	0.03 ± 0.00 *	10.03 ± 0.79 *	434.03 ± 153.05	0.003 ± 0.001 *
LA1420	Initial value	55.2 ± 3.3	205.32 ± 44.00	28.36 ± 0.83	11.40 ± 0.18	0.01 ± 0.00	8.34 ± 0.59	584.07 ± 34.25	0.002 ± 0.000
LA1420	Stress	12.7 ± 1.7 *	133.00 ± 20.07 *	28.39 ± 0.95	13.44 ± 0.44 *	0.03 ± 0.00 *	11.82 ± 1.37 *	425.54 ± 32.04 *	0.002 ± 0.000
Plovdiv	Initial value	57.1 ± 1.9	182.20 ± 18.61	31.22 ± 0.87	14.60 ± 0.42	0.02 ± 0.00	6.92 ± 0.51	411.45 ± 14.90	0.002 ± 0.000
Plovdiv	Stress	16.7 ± 0.9 *	61.81 ± 8.38 *	33.79 ± 0.74 *	22.46 ± 0.90 *	0.04 ± 0.01 *	11.63 ± 0.46 *	310.13 ± 59.16	0.004 ± 0.001 *
Levovil	Initial value	58.7 ± 3.7	229.10 ± 17.80	32.76 ± 0.97	13.84 ± 0.49	0.02 ± 0.00	6.17 ± 0.81	389.04 ± 21.14	0.003 ± 0.000
Levovil	Stress	14.1 ± 2.1 *	124.65 ± 21.51 *	34.97 ± 0.79 *	16.12 ± 1.04 *	0.05 ± 0.00 *	13.22 ± 0.81 *	281.36 ± 10.70	0.004 ± 0.000 *

Genotype	Treatment	Fo (UA)	Fk (UA)	Fj (UA)	Fm (UA)	Fv (UA)	Fv/Fm (UA)	Sm (UA)	N (UA)
Cervil	Initial value	306.75 ± 10.15	638.00 ± 20.56	958.75 ± 26.17	1936.5 ± 47.1	1629.75 ± 37.27	0.842 ± 0.002	111.60 ± 49.61	230.60 ± 104.19
Cervil	Stress	352.80 ± 19.46 *	709.60 ± 28.42	1112.40 ± 32.72	2108.2 ± 104.3	1755.40 ± 91.66	0.832 ± 0.007	31.73 ± 1.71 *	59.50 ± 3.23 *
LA1420	Initial value	309.40 ± 10.84	736.20 ± 28.39	1095.20 ± 33.07	1943.8 ± 48.3	1634.40 ± 41.00	0.841 ± 0.004	32.61 ± 1.93	70.53 ± 3.15
LA1420	Stress	376.20 ± 30.59 *	802.20 ± 84.66	1138.80 ± 73.86	1810.0 ± 121.6	1433.80 ± 136.18	0.787 ± 0.027	21.88 ± 3.41 *	45.89 ± 3.75 *
Plovdiv	Initial value	339.20 ± 19.20	741.40 ± 25.70	1052.20 ± 35.01	1941.2 ± 90.2	1602.00 ± 75.80	0.825 ± 0.006	38.35 ± 3.14	86.37 ± 6.54
Plovdiv	Stress	333.80 ± 16.28	740.40 ± 41.18	1107.40 ± 44.12	1878.0 ± 51.6	1544.20 ± 37.16	0.823 ± 0.005	25.24 ± 0.72 *	52.78 ± 1.46 *
Levovil	Initial value	313.00 ± 9.92	690.40 ± 22.63	991.60 ± 32.44	1789.8 ± 74.7	1476.80 ± 65.54	0.825 ± 0.003	36.18 ± 1.46	80.45 ± 3.15
Levovil	Stress	345.20 ± 20.79 *	695.60 ± 48.32	1085.60 ± 61.58	1923.6 ± 119.7	1578.40 ± 99.44	0.820 ± 0.002	25.96 ± 1.32 *	48.76 ± 2.74 *

Genotype	Treatment	Vk	Vk/Vj	RC/ABS (UA)	Fv/Fo (UA)	(1-Vj)/Vj (UA)	PI (UA)	PI_{ABS}^{TOT}	$PI_{CS_0}^{TOT}$
Cervil	Initial value	0.20 ± 0.01	0.51 ± 0.01	1.04 ± 0.03	5.32 ± 0.07	1.50 ± 0.01	3.33 ± 0.13	6.12 ± 0.34	1017.62 ± 42.53
Cervil	Stress	0.20 ± 0.01	0.47 ± 0.01	1.03 ± 0.06	5.00 ± 0.26	1.31 ± 0.10	2.96 ± 0.37	2.05 ± 0.43 *	1040.37 ± 134.44
LA1420	Initial value	0.26 ± 0.01	0.54 ± 0.01	0.81 ± 0.03	5.30 ± 0.15	1.08 ± 0.04	2.25 ± 0.19	2.24 ± 0.21	688.63 ± 43.75
LA1420	Stress	0.32 ± 0.06	0.55 ± 0.05	0.75 ± 0.17	3.98 ± 0.60 *	0.92 ± 0.24	2.79 ± 0.40	1.21 ± 0.58	600.99 ± 227.16
Plovdiv	Initial value	0.25 ± 0.01	0.56 ± 0.01	0.82 ± 0.04	4.75 ± 0.18	1.24 ± 0.07	2.17 ± 0.19	2.90 ± 0.52	738.70 ± 77.37
Plovdiv	Stress	0.26 ± 0.01	0.52 ± 0.01	0.79 ± 0.04	4.65 ± 0.15	1.00 ± 0.03	1.85 ± 0.16	1.01 ± 0.13 *	608.90 ± 32.86
Levovil	Initial value	0.26 ± 0.00	0.56 ± 0.00	0.80 ± 0.01	4.71 ± 0.10	1.17 ± 0.03	2.06 ± 0.09	1.87 ± 0.14	645.52 ± 45.08
Levovil	Stress	0.22 ± 0.01	0.47 ± 0.01	0.94 ± 0.04	4.57 ± 0.07	1.14 ± 0.05	2.26 ± 0.10	1.29 ± 0.16	781.60 ± 63.57

Genotype	Treatment	J_0^{ABS}/RC (UA)	J_0^{TR}/RC (UA)	J_0^{DI}/RC (UA)	J_0^{ET2}/RC (UA)	J_0^{RE1}/RC	J_0^{TR}/CS_0	J_0^{DI}/CS_0	J_0^{ET2}/CS_0
Cervil	Initial value	2.41 ± 0.07	2.03 ± 0.06	0.38 ± 0.01	1.22 ± 0.03	0.79 ± 0.02	258.13 ± 8.09	48.62 ± 2.07	154.87 ± 4.68
Cervil	Stress	2.26 ± 0.05	1.87 ± 0.03 *	0.38 ± 0.02	1.06 ± 0.03	0.42 ± 0.03 *	293.36 ± 15.25 *	59.44 ± 4.81 *	166.02 ± 12.89
LA1420	Initial value	2.58 ± 0.05	2.17 ± 0.03	0.41 ± 0.02	1.13 ± 0.01	0.56 ± 0.00	260.04 ± 8.31	49.36 ± 2.71	134.78 ± 3.61
LA1420	Stress	2.85 ± 0.33	2.20 ± 0.18	0.65 ± 0.15	0.93 ± 0.08	0.33 ± 0.05 *	292.96 ± 16.08 *	83.24 ± 16.57 *	127.81 ± 17.10
Plovdiv	Initial value	2.74 ± 0.05	2.26 ± 0.04	0.48 ± 0.02	1.25 ± 0.02	0.69 ± 0.03	279.66 ± 14.64	59.54 ± 4.97	155.09 ± 10.73
Plovdiv	Stress	2.55 ± 0.08	2.10 ± 0.06 *	0.45 ± 0.03	1.04 ± 0.02	0.36 ± 0.02 *	274.31 ± 12.00	59.49 ± 4.35	136.71 ± 4.55
Levovil	Initial value	2.70 ± 0.02	2.22 ± 0.01	0.47 ± 0.01	1.20 ± 0.01	0.58 ± 0.02	258.20 ± 8.63	54.80 ± 1.59	139.47 ± 5.88
Levovil	Stress	2.29 ± 0.06	1.88 ± 0.06 *	0.41 ± 0.01	1.00 ± 0.03*	0.36 ± 0.02 *	283.21 ± 17.12 *	61.99 ± 3.77 *	150.48 ± 11.30

Genotype	Treatment	J_0^{RE1}/CS_0	J_O^{RE1}/J_O^{ET2}	J_0^{TR}/J^{ABS} (UA)	J_0^{DI}/J^{ABS} (UA)	J_0^{ET2}/J^{ABS}	J_0^{RE1}/J^{ABS}
Cervil	Initial value	99.99 ± 2.23	0.65 ± 0.01	0.84 ± 0.00	0.16 ± 0.00	0.51 ± 0.00	0.33 ± 0.01
Cervil	Stress	66.25 ± 6.59*	0.40 ± 0.02 *	0.83 ± 0.01	0.17 ± 0.01	0.47 ± 0.02	0.19 ± 0.01 *
LA1420	Initial value	67.18 ± 1.75	0.50 ± 0.01	0.84 ± 0.00	0.16 ± 0.00	0.44 ± 0.01	0.22 ± 0.01
LA1420	Stress	46.95 ± 8.56	0.36 ± 0.03 *	0.78 ± 0.03	0.21 ± 0.03	0.36 ± 0.07	0.13 ± 0.03 *
Plovdiv	Initial value	87.28 ± 7.99	0.56 ± 0.02	0.82 ± 0.01	0.17 ± 0.01	0.45 ± 0.01	0.25 ± 0.02
Plovdiv	Stress	47.54 ± 1.93*	0.35 ± 0.02 *	0.82 ± 0.00	0.18 ± 0.00	0.41 ± 0.01	0.14 ± 0.01 *
Levovil	Initial value	66.39 ± 3.69	0.47 ± 0.01	0.83 ± 0.00	0.18 ± 0.00	0.44 ± 0.01	0.21 ± 0.01
Levovil	Stress	54.52 ± 6.90	0.36 ± 0.02 *	0.82 ± 0.00	0.18 ± 0.00	0.44 ± 0.01	0.15 ± 0.01 *

Supplementary data table 1: Substrate humidity, stomatal conductance, chlorophyll content extrapolated from SPAD units, dry matter content, dry weight, leaf surface area, specific leaf area (SLA), mass per surface and JIP-test parameters measured on vegetative plants of Cervil, LA1420, Plovdiv and Levovil genotypes. Stress treatment corresponds to measurements on the last day of soil drying for each experiment. Data are means of the two trials ± SE ($n \geq 5$). Only two significant differences were found between repetitions for chlorophyll content, due to a climate chamber effects. Significant differences between treatments are indicated using * symbol at the threshold $P < 0.05$ according to Tukey test or Kruskal-Wallis test. Tukey test was used for SPAD units, F_0, F_M, J_0^{TR}/CS_0, J_0^{ET2}/CS_0, J_0^{RE1}/CS_0, leaf surface area and SLA only.

Genotype	Treatment	Substrate humidity (%)	Stem Water Potential at predawn (MPa)	Leaf Water Potential at midday (MPa)	Stomatal conductance (mmol m^{-2} s^{-1})	SPAD units (UA)	Dry matter content (g 100g FW^{-1})	Dry mass (g)
Cervil	Initial value	29.9 ± 2.6	-0.12 ± 0.02	-0.34 ± 0.03	148.00 ± 41.25	37.34 ± 0.67	14.65 ± 0.62	3.01 ± 0.19
Cervil	Stress	12.3 ± 1.5 *	-1.31 ± 0.03 *	-1.20 ± 0.13 *	16.37 ± 2.62 *	42.70 ± 1.12 *	18.77 ± 0.40 *	3.79 ± 0.27 *
LA1420	Initial value	28.8 ± 1.8	-0.10 ± 0.01	-0.20 ± 0.03	114.79 ± 18.09	37.73 ± 0.90	14.74 ± 0.20	3.69 ± 0.23
LA1420	Stress	11.2 ± 1.2 *	-1.05 ± 0.04 *	-1.00 ± 0.07 *	38.52 ± 4.96 *	45.48 ± 1.47 *	16.85 ± 0.72	2.54 ± 0.22 *
Plovdiv	Initial value	27.3 ± 3.3	-0.19 ± 0.03	-0.28 ± 0.03	156.18 ± 35.01	39.49 ± 1.09	11.38 ± 0.17	4.99 ± 0.23
Plovdiv	Stress	10.0 ± 0.5 *	-0.72 ± 0.12 *	-1.18 ± 0.08 *	37.45 ± 8.91 *	44.44 ± 1.25 *	18.93 ± 0.77 *	2.55 ± 0.19 *
Levovil	Initial value	34.3 ± 3.4	-0.12 ± 0.01	-0.20 ± 0.02	137.58 ± 17.10	42.26 ± 1.10	9.33 ± 0.09	3.88 ± 0.28
Levovil	Stress	9.8 ± 0.7 *	-0.77 ± 0.07 *	-1.12 ± 0.04 *	46.94 ± 9.26 *	45.83 ± 1.58 *	15.44 ± 0.58 *	2.09 ± 0.18 *

Genotype	Treatment	Fo (UA)	Fk (UA)	Fj (UA)	Fm (UA)	Fv (UA)	Fv/Fm (UA)	Sm (UA)
Cervil	Initial value	335.17 ± 13.93	680.67 ± 32.49	1032.67 ± 45.81	2158.3 ± 106.6	1823.17 ± 96.86	0.84 ± 0.01	25.22 ± 0.81
Cervil	Stress	319.17 ± 14.49	708.67 ± 38.91	1067.83 ± 43.18	1947.0 ± 110.0	1627.83 ± 97.96	0.83 ± 0.00	24.82 ± 2.55
LA1420	Initial value	346.08 ± 14.62	693.25 ± 26.31	1052.17 ± 36.88	2198.8 ± 96.5	1852.75 ± 83.68	0.84 ± 0.00	24.43 ± 0.99
LA1420	Stress	344.50 ± 18.17	703.50 ± 14.15	1113.17 ± 30.30	2250.2 ± 123.2	1905.67 ± 106.32	0.85 ± 0.00	28.81 ± 1.95
Plovdiv	Initial value	300.08 ± 7.07	610.33 ± 20.97	939.83 ± 29.09	2111.0 ± 44.6	1810.92 ± 38.40	0.86 ± 0.01	28.10 ± 1.38
Plovdiv	Stress	306.00 ± 14.49	644.33 ± 32.31	1000.83 ± 42.87	1967.7 ± 94.5	1661.67 ± 80.34	0.84 ± 0.01	25.49 ± 2.09
Levovil	Initial value	307.42 ± 14.39	616.08 ± 25.88	895.42 ± 34.76	2033.8 ± 90.2	1726.33 ± 76.22	0.85 ± 0.01	28.04 ± 0.78
Levovil	Stress	317.17 ± 13.02	677.83 ± 30.55	997.67 ± 42.16	1909.7 ± 61.3	1592.50 ± 50.06	0.83 ± 0.01	28.15 ± 1.79

Genotype	Treatment	N (UA)	Vk	Vk/Vj	RC/ABS (UA)	Fv/Fo (UA)	(1-Vj)/Vj (UA)	PI (UA)
Cervil	Initial value	49.65 ± 1.87	0.19 ± 0.01	0.49 ± 0.01	1.13 ± 0.04	5.46 ± 0.22	1.62 ± 0.07	3.84 ± 0.27
Cervil	Stress	50.77 ± 4.86	0.24 ± 0.03	0.52 ± 0.02	0.90 ± 0.08	5.10 ± 0.20	1.18 ± 0.13 *	2.56 ± 0.39 *
LA1420	Initial value	47.75 ± 1.73	0.19 ± 0.01	0.49 ± 0.01	1.14 ± 0.05	5.37 ± 0.12	1.63 ± 0.08	3.80 ± 0.27
LA1420	Stress	53.44 ± 2.33	0.19 ± 0.01	0.47 ± 0.01	1.13 ± 0.08	5.54 ± 0.11	1.48 ± 0.11	3.75 ± 0.41
Plovdiv	Initial value	53.87 ± 2.06	0.17 ± 0.01	0.48 ± 0.01	1.28 ± 0.05	6.05 ± 0.07	1.85 ± 0.07	5.04 ± 0.29
Plovdiv	Stress	49.01 ± 3.25	0.20 ± 0.01	0.49 ± 0.02	1.06 ± 0.07	5.43 ± 0.07	1.41 ± 0.10	3.36 ± 0.32 *
Levovil	Initial value	58.81 ± 1.76	0.18 ± 0.00	0.52 ± 0.01	1.19 ± 0.02	5.63 ± 0.07	1.93 ± 0.05	4.40 ± 0.13
Levovil	Stress	59.64 ± 4.18	0.23 ± 0.01	0.53 ± 0.01	0.93 ± 0.03 *	5.04 ± 0.11	1.35 ± 0.05 *	2.69 ± 0.14 *

Genotype	Treatment	PI_{ABS}^{TOT}	PI_{CSo}^{TOT}	J_0^{ABS}/RC (UA)	J_0^{TR}/RC (UA)	J_0^{DI}/RC (UA)	J_0^{ET2}/RC (UA)	J_0^{RE1}/RC
Cervil	Initial value	2.02 ± 0.21	1279.47 ± 95.65	2.34 ± 0.04	1.97 ± 0.04	0.37 ± 0.02	1.21 ± 0.02	0.41 ± 0.02
Cervil	Stress	1.21 ± 0.27	818.14 ± 123.51	2.48 ± 0.11	2.07 ± 0.08	0.41 ± 0.03	1.09 ± 0.06	0.33 ± 0.04
LA1420	Initial value	2.04 ± 0.24	1327.54 ± 124.40	2.33 ± 0.05	1.96 ± 0.04	0.37 ± 0.01	1.21 ± 0.02	0.41 ± 0.02
LA1420	Stress	1.94 ± 0.35	1323.19 ± 202.74	2.21 ± 0.07	1.87 ± 0.06	0.34 ± 0.01	1.10 ± 0.01	0.37 ± 0.02
Plovdiv	Initial value	3.23 ± 0.35	1502.83 ± 80.18	2.25 ± 0.05	1.93 ± 0.04	0.32 ± 0.01	1.25 ± 0.03	0.47 ± 0.02
Plovdiv	Stress	1.52 ± 0.29 *	1037.83 ± 138.45	2.30 ± 0.07	1.94 ± 0.06	0.36 ± 0.01	1.12 ± 0.02	0.34 ± 0.03 *
Levovil	Initial value	2.55 ± 0.18	1355.77 ± 82.42	2.47 ± 0.02	2.10 ± 0.02	0.37 ± 0.00	1.38 ± 0.02	0.50 ± 0.02
Levovil	Stress	1.27 ± 0.13 *	845.35 ± 24.81 *	2.54 ± 0.04	2.12 ± 0.03	0.42 ± 0.01	1.21 ± 0.02	0.38 ± 0.03 *

Genotype	Treatment	J_0^{TR}/CS_0	J_0^{DI}/CS_0	J_0^{ET2}/CS_0	J_0^{RE1}/CS_0	J_O^{RE1}/J_O^{ET2}	J_0^{TR}/J^{ABS} (UA)	J_0^{DI}/J^{ABS} (UA)
Cervil	Initial value	282.36 ± 11.65	52.81 ± 3.18	174.03 ± 8.15	58.95 ± 3.93	0.34 ± 0.01	0.84 ± 0.01	0.16 ± 0.01
Cervil	Stress	266.57 ± 12.45	52.60 ± 2.74	142.73 ± 12.70	43.76 ± 6.38	0.30 ± 0.03	0.83 ± 0.00	0.17 ± 0.00
LA1420	Initial value	291.38 ± 12.19	54.71 ± 2.71	180.15 ± 9.64	62.35 ± 4.83	0.34 ± 0.01	0.84 ± 0.00	0.16 ± 0.00
LA1420	Stress	291.67 ± 15.33	52.83 ± 3.03	173.94 ± 13.98	58.49 ± 7.16	0.33 ± 0.02	0.85 ± 0.00	0.15 ± 0.00
Plovdiv	Initial value	257.38 ± 5.88	42.71 ± 1.27	166.36 ± 3.84	63.10 ± 3.12	0.38 ± 0.02	0.86 ± 0.01	0.14 ± 0.00
Plovdiv	Stress	258.39 ± 12.23	47.61 ± 2.31	150.31 ± 10.43	45.58 ± 6.13	0.30 ± 0.02	0.84 ± 0.01	0.16 ± 0.00
Levovil	Initial value	260.90 ± 12.07	46.52 ± 2.37	171.98 ± 8.88	62.12 ± 3.33	0.36 ± 0.01	0.85 ± 0.01	0.15 ± 0.00
Levovil	Stress	264.40 ± 10.19	52.77 ± 2.97	151.48 ± 5.19	48.69 ± 4.90	0.32 ± 0.02	0.83 ± 0.01	0.17 ± 0.00

Genotype	Treatment	J_0^{ET2}/J^{ABS} (UA)	J_0^{RE1}/J^{ABS}	J_0^{TR}/CS_m	J_0^{DI}/CS_m	J_0^{ET2}/CS_m	RC/CS_m
Cervil	Initial value	0.52 ± 0.01	0.18 ± 0.01	1823.17 ± 96.86	1875.98 ± 97.02	1125.67 ± 66.20	924.92 ± 44.18
Cervil	Stress	0.45 ± 0.03	0.14 ± 0.02 *	1627.83 ± 97.96	1680.43 ± 98.72	879.17 ± 91.60	794.15 ± 58.65
LA1420	Initial value	0.52 ± 0.01	0.18 ± 0.01	1852.75 ± 83.68	1907.46 ± 85.27	1146.67 ± 66.56	949.26 ± 49.65
LA1420	Stress	0.50 ± 0.02	0.17 ± 0.01	1905.67 ± 106.32	1958.49 ± 108.60	1137.00 ± 96.06	1029.70 ± 83.53
Plovdiv	Initial value	0.55 ± 0.01	0.21 ± 0.01	1810.92 ± 38.40	1853.62 ± 39.24	1171.17 ± 27.81	941.61 ± 26.31
Plovdiv	Stress	0.49 ± 0.02	0.15 ± 0.02 *	1661.67 ± 80.34	1709.28 ± 82.43	966.83 ± 68.36	861.54 ± 52.01
Levovil	Initial value	0.56 ± 0.01	0.20 ± 0.01	1726.33 ± 76.22	1772.85 ± 78.39	1138.33 ± 57.17	822.30 ± 34.58
Levovil	Stress	0.48 ± 0.01 *	0.16 ± 0.01 *	1592.50 ± 50.06	1645.27 ± 52.05	912.00 ± 20.82	752.76 ± 22.38

Supplementary data table 2: Substrate humidity, predawn water potential, leaf water potential at midday, stomatal conductance, chlorophyll content extrapolated from SPAD units, dry matter content, dry weight and JIP-test parameters measured on reproductive plants of Cervil, LA1420, Plovdiv and Levovil genotypes. Stress treatment corresponds to measurements on the last day of soil drying for each experiment. Data are means of the two trials ± SE ($n \geq 5$). Only two significant differences were found between repetitions for chlorophyll content, due to a climate chamber effects. Significant differences between treatments are indicated using * symbol at the threshold $P < 0.05$ according to Tukey test or Kruskal-Wallis test. Tukey test was used for SPAD units, J_0^{ABS}/RC, J_0^{TR}/CS_0, J_0^{ET2}/CS_0, J_0^{RE1}/CS_0, J_0^{TR}/CS_M, J_0^{RE1}/J^{ABS}, PI and dry weight only.

Annexes du Chapitre V

	Génotype	Momor			Monalbo		
	Déficit hydrique	0	-60	-80	0	-60	-80
Date de récolte	infection sur tige						
J0 Surface foliaire infectée (cm^2)	non	3.53 ± 0.18 **a**	4.26 ± 0.10 **b**	3.60 ± 0.16 **a**	3.90 ± 0.31 **a**	3.66 ± 0.07 **a**	3.79 ± 0.18 **a**
J3 Surface foliaire infectée (cm^2)	non	3.79 ± 0.19 **a**	3.19 ± 0.06 **a**	3.07 ± 0.34 **a**	3.42 ± 0.29 **a**	3.60 ± 0.21 **a**	3.23 ± 0.19 **a**
Surface foliaire infectée (cm^2)	oui	3.47 ± 0.21 **b**	3.57 ± 0.18 **b**	3.04 ± 0.23 **b**	3.35 ± 0.16 **b**	3.64 ± 0.13 **b**	2.64 ± 0.16 **a**
J7 Surface foliaire infectée (cm^2)	non	4.30 ± 0.30 **a**	5.02 ± 0.39 **a**	4.51 ± 0.50 **a**	4.25 ± 0.19 **a**	5.25 ± 0.20 **a**	4.65 ± 0.31 **a**
Surface foliaire infectée (cm^2)	oui	5.13 ± 0.18 **b**	4.67 ± 0.36 **ab**	3.85 ± 0.27 **a**	4.56 ± 0.36 **a**	4.60 ± 0.24 **a**	3.82 ± 0.45 **a**

Annexe 1: Surfaces foliaires infectées mesurées 72 h après dépôts des pastilles de gel avec mycélium de la souche BC1, moyenne pour deux folioles par plante, pour la première expérimentation. Les données sont les moyennes de 5 plantes par traitement et par génotype ± SE. Les lettres indiquent des différences significatives entre traitements hydriques pour un génotype donné à $P < 0.05$ (Test de Tukey).

Feuilles	Génotype	Momor			Monalbo			Interaction
	Déficit hydrique	0	-60	-80	0	-60	-80	
Date de récolte	Infection sur tige							
J0								
Glucose (g 100g^{-1} MS)	non	0.74 ± 0.09 **a**	0.84 ± 0.15 **a**	2.77 ± 0.45 **b**	1.20 ± 0.16 **a**	1.61 ± 0.25 **a**	3.77 ± 0.32 **b**	NS
Fructose (g 100g^{-1} MS)	non	1.56 ± 0.14 **a**	1.98 ± 0.17 **a**	4.08 ± 0.38 **b**	1.81 ± 0.09 **a**	3.13 ± 0.23 **b**	5.07 ± 0.17 **c**	NS
Saccharose (g 100g^{-1} MS)	non	0.29 ± 0.04 **a**	0.37 ± 0.01 **a**	0.32 ± 0.05 **a**	0.34 ± 0.02 **a**	0.37 ± 0.01 **a**	0.40 ± 0.05 **a**	NS
Total sucres(g 100g^{-1} MS)	non	2.58 ± 0.19 **a**	3.19 ± 0.31 **b**	7.18 ± 0.72 **c**	3.34 ± 0.17 **a**	5.11 ± 0.40 **a**	9.24 ± 0.46 **b**	NS
Acide malique (g 100g^{-1} MS)	non	1.17 ± 0.13 **a**	1.33 ± 0.09 **a**	1.09 ± 0.16 **a**	1.09 ± 0.08 **a**	1.29 ± 0.07 **a**	1.14 ± 0.11 **a**	NS
Acide quinique (g 100g^{-1} MS)	non	0.98 ± 0.09 **a**	1.27 ± 0.11 **a**	1.74 ± 0.12 **b**	1.37 ± 0.15 **a**	1.56 ± 0.11 **a**	2.44 ± 0.18 **b**	NS
Acide citrique (g 100g^{-1} MS)	non	1.99 ± 0.12 **a**	2.22 ± 0.18 **a**	2.43 ± 0.10 **a**	2.27 ± 0.08 **a**	2.56 ± 0.15 **ab**	2.99 ± 0.12 **b**	NS
Total acides (g 100g^{-1} MS)	non	4.14 ± 0.25 **a**	4.83 ± 0.24 **a**	5.25 ± 0.13 **b**	4.73 ± 0.16 **a**	5.41 ± 0.26 **ab**	6.57 ± 0.24 **b**	NS
N (g 100g^{-1} MS)	non	1.50 ± 0.06 **a**	1.76 ± 0.07 **ab**	1.99 ± 0.06 **b**	1.58 ± 0.05 **a**	2.04 ± 0.08 **b**	2.25 ± 0.10 **b**	NS
C (g 100g^{-1} MS)	non	35.60 ± 1.22 **a**	36.97 ± 0.39 **a**	35.76 ± 1.20 **a**	36.17 ± 0.63 **a**	37.26 ± 0.70 **a**	36.68 ± 0.73 **a**	NS
J3								
Glucose (g 100g^{-1} MS)	non	1.08 ± 0.20 **a**	1.56 ± 0.25 **a**	5.04 ± 0.52 **b**	1.65 ± 0.19 **a**	2.56 ± 0.19 **a**	6.54 ± 0.55 **b**	NS
Fructose (g 100g^{-1} MS)	non	1.94 ± 0.18 **a**	2.81 ± 0.30 **a**	7.12 ± 0.66 **b**	2.23 ± 0.25 **a**	3.57 ± 0.25 **a**	7.40 ± 0.55 **b**	NS
Saccharose (g 100g^{-1} MS)	non	0.39 ± 0.07 **a**	0.36 ± 0.03 **a**	0.54 ± 0.06 **a**	0.30 ± 0.02 **a**	0.37 ± 0.06 **a**	0.68 ± 0.08 **b**	NS
Total sucres(g 100g^{-1} MS)	non	3.39 ± 0.32 **a**	4.73 ± 0.54 **a**	12.70 ± 1.20 **b**	4.19 ± 0.44 **a**	6.49 ± 0.40 **a**	14.62 ± 1.02 **b**	NS
Acide malique (g 100g^{-1} MS)	non	1.48 ± 0.00 **a**	1.40 ± 0.11 **a**	1.51 ± 0.07 **a**	0.92 ± 0.04 **a**	1.32 ± 0.21 **b**	1.22 ± 0.07 **ab**	NS
Acide quinique (g 100g^{-1} MS)	non	1.28 ± 0.03 **a**	1.68 ± 0.09 **a**	1.99 ± 0.15 **a**	1.94 ± 0.25 **a**	2.64 ± 0.30 **a**	2.45 ± 0.28 **a**	NS
Acide citrique (g 100g^{-1} MS)	non	3.23 ± 0.01 **b**	2.67 ± 0.18 **ab**	2.54 ± 0.15 **a**	2.59 ± 0.21 **a**	2.88 ± 0.19 **ab**	3.36 ± 0.19 **b**	*
Total acides (g 100g^{-1} MS)	non	5.98 ± 0.02 **a**	5.74 ± 0.33 **a**	6.05 ± 0.33 **a**	5.45 ± 0.29 **a**	6.84 ± 0.58 **b**	7.03 ± 0.34 **b**	NS
N (g 100g^{-1} MS)	non	1.44 ± 0.03 **a**	1.83 ± 0.04 **b**	2.10 ± 0.15 **b**	1.73 ± 0.06 **a**	2.23 ± 0.15 **b**	2.31 ± 0.07 **b**	NS
C (g 100g^{-1} MS)	non	37.09 ± 0.28 **a**	36.31 ± 0.04 **a**	37.01 ± 0.32 **a**	36.63 ± 0.24 **b**	34.38 ± 1.02 **a**	37.29 ± 0.33 **b**	NS
Glucose (g 100g^{-1} MS)	oui	0.96 ± 0.10 **a**	2.68 ± 0.49 **b**	3.13 ± 0.32 **b**	1.52 ± 0.27 **a**	2.94 ± 0.17 **b**	4.99 ± 0.39 **c**	NS
Fructose (g 100g^{-1} MS)	oui	1.61 ± 0.12 **a**	4.09 ± 0.51 **b**	4.48 ± 0.45 **b**	2.08 ± 0.18 **a**	4.49 ± 0.36 **b**	6.24 ± 0.31 **c**	NS
Saccharose (g 100g^{-1} MS)	oui	0.26 ± 0.02 **a**	0.52 ± 0.04 **b**	0.57 ± 0.07 **b**	0.27 ± 0.02 **a**	0.36 ± 0.02 **a**	0.55 ± 0.03 **b**	NS
Total sucres(g 100g^{-1} MS)	oui	2.83 ± 0.20 **a**	7.29 ± 1.00 **b**	8.18 ± 0.81 **b**	3.87 ± 0.43 **a**	7.78 ± 0.54 **b**	11.77 ± 0.60 **c**	NS
Acide malique (g 100g^{-1} MS)	oui	1.16 ± 0.12 **a**	1.49 ± 0.06 **b**	1.51 ± 0.08 **b**	1.03 ± 0.10 **a**	1.31 ± 0.05 **b**	0.97 ± 0.04 **a**	*
Acide quinique (g 100g^{-1} MS)	oui	1.48 ± 0.11 **a**	1.94 ± 0.11 **ab**	2.25 ± 0.26 **b**	1.80 ± 0.20 **a**	2.04 ± 0.08 **a**	2.10 ± 0.19 **a**	NS
Acide citrique (g 100g^{-1} MS)	oui	2.80 ± 0.14 **a**	2.54 ± 0.19 **a**	2.92 ± 0.17 **a**	2.63 ± 0.24 **a**	3.22 ± 0.24 **a**	2.81 ± 0.09 **a**	NS
Total acides (g 100g^{-1} MS)	oui	5.44 ± 0.20 **a**	5.97 ± 0.16 **ab**	6.68 ± 0.27 **b**	5.46 ± 0.43 **a**	6.57 ± 0.27 **b**	5.88 ± 0.21 **ab**	NS
N (g 100g^{-1} MS)	oui	1.91 ± 0.07 **a**	1.96 ± 0.08 **a**	1.94 ± 0.10 **a**	2.00 ± 0.10 **a**	2.22 ± 0.02 **ab**	2.40 ± 0.04 **b**	NS
C (g 100g^{-1} MS)	oui	35.25 ± 0.67 **a**	35.97 ± 0.21 **a**	36.26 ± 0.21 **a**	36.15 ± 0.42 **a**	35.90 ± 0.39 **a**	37.05 ± 0.27 **a**	NS
J7								
Glucose (g 100g^{-1} MS)	non	0.97 ± 0.19 **a**	2.32 ± 0.18 **b**	3.07 ± 0.33 **b**	1.23 ± 0.15 **a**	3.21 ± 0.52 **b**	3.83 ± 0.42 **b**	NS
Fructose (g 100g^{-1} MS)	non	1.89 ± 0.30 **a**	3.56 ± 0.17 **b**	5.09 ± 0.38 **c**	1.71 ± 0.25 **a**	4.32 ± 0.36 **b**	5.37 ± 0.64 **b**	NS
Saccharose (g 100g^{-1} MS)	non	0.39 ± 0.05 **a**	0.25 ± 0.07 **a**	0.49 ± 0.04 **a**	0.14 ± 0.09 **a**	0.26 ± 0.07 **a**	0.28 ± 0.12 **a**	NS
Total sucres(g 100g^{-1} MS)	non	3.25 ± 0.52 **a**	6.14 ± 0.26 **b**	8.64 ± 0.71 **c**	3.08 ± 0.46 **a**	7.80 ± 0.86 **b**	9.47 ± 1.09 **b**	NS
Acide malique (g 100g^{-1} MS)	non	1.37 ± 0.25 **a**	1.48 ± 0.25 **a**	1.29 ± 0.08 **a**	0.79 ± 0.24 **a**	1.10 ± 0.19 **a**	1.05 ± 0.28 **a**	NS
Acide quinique (g 100g^{-1} MS)	non	1.57 ± 0.31 **a**	1.78 ± 0.17 **a**	1.77 ± 0.16 **a**	1.45 ± 0.34 **a**	1.87 ± 0.28 **a**	2.06 ± 0.28 **a**	NS
Acide citrique (g 100g^{-1} MS)	non	2.62 ± 0.15 **a**	1.83 ± 0.24 **a**	2.04 ± 0.10 **a**	1.52 ± 0.62 **a**	2.13 ± 0.14 **a**	1.80 ± 0.36 **a**	NS
Total acides (g 100g^{-1} MS)	non	5.56 ± 0.63 **a**	5.09 ± 0.49 **a**	5.10 ± 0.26 **a**	3.77 ± 1.15 **a**	5.10 ± 0.54 **a**	4.91 ± 0.80 **a**	NS
N (g 100g^{-1} MS)	non	2.08 ± 0.20 **a**	1.99 ± 0.09 **a**	2.17 ± 0.14 **a**	1.72 ± 0.28 **a**	2.44 ± 0.20 **a**	2.24 ± 0.29 **a**	NS
C (g 100g^{-1} MS)	non	33.97 ± 0.82 **a**	34.86 ± 1.86 **a**	35.99 ± 0.40 **a**	25.66 ± 4.20 **a**	34.54 ± 1.44 **a**	31.42 ± 4.12 **a**	NS
Glucose (g 100g^{-1} MS)	oui	0.78 ± 0.13 **a**	1.45 ± 0.18 **ab**	2.64 ± 0.63 **b**	0.88 ± 0.27 **a**	2.88 ± 0.21 **b**	4.17 ± 0.55 **b**	NS
Fructose (g 100g^{-1} MS)	oui	1.54 ± 0.16 **a**	2.80 ± 0.21 **ab**	4.07 ± 1.09 **b**	1.02 ± 0.36 **a**	3.96 ± 0.27 **b**	5.97 ± 0.64 **c**	NS
Saccharose (g 100g^{-1} MS)	oui	0.32 ± 0.03 **a**	0.36 ± 0.10 **a**	0.45 ± 0.17 **a**	0.04 ± 0.04 **a**	0.53 ± 0.05 **b**	0.38 ± 0.16 **ab**	NS
Total sucres(g 100g^{-1} MS)	oui	2.64 ± 0.24 **a**	4.61 ± 0.42 **ab**	7.15 ± 1.82 **b**	1.94 ± 0.61 **a**	7.37 ± 0.48 **b**	10.51 ± 1.28 **b**	NS
Acide malique (g 100g^{-1} MS)	oui	1.46 ± 0.21 **a**	1.68 ± 0.27 **a**	1.47 ± 0.28 **a**	0.70 ± 0.23 **a**	1.37 ± 0.09 **a**	1.19 ± 0.23 **a**	NS
Acide quinique (g 100g^{-1} MS)	oui	1.25 ± 0.05 **a**	1.74 ± 0.26 **a**	2.01 ± 0.39 **a**	0.91 ± 0.23 **a**	2.59 ± 0.16 **b**	1.86 ± 0.19 **b**	*
Acide citrique (g 100g^{-1} MS)	oui	2.59 ± 0.17 **a**	1.95 ± 0.31 **a**	2.03 ± 0.56 **a**	0.61 ± 0.27 **a**	2.18 ± 0.17 **b**	2.52 ± 0.46 **b**	*
Total acides (g 100g^{-1} MS)	oui	5.30 ± 0.17 **a**	5.36 ± 0.80 **a**	5.51 ± 1.12 **a**	2.23 ± 0.68 **a**	6.15 ± 0.33 **b**	5.56 ± 0.84 **b**	*
N (g 100g^{-1} MS)	oui	1.89 ± 0.10 **a**	1.93 ± 0.21 **a**	1.66 ± 0.17 **a**	1.62 ± 0.30 **a**	2.42 ± 0.08 **b**	2.30 ± 0.13 **b**	*
C (g 100g^{-1} MS)	oui	32.40 ± 0.50 **a**	31.42 ± 3.45 **a**	30.37 ± 3.31 **a**	22.73 ± 3.86 **a**	34.86 ± 0.67 **b**	33.08 ± 2.23 **b**	*

Annexe 2: Teneurs des feuilles en glucose, en fructose, en saccharose, en acide malique, en acide quinique, en acide citrique, en azote et en carbone pour les génotypes Momor et Monalbo, aux trois dates de récolte en fonction des traitements hydriques et des infections sur tige. Les données sont les moyennes ± SE de 5 répétitions (plantes) par traitement et par génotype. Les lettres indiquent des différences significatives entre traitements hydriques pour un génotype donné à $P < 0.05$ (Test de Tukey). Les interactions entre génotype et traitement sont indiquées à l'aide du symbole *, dans le cas contraire elles sont non significatives (NS).

Tiges	Génotype	Momor			Monalbo			Interaction
	Déficit hydrique	0	-60	-80	0	-60	-80	
Date de récolte	Infection sur tige							
J0								
Glucose (g 100g^{-1} MS)	non	9,91 ± 0,19 **a**	9,84 ± 0,33 **a**	12,36 ± 0,58 **a**	8,65 ± 1,00 **a**	8,29 ± 0,56 **a**	9,88 ± 0,55 **a**	NS
Fructose (g 100g^{-1} MS)	non	1,46 ± 0,07 **a**	1,45 ± 0,08 **a**	1,82 ± 0,12 **a**	1,32 ± 0,12 **a**	1,35 ± 0,10 **a**	1,44 ± 0,09 **a**	NS
Saccharose (g 100g^{-1} MS)	non	5,19 ± 0,40 **a**	5,39 ± 0,19 **a**	6,16 ± 0,50 **a**	5,64 ± 0,13 **a**	6,31 ± 0,50 **a**	7,60 ± 0,52 **a**	NS
Total sucres(g 100g^{-1} MS)	non	16,56 ± 0,48 **a**	16,67 ± 0,40 **a**	20,34 ± 0,92 **b**	15,60 ± 1,21 **a**	15,94 ± 0,97 **a**	18,92 ± 0,84 **b**	NS
Acide malique (g 100g^{-1} MS)	non	0,49 ± 0,03 **a**	0,61 ± 0,05 **ab**	0,80 ± 0,06 **b**	0,66 ± 0,07 **a**	1,04 ± 0,08 **b**	1,06 ± 0,05 **b**	NS
Acide quinique (g 100g^{-1} MS)	non	3,99 ± 0,33 **a**	4,29 ± 0,10 **a**	4,85 ± 0,17 **a**	2,63 ± 0,15 **a**	3,16 ± 0,18 **a**	3,78 ± 0,11 **a**	NS
Acide citrique (g 100g^{-1} MS)	non	0,94 ± 0,07 **a**	0,88 ± 0,08 **a**	1,31 ± 0,08 **b**	0,89 ± 0,04 **a**	1,18 ± 0,11 **b**	1,46 ± 0,16 **b**	NS
Total acides (g 100g^{-1} MS)	non	5,41 ± 0,33 **a**	5,77 ± 0,16 **b**	6,96 ± 0,18 **c**	4,18 ± 0,21 **a**	5,38 ± 0,16 **a**	6,31 ± 0,17 **b**	NS
N (g 100g^{-1} MS)	non	1,02 ± 0,03 **a**	1,18 ± 0,04 **b**	1,12 ± 0,05 **ab**	0,93 ± 0,03 **a**	1,16 ± 0,03 **a**	1,14 ± 0,03 **a**	NS
C (g 100g^{-1} MS)	non	35,32 ± 0,50 **a**	35,90 ± 0,15 **a**	36,21 ± 0,86 **a**	37,18 ± 0,39 **a**	37,09 ± 0,32 **a**	36,49 ± 0,28 **a**	NS
J3								
Glucose (g 100g^{-1} MS)	non	10,49 ± 0,58 **b**	8,28 ± 0,28 **a**	8,34 ± 0,66 **a**	6,29 ± 0,61 **a**	7,42 ± 0,45 **ab**	8,22 ± 0,43 **b**	*
Fructose (g 100g^{-1} MS)	non	1,39 ± 0,15 **ab**	1,12 ± 0,06 **a**	1,53 ± 0,10 **b**	0,92 ± 0,07 **a**	1,04 ± 0,06 **a**	1,22 ± 0,08 **a**	NS
Saccharose (g 100g^{-1} MS)	non	4,81 ± 0,71 **ab**	4,47 ± 0,27 **a**	7,18 ± 1,07 **b**	3,09 ± 0,23 **a**	5,44 ± 0,62 **ab**	7,71 ± 0,71 **b**	NS
Total sucres(g 100g^{-1} MS)	non	16,70 ± 1,23 **a**	13,86 ± 0,52 **b**	17,05 ± 1,71 **a**	10,30 ± 0,88 **a**	13,90 ± 0,85 **a**	17,15 ± 0,92 **b**	NS
Acide malique (g 100g^{-1} MS)	non	0,79 ± 0,04 **a**	0,70 ± 0,05 **a**	0,71 ± 0,05 **a**	0,89 ± 0,09 **a**	0,86 ± 0,06 **b**	0,95 ± 0,09 **ab**	NS
Acide quinique (g 100g^{-1} MS)	non	4,00 ± 0,22 **a**	3,41 ± 0,10 **a**	3,08 ± 0,20 **a**	1,88 ± 0,09 **a**	2,77 ± 0,25 **a**	2,87 ± 0,16 **a**	NS
Acide citrique (g 100g^{-1} MS)	non	0,97 ± 0,13 **b**	0,80 ± 0,06 **ab**	1,22 ± 0,15 **a**	0,69 ± 0,05 **a**	0,92 ± 0,09 **ab**	1,26 ± 0,10 **b**	*
Total acides (g 100g^{-1} MS)	non	5,76 ± 0,20 **a**	4,91 ± 0,08 **a**	5,01 ± 0,32 **a**	3,46 ± 0,21 **a**	4,55 ± 0,30 **b**	5,08 ± 0,21 **b**	NS
N (g 100g^{-1} MS)	non	0,87 ± 0,02 **a**	0,98 ± 0,03 **b**	1,25 ± 0,06 **b**	0,84 ± 0,03 **a**	1,00 ± 0,02 **b**	1,07 ± 0,01 **b**	NS
C (g 100g^{-1} MS)	non	35,69 ± 0,17 **a**	36,03 ± 0,28 **a**	36,68 ± 0,28 **a**	36,15 ± 0,17 **a**	36,58 ± 0,37 **a**	36,80 ± 0,21 **a**	NS
Glucose (g 100g^{-1} MS)	oui	10,15 ± 0,32 **a**	10,86 ± 1,42 **a**	9,55 ± 0,61 **a**	8,53 ± 1,21 **a**	8,22 ± 0,61 **a**	8,94 ± 0,90 **a**	NS
Fructose (g 100g^{-1} MS)	oui	1,21 ± 0,08 **a**	1,87 ± 0,29 **a**	1,62 ± 0,13 **a**	1,11 ± 0,08 **a**	1,68 ± 0,06 **b**	1,75 ± 0,23 **b**	NS
Saccharose (g 100g^{-1} MS)	oui	3,65 ± 0,23 **a**	6,51 ± 0,61 **b**	6,70 ± 0,60 **b**	4,09 ± 0,16 **a**	7,03 ± 0,28 **b**	7,63 ± 0,62 **b**	NS
Total sucres(g 100g^{-1} MS)	oui	15,00 ± 0,46 **a**	19,24 ± 2,17 **b**	17,87 ± 1,13 **b**	13,73 ± 1,33 **a**	16,93 ± 0,63 **b**	18,32 ± 1,72 **b**	NS
Acide malique (g 100g^{-1} MS)	oui	0,74 ± 0,03 **a**	0,83 ± 0,05 **a**	1,28 ± 0,06 **b**	0,92 ± 0,07 **a**	1,25 ± 0,07 **b**	1,17 ± 0,13 **ab**	*
Acide quinique (g 100g^{-1} MS)	oui	4,52 ± 0,36 **a**	4,33 ± 0,28 **a**	3,86 ± 0,29 **a**	2,70 ± 0,37 **a**	2,99 ± 0,24 **a**	2,54 ± 0,14 **a**	NS
Acide citrique (g 100g^{-1} MS)	oui	0,77 ± 0,06 **a**	1,10 ± 0,17 **a**	1,07 ± 0,11 **a**	0,82 ± 0,05 **a**	1,13 ± 0,04 **ab**	1,19 ± 0,07 **b**	NS
Total acides (g 100g^{-1} MS)	oui	6,04 ± 0,30 **a**	6,25 ± 0,34 **a**	6,21 ± 0,32 **a**	4,44 ± 0,44 **a**	5,37 ± 0,33 **a**	4,90 ± 0,16 **a**	NS
N (g 100g^{-1} MS)	oui	0,94 ± 0,01 **a**	1,18 ± 0,07 **b**	1,16 ± 0,03 **b**	0,94 ± 0,03 **a**	1,12 ± 0,03 **b**	1,23 ± 0,04 **b**	NS
C (g 100g^{-1} MS)	oui	35,15 ± 0,47 **a**	36,00 ± 0,30 **ab**	36,10 ± 0,14 **b**	35,69 ± 0,39 **a**	36,60 ± 0,37 **ab**	36,85 ± 0,31 **b**	NS
J7								
Glucose (g 100g^{-1} MS)	non	8,51 ± 0,46 **ab**	8,06 ± 0,43 **a**	10,03 ± 0,55 **b**	6,07 ± 0,41 **a**	5,57 ± 0,58 **a**	7,91 ± 0,51 **b**	NS
Fructose (g 100g^{-1} MS)	non	1,26 ± 0,05 **a**	1,64 ± 0,10 **b**	2,11 ± 0,20 **c**	1,07 ± 0,06 **a**	1,44 ± 0,05 **ab**	1,64 ± 0,09 **b**	NS
Saccharose (g 100g^{-1} MS)	non	2,98 ± 0,28 **a**	5,50 ± 0,10 **b**	8,24 ± 0,77 **c**	2,62 ± 0,15 **a**	5,20 ± 0,58 **b**	7,24 ± 0,50 **c**	NS
Total sucres(g 100g^{-1} MS)	non	12,74 ± 0,66 **a**	15,20 ± 0,52 **a**	20,38 ± 1,34 **b**	9,76 ± 0,50 **a**	12,21 ± 1,14 **a**	16,79 ± 0,67 **b**	NS
Acide malique (g 100g^{-1} MS)	non	0,81 ± 0,09 **a**	1,77 ± 0,30 **b**	1,21 ± 0,08 **ab**	0,84 ± 0,07 **a**	1,90 ± 0,18 **b**	1,40 ± 0,13 **ab**	NS
Acide quinique (g 100g^{-1} MS)	non	3,65 ± 0,34 **a**	2,85 ± 0,23 **a**	2,89 ± 0,13 **a**	2,31 ± 0,21 **a**	1,97 ± 0,17 **a**	2,71 ± 0,32 **a**	NS
Acide citrique (g 100g^{-1} MS)	non	0,64 ± 0,03 **a**	1,59 ± 0,23 **b**	1,44 ± 0,10 **b**	0,53 ± 0,04 **a**	1,28 ± 0,06 **b**	1,24 ± 0,05 **b**	NS
Total acides (g 100g^{-1} MS)	non	5,10 ± 0,34 **a**	6,21 ± 0,62 **a**	5,55 ± 0,19 **a**	3,67 ± 0,22 **a**	5,15 ± 0,33 **b**	5,34 ± 0,25 **b**	NS
N (g 100g^{-1} MS)	non	1,30 ± 0,03 **a**	1,28 ± 0,12 **a**	1,16 ± 0,06 **a**	1,19 ± 0,03 **a**	1,12 ± 0,06 **a**	1,26 ± 0,10 **a**	NS
C (g 100g^{-1} MS)	non	35,69 ± 0,42 **a**	36,98 ± 0,20 **ab**	37,38 ± 0,26 **b**	36,80 ± 0,41 **a**	38,40 ± 0,41 **b**	37,63 ± 0,59 **ab**	NS
Glucose (g 100g^{-1} MS)	oui	8,62 ± 0,21 **a**	11,16 ± 0,65 **b**	11,19 ± 0,82 **b**	6,66 ± 0,25 **a**	9,29 ± 0,23 **b**	9,21 ± 0,19 **b**	NS
Fructose (g 100g^{-1} MS)	oui	1,66 ± 0,07 **a**	1,98 ± 0,16 **a**	1,84 ± 0,07 **a**	1,69 ± 0,10 **a**	2,19 ± 0,07 **b**	2,05 ± 0,12 **ab**	NS
Saccharose (g 100g^{-1} MS)	oui	2,57 ± 0,05 **a**	4,29 ± 0,36 **b**	6,05 ± 0,38 **c**	2,59 ± 0,15 **a**	5,25 ± 0,60 **b**	5,98 ± 0,39 **b**	NS
Total sucres(g 100g^{-1} MS)	oui	12,85 ± 0,30 **a**	17,44 ± 0,98 **b**	19,08 ± 0,78 **b**	10,94 ± 0,45 **a**	16,72 ± 0,80 **b**	17,25 ± 0,52 **b**	NS
Acide malique (g 100g^{-1} MS)	oui	0,92 ± 0,04 **a**	1,40 ± 0,04 **b**	1,55 ± 0,07 **b**	0,98 ± 0,04 **a**	1,49 ± 0,06 **b**	1,57 ± 0,04 **b**	NS
Acide quinique (g 100g^{-1} MS)	oui	2,94 ± 0,09 **a**	4,55 ± 0,20 **c**	3,78 ± 0,21 **b**	2,66 ± 0,12 **a**	3,86 ± 0,21 **b**	3,01 ± 0,08 **a**	NS
Acide citrique (g 100g^{-1} MS)	oui	0,75 ± 0,04 **a**	0,84 ± 0,07 **a**	1,09 ± 0,04 **b**	0,69 ± 0,06 **a**	0,97 ± 0,05 **b**	1,15 ± 0,03 **c**	NS
Total acides (g 100g^{-1} MS)	oui	4,61 ± 0,10 **a**	6,80 ± 0,30 **b**	6,42 ± 0,24 **b**	4,33 ± 0,10 **a**	6,32 ± 0,22 **b**	5,73 ± 0,11 **b**	NS
N (g 100g^{-1} MS)	oui	1,46 ± 0,03 **a**	1,40 ± 0,04 **a**	1,31 ± 0,06 **a**	1,47 ± 0,07 **a**	1,47 ± 0,06 **a**	1,32 ± 0,07 **a**	NS
C (g 100g^{-1} MS)	oui	36,42 ± 0,46 **a**	36,89 ± 0,39 **a**	36,55 ± 0,22 **a**	36,68 ± 0,40 **a**	36,85 ± 0,29 **a**	38,03 ± 0,51 **a**	NS

Annexe 3: Teneurs des tiges en glucose, en fructose, en saccharose, en acide malique, en acide quinique, en acide citrique, en azote et en carbone pour les génotypes Momor et Monalbo, aux trois dates de récolte en fonction des traitements hydrique et des infections sur tige. Les données sont les moyennes ± SE de 5 répétitions (plantes) par traitement et par génotype. Les lettres indiquent des différences significatives entre traitements hydriques pour un génotype donné à $P < 0.05$ (Test de Tukey). Les interactions entre génotype et traitement sont indiquées à l'aide du symbole *, dans le cas contraire elles sont non significatives (NS).

Abstract

In horticulture, yield losses associated to water deficit (WD) could be compensated by positive effects on fruit quality and stimulation of plant to others stresses. The aim of the thesis was to explore a number of questions to assess the positive and negative effects of the WD, and to understand factors variations after setting a current knowledge of the effects of WD quality fruit. The questions were: 1) to characterize the effects of WD applied at specific stages of development of plant or fruit, 2) to understand the stress memory effect by alternating period of WD and recovery, 3) to test the stimulation of plant defences by WD during an infection by a pathogen, and 4) to study the genetic variability associated with responses to WD through the previous objectives. For this, several experiments were conducted in greenhouses and growth chambers. The parents of the MAGIC population of tomato (with rich allelic variability) were studied, as well as two commercial tomatoes (for the study of the interaction between WD and *Botrytis cinerea*). Plant response mechanisms induced during a WD were characterized in both plant vegetative and reproductive stages, and then during the fruit development stages (cell division, cell expansion and maturation). The plant vegetative and reproductive stages are distinguished by different mechanisms of oxidative stress management generated by WD. Vegetative plants are more affected at the photosystem II while reproductive plants rather modulate their water needs and responses at the photosystem I. The impact of WD on fruit quality differs according to fruit developmental stages. Strong genotypes effects were observed with an important increase in fruit taste quality (according to the concentrations in sugars and acids) for one genotype, which had poorer initial fruit quality than the second. Alternating periods of WD and recovery is a good compromise between water saving and plant adaptation (through proper management of oxidative stress). This alternation of stress has neutral to positive effects on fruit sugar content according to the genotypes and highly variable responses for contents of organic acids, carotenoids and ascorbic acid according to the genotypes. The interaction between a WD and *B. cinerea* generates a decrease of C/N ratio that can be associated to a stimulation of the pool of plant defence. However, the interaction between *B. Cinerea* and this WD promote pathogen development in stems. This deleterious effect of interaction seems attributable to hormonal regulation with the stimulation of the ABA pathway (by WD) and the SA pathway (by *B. cinerea*), which are antagonistic to the JA / ET pathway involved in response to necrotroph pathogens. However, a positive effect was observed for infections in detached leaves of infected plants, detached leaves represent a snapshot of plant defences and these results raise new questions. Overall, these data provide that WD improve fruit taste (sugars and acids) without yield losses but had variables effects on health promoting compounds. WD could permit the stimulation of plant defence but more research is needed. To finish, the genetic variability induced important variability in plant and fruit responses and interact with WD.

Key words: Adaptation, *B. cinerea*, Ecophysiology, Fruit quality, Genetic variability, OJIP transient, Water deficit, *Solanum lycopersicum* L.

Résumé

En horticulture, les effets négatifs du déficit hydrique (WD) sur le rendement peuvent être en partie compensés par des effets positifs sur la qualité des fruits et la stimulation des défenses de la plante face à d'autres stress. L'objectif de la thèse était d'explorer un certain nombre de pistes permettant d'évaluer les effets positifs et négatifs du WD, et d'appréhender les facteurs de variations. Après avoir établi un état des connaissances actuelles des effets du WD sur la qualité des fruits, plusieurs questions ont été abordées: 1) quels sont les effets de WD appliqués à des stades précis du développement de la plante ou du fruit, 2) existe-t-il une mémoire du stress en cas d'alternance de période de WD et de récupération, 3) les défenses de la plante sont-elles stimulées par un WD lors d'une infection par un pathogène, et enfin 4) quelle est la variabilité génétique associée aux réponses au WD au travers des objectifs précédents. Pour cela, plusieurs expérimentations ont été réalisées en serres et en phytotrons. Les parents de la population MAGIC de la tomate (ayant une forte variabilité allélique) ont été étudiés, ainsi que deux tomates commerciales (pour l'étude de l'interaction WD – *Botrytis cinerea*). Les mécanismes de réponses induits lors du WD ont été caractérisés aux stades végétatif et reproductif à l'échelle de la plante, puis lors des phases de développement du fruit (division cellulaire, expansion cellulaire et maturation). Les stades végétatif et reproductif se distinguent par les mécanismes de gestion du stress oxydatif engendré par le WD. Les plantes végétatives seraient plus affectées au niveau des photosystèmes II alors que les plantes reproductives modulent plutôt leur besoin en eau et sont plus sensibles à l'approche des photosystèmes I. L'impact du WD sur la qualité des fruits diffère en fonction de leurs phases de développement. De forts effets génotypes ont été observés avec une amélioration de la qualité dite gustative (par rapport aux teneurs en sucres et acides) pour un génotype dont les caractéristiques initiales étaient plus faibles que le second génotype testé. L'alternance de périodes de WD et de récupérations constitue un bon compromis entre économie d'eau et adaptation des plantes (via une bonne gestion du stress oxydatif). Cette alternance de stress a des effets neutres à positifs sur les teneurs en sucres des fruits en fonction des génotypes et des réponses très variables pour les teneurs en acides organiques, caroténoïdes et acide ascorbique en fonction des génotypes. L'interaction entre un WD et *B. cinerea* génère une diminution du ratio C/N associé à une stimulation des défenses de la plante. Cependant l'interaction favorise le développement du pathogène dans les tiges. Cet effet délétère de l'interaction semble lié aux régulations hormonales mises en jeu avec la stimulation de l'ABA (par le WD) et de la voie SA (par *B. cinerea* et indirectement par le WD) qui sont antagonistes aux voies JA/ET de réponse aux pathogènes nécrotrophes. Toutefois, un effet positif a été observé pour les infections sur feuilles détachées qui constituent un instantané des défenses de la plante, et ce, uniquement pour les plantes déjà infectées sur tige. Ces résultats suscitent de nouvelles interrogations sur la stimulation des défenses de la plante. Ainsi, le WD permet une augmentation du pool de sucres solubles, sans impact notable sur le rendement, avec des effets variables sur les composés secondaires. Le WD permet également dans une certaine mesure de stimuler les défenses de la plante mais de nouvelles recherches sont nécessaires. Enfin, la variabilité génétique des réponses à l'échelle de la plante et du fruit est importante et interagit avec le WD.

Mots clés : Adaptation, *B. cinerea*, Déficit hydrique, Ecophysiologie, Modèle OJIP, Qualité du fruit, *Solanum lycopersicum* L., Variabilité génétique