

Rôle joué par le potassium dans la réponse au déficit hydrique du maïs (Zea mays L.): des mécanismes physiologiques au fonctionnement intégré du peuplement

Elsa Martineau

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Par Elsa MARTINEAU

Rôle joué par le potassium dans la réponse au déficit hydrique du maïs (*Zea mays* L.) : des mécanismes physiologiques au fonctionnement intégré du peuplement

Sous la direction de Jean-Christophe DOMEC et Lionel JORDAN-MEILLE

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Rôle joué par le potassium dans la réponse au déficit hydrique du maïs (*Zea mays* L.) : des mécanismes physiologiques au fonctionnement intégré du peuplement

Résumé

Le potassium (K) est un élément majeur connu pour contribuer à la résistance des plantes à la sècheresse. L'étude de son influence sur la réponse physiologique du maïs (*Zea mays* L.) sous contrainte hydrique est essentielle pour prédire la future productivité dans un contexte de changements climatiques, en particulier de la diminution des précipitations.

Des modalités d'apports en K et en eau ont été croisées et soumises à des plants de maïs, élevés en condition contrôlées ou cultivés au champ. La croissance (biomasses aériennes et racinaires, rendements en grain) ainsi que les mécanismes écophysiologiques du métabolisme carboné (photosynthèse, transport des sucres) et du statut hydrique (transpiration, conductance stomatique, potentiels hydriques) ont été étudiés.

L'apport de K a contribué à l'augmentation de la croissance, le développement et le rendement grain quel que soit le régime hydrique imposé au maïs et les conditions d'expérimentation. Les résultats attendus sur la meilleure régulation stomatique en cas de déficit hydrique sont moins évidents. L'effet du stress hydrique ou de la déficience en K tendent à diminuer la photosynthèse. Cependant, ces effets ressortent plus sur les feuilles âgées que sur les feuilles jeunes. Dans ces mêmes conditions, le transport des sucres ne semble pas être un élément limitant de la croissance.

Plusieurs résultats convergent pour attribuer au K un rôle dans la maîtrise des pertes en eau (par unité de surface foliaire) et sur la meilleure efficience d'utilisation de l'eau. Néanmoins, cette efficience est imputée à des meilleurs rendements, liés à une surface foliaire plus importante, et non pas à une moindre consommation de l'eau.

Mots clés : Potassium, déficit hydrique, maïs (*Zea mays* L.), croissance, conductance stomatique, transport des sucres, rendement, efficience d'utilisation de l'eau

Quantifying the role of potassium in maize (*Zea mays* L.) resistance to water stress: from leaf-level physiological mechanisms to whole-plant functioning

Abstract

Potassium (K) is a major nutrient known to help plants resist drought. In the context of climate change, quantifying the role of K on maize physiological acclimation to reduced precipitations is essential to better predict future productivity.

Maize (*Zea mays* L.) plants grown under controlled or field conditions were submitted to different K and water levels. Plant growth (shoot and root biomass, grain yield) as well as plant water status (transpiration, stomatal conductance, water potential) and ecophysiological mechanisms of Carbon metabolism (photosynthesis, sugar transport) were studied.

Regardless of the water regime and experimental conditions, K nutrition increased growth and whole-plant development and improved grain yield. The effect of water stress on stomatal regulation was not straightforward and depended on the level of K fertilization. The effects of water or K deficit tend to decrease photosynthesis. Drought or K nutrition affected more leaf photosynthesis in old than in young leaves, and sugar transport did not seem to be a growth limiting factor.

Our results demonstrated a strong effect of K on biomass production and a higher water use efficiency with less of an impact on leaf-level physiology. This better water use was mainly the consequence of the positive effect of leaf area on yield, and not due to a reduce water use.

Keywords: Potassium, drought, maize (Zea mays L.), growth, stomatal conductance, sugar transport, yield, water use efficiency

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interaction (W x K) were tested using non-parametric two-way analysis of variance

Chapitre I.

Introduction générale

I. <u>La filière maïs, au cœur du débat sur l'utilisation des ressources en</u> <u>eau</u>

Les modèles climatiques prédisent une augmentation de la température de 1°C à 3.7°C selon les scénarios, à la surface du globe d'ici 2100 (GIEC, 2014). Le rapport du groupe d'experts intergouvernemental sur l'évolution du climat (GIEC) prévoit, avec une forte probabilité, que la fréquence et la durée d'événements climatiques extrêmes (ECE) s'ajouteront à cette hausse de température. Les ECE tels que les vagues de chaleurs, augmenteront sur une grande partie de l'Europe au cours du XXIe siècle (GIEC, 2014; Meehl and Tebaldi, 2004). Ces évènements, dus en majorité à l'activité humaine (démographique et économique), ne cessent d'augmenter depuis l'ère industrielle. Les activités anthropiques entraînent l'augmentation des émissions de gaz à effet de serre (GES), qui est la principale cause du réchauffement climatique.

Les menaces relatives aux changements climatiques risquent d'impacter la production alimentaire mondiale (GIEC, 2014). Dans les régions tempérées, l'augmentation de température et la diminution des précipitations auront des conséquences négatives sur la production du blé et du maïs. Ces conséquences varient selon les cultures, les régions et le scénario d'adaptation. Environ 10% des projections, sur la période 2030–2049, concluent à une baisse de rendement de plus de 25% par rapport aux observations du XXe siècle (GIEC, 2014).

En France, dans un horizon lointain (2071-2100) les scénarios (Ouzeau et al., 2014) prévoient une hausse des températures moyennes estivales comprises entre 2.6°C et 5.3°C. S'ajoutent à cela une augmentation du nombre de jours de vagues de chaleur estivale et une augmentation des épisodes de sècheresse sur une grande partie Sud du pays. Pour finir, une réduction des cumuls de précipitations est attendue, représentant une diminution relative de 12.5% par rapport à la période 1976-2005 (Fig. I.1). Tous ces phénomènes mis conjointement n'apparaissent pas comme favorables à l'agriculture de demain.



Fig. I.1 Projection de l'évolution des cumuls des précipitations moyennes annuelles sur un horizon lointain (entre 1976-2005 et 2071-2100) en mm, pour les scénarios du RCP2,6 (haut), RCP4.5(milieu) et du RCP8,5 (bas). Météo-France/CNRM2014 : modèle Aladin de Météo-France. Source : Drias, 2016

En parallèle, le modèle ISBA (Drias, 2016) prévoit une augmentation variable, selon les saisons, d'un état de dessiccation avancé des sols en France autour de 2055 (Fig. I.2). Pour le printemps et l'été, l'indice de sècheresse des sols (SWI) dans le Sud-Ouest passerait de normal à extrêmement sec. Le SWI est un indice de probabilité qui permet d'évaluer les sècheresses agricoles, basé sur les précipitations et la teneur en eau du sol au point de flétrissement et à la capacité au champ (http://www.drias-climat.fr). Ces variations climatiques, couplées à l'augmentation de la demande alimentaire, présentent un risque majeur en termes de sécurité alimentaire à l'échelle mondiale, mais aussi régionale. Dans ces conditions particulièrement désavantageuses l'enjeu majeur pour l'agriculture est de s'adapter.



Fig. I.2 Indice de sècheresse des sols (Modèle ISBA). Scénario d'évolution socio-économique intermédiaire (A1B). Météo France/CLIMSEC-CERFACS/SCRATCH08 : modèle Arpege-V4.6 de Météo France. Source : Drias, 2016

En France, le maïs est la deuxième céréale cultivée, après le blé tendre, avec une production de 18.5 millions de tonnes en 2014 (http:/agreste.agriculture.gouv.fr). L'ancienne région Aquitaine représentait 40% des surfaces de maïs (Brisson and Levrault, 2010) avec une production de 3.2 millions de tonnes pour 3261 km² en 2014, soit un rendement moyen de 98 quintaux par ha (Agreste, 2015). En Aquitaine, sur les trente dernières années, l'évapotranspiration moyenne du maïs a été de 410 mm (http://www.landes.chambagri.fr). L'irrigation s'impose donc pour cette culture dont le rendement décroît rapidement dès que la disponibilité en eau du sol diminue. Le maïs consomme ainsi 2/3 des volumes d'eau utilisés pour l'irrigation (http://agreste.agriculture.gouv.fr), apparaissant ainsi légitimement comme une culture consommatrice d'eau. En France, seules 50% des surfaces cultivées en France (maïs grain ou ensilage) sont irriguées (Brisson and Levrault, 2010).

Le manque d'eau induit par les sècheresses, réduit les rendements du maïs en diminuant l'absorption du rayonnement photosynthétique actif (PAR) par le couvert végétal via une diminution de la surface foliaire et sa conversion en biomasse. De fait, la part allouée aux parties récoltées est aussi réduite (Çakir, 2004; Earl and Davis, 2003). Face à la répétition des épisodes de sècheresses estivales, de nouvelles stratégies culturales s'imposent pour limiter les

pertes de rendements (Amigues et al., 2006). A l'échelle de l'itinéraire technique, une modification de la période ou de la durée culturale peuvent s'avérer nécessaire pour s'adapter à la disponibilité de la ressource en eau. Pour cela, les dates de semis peuvent être décalées, ou une variété plus précoce choisie. Une deuxième stratégie consiste à diminuer la demande en eau, en réduisant la densité du peuplement ou / et la fertilisation azotée. Cette diminution permet de limiter le développement et la surface foliaire et donc la perte en eau via la transpiration par la culture. A l'échelle du système de culture, une solution consisterait à semer des espèces végétales plus tolérantes à la sècheresse (Amigues et al., 2006).

II. Les plantes face au déficit hydrique

1. Qu'est-ce qu'un stress hydrique ?

En biologie, un **stress** peut être caractérisé de plusieurs manières. Dans notre contexte, il est défini comme un facteur de l'environnement défavorable à un organisme vivant (Levitt, 1980), ce qui introduit aussi la notion de **résistance** considérée comme la capacité de cet organisme à survivre à ce facteur environnemental (Levitt, 1980).

Lorsque le stress concerne la ressource en eau, il s'agit d'un stress de déficit hydrique, qu'on appellera plus communément un **déficit hydrique**. Cet état hydrique correspond à un niveau d'eau dans le sol qui ne permet plus à la plante de répondre à la demande évaporative extérieure. Les phénomènes de sècheresse introduits précédemment (ECE) peuvent entrainer une situation de déficit hydrique et donc de stress hydrique au sein des processus de fonctionnement de la plante.

Selon l'occurrence du stress hydrique, son intensité et sa durée, ses effets sur la culture de maïs seront différents (Gaspar et al., 2002). Si celui-ci apparaît au moment de la floraison ou du remplissage des grains, on peut s'attendre à avoir des répercussions négatives sur le rendement (Fig. I.3).



Fig. I.3 Evolution de la sensibilité au stress hydrique du maïs tout au long de son cycle. Source : Arvalis, 2016

2. Les stratégies d'adaptations des plantes au déficit hydrique

Face à ce déficit hydrique, les plantes mettent en place des stratégies d'adaptation à la sècheresse. On distingue quatre stratégies : l'esquive, l'évitement, la tolérance et la résistance. L'**esquive** est la première stratégie à être mise en place. Elle consiste pour les plantes à avancer leur stade phénologique en particulier la floraison (Levitt, 1980).

La stratégie d'évitement réduit l'impact du déficit hydrique en minimisant ses pertes en eau, et en optimisant son absorption (Gaspar et al., 2002; Hopkins, 2013). Les pertes d'eau peuvent être minimisées *via* une fermeture stomatique et une réduction de la surface foliaire (arrêt de croissance, enroulement foliaire, sénescence) (Tardieu, 2013; Yi et al., 2010). Le développement du système racinaire permet quant à lui d'augmenter l'absorption de l'eau en prospectant plus en profondeur (Levitt, 1980).

La stratégie de **tolérance** quant à elle, permet aux plantes de subir le stress sans que celui-ci n'impacte leur fonctionnement physiologique. Avec cette stratégie, la surface foliaire est maintenue ainsi que la floraison. De plus, la sénescence est retardée ce qui permet la translocation des réserves vers les organes grâce au maintien de la photosynthèse. L'ajustement osmotique est un mécanisme principal qui permet le maintien de la teneur en eau des plantes en condition hydrique limitante. La turgescence cellulaire permet d'assurer l'activité métabolique de la plante et par conséquent, la croissance et la productivité (Turner and Jones 1980). En cas de pluie, ces plantes sont capables de reprendre un fonctionnement normal, on les appelle les plantes reviviscentes. Au sein de cette stratégie, on peut distinguer deux autres phénomènes : l'adaptation ou l'acclimatation. L'**adaptation** engendre des modifications structurelles ou fonctionnelles (fonction héritable), telles que la morphologie ou la physiologie de la plante. L'**acclimatation** entraine une modification physiologique qui intervient au cours du cycle de vie (caractère non héritable). Lorsque les végétaux arrivent à s'acclimater sur une période longue et intense de stress, on parle de **résistance**.

3. Mécanismes de résistance des plantes au déficit hydrique

a. Fermeture stomatique

La transpiration (E), qui est directement proportionnelle au déficit de pression de vapeur (VPD), peut être réduite via la conductance stomatique (g_s) . Cette régulation est une réponse commune des plantes au stress hydrique (Hsiao, 1973; Levitt, 1980). Elle procure également une opportunité d'augmenter l'efficience d'utilisation de l'eau (WUE) des plantes (Singh and Raja Reddy, 2011). Il s'agit d'une des premières réponses rapides au stress hydrique, essentielle dans la limitation des pertes en eau. L'ouverture et la fermeture stomatique résultent d'une différence de turgescence entre les cellules de gardes et les cellules annexes et épidermiques (Hsiao, 1973). Ce contrôle stomatique peut être induit soit par un signal hormonal de l'acide abscissique (ABA), soit par un contrôle hydraulique passif lié à la turgescence cellulaire (Levitt, 1980; Tardieu and Davies, 1993). Il existe deux comportements de contrôle stomatique dépendant de leur potentiel hydrique (Attia et al., 2015). On distingue les espèces dites anisohydriques (tournesol, orge) et isohydriques (peuplier, maïs). Sous déficit hydrique, les espèces anisohydriques maintiennent une forte transpiration ce qui amène leur potentiel hydrique foliaire (Ψ_h) à des valeurs très négatives. A l'inverse les espèces isohydriques réduisent leur transpiration et leur conductance stomatique qui permet le maintien de leur potentiel hydrique. Ce contrôle stomatique permet de diminuer le flux d'eau sortant via la transpiration, et par conséquent les pertes en eau au détriment de l'assimilation net de carbone (A_n) qui se retrouve fortement réduite voire même stoppée.

b. Ajustement de la surface transpirante

C'est au niveau foliaire que les premiers symptômes du déficit hydrique se font ressentir chez les végétaux. Face à ce déficit, une des réponses importantes des plantes repose sur la réduction de leur surface foliaire (Hsiao, 1973). Cette réduction s'opère à travers la diminution de la taille des feuilles, de leur nombre (arrêt de croissance et senescence) et de leur **enroulement** (Fang and Xiong, 2015). Par conséquent, la surface d'échange plante-atmosphère se retrouve réduite et les pertes en eau sont ainsi limitées.

Cependant, cette restriction de surface d'échange induit aussi, tout comme la fermeture stomatique, une diminution de l'assimilation nette de carbone par la photosynthèse (Blum, 1996; Hsiao, 1973; Yordanov et al., 2000). La photosynthèse est directement affectée par les effets négatifs du déficit hydrique, d'une part à travers la fermeture stomatique, et d'autre part *via* l'altération de sa chaîne photosynthétique. La réduction de surface foliaire a pour conséquences directes la diminution de la biomasse et du rendement qui en découlent (Hsiao, 1973).

c. Accroissement racinaire

En cas de sécheresse, une réponse morphologique courante est l'augmentation relative de la biomasse racinaire par rapport à la biomasse aérienne (couramment utilisée sous le terme du ratio **Root/Shoot**) (Blum, 1996; Lu et al., 2014; Xu et al., 2015). Ce phénomène a pour conséquence d'augmenter la prospection du sol et donc l'accès à la ressource en eau, conférant ainsi aux plantes la possibilité de maintenir leur potentiel hydrique élevé en cas de déficit hydrique (Sharp et al., 1988).

d. Ajustement osmotique et production d'espèces réactives de l'oxygène

L'accumulation de la teneur en solutés (tel que ions, sucres, acides aminés) dans les vacuoles des cellules est appelée l'**ajustement osmotique** (Morgan, 1984). Il s'agit d'une des caractéristiques la plus distincte de la réponse adaptative des végétaux au déficit hydrique. La diminution du **potentiel osmotique** (Ψ_s) des plantes entraîne une moindre déshydratation et limite l'effet du stress hydrique dans les tissus. Chez la vigne ce potentiel osmotique peut passer de -1.37 MPa à -1.82 MPa (Rodrigues et al., 1993) ou encore chez le maïs passant de - 0.9 MPa à -2 MPa (Voetberg and Sharp, 1991). Cet ajustement osmotique est connu pour

maintenir la photosynthèse sous faible potentiel hydrique, mais aussi de retarder la sénescence foliaire et pour finir, d'améliorer la croissance racinaire (Fang and Xiong, 2015). En diminuant le Ψ_h , le Ψ_s permet ainsi de maintenir la teneur en eau dans les feuilles et donc leur turgescence. En permettant à la feuille de maintenir sa turgescence le faible Ψ_h permet de garder les stomates ouverts et d'assimiler du carbone dans ces conditions limitantes en eau. Munns (1988) souligne toutefois que le mainteni d'un faible potentiel hydrique peut être la simple conséquence d'un ralentissement de la croissance, et non pas le résultat.

Lors d'un déficit hydrique, un déséquilibre dans la chaîne de réaction de la photosynthèse apparaît. Le transport des électrons est altéré (Cakmak, 2005) et il en résulte une accumulation d'énergie non dissipée, sous forme chimique: les **espèces réactives de l'oxygène** (ROS). Leur présence crée des nécroses sur les tissus foliaires qui peuvent conduire à la mort des cellules (Munné-Bosch and Alegre, 2004) et donc à terme de la feuille.

Les processus de résistance au déficit hydrique présentés précédemment sont résumés sous la forme d'un schéma (Fig. I.4).



Fig. I.4 Schéma récapitulatif de la réponse physiologique des plantes sous contrainte hydrique.

III. Propriété et rôle du potassium dans le fonctionnement des plantes

1. Origines géologiques et géographiques du potassium

Le **potassium** (K) représente 2,6% de la croûte terrestre (Schroeder, 1978). Il abonde dans les sédiments sodiques et dans quelques minéraux comme les feldspaths ou les micas. Les ressources naturelles se situent en grande majorité à des niveaux variant de 600 à 1200 mètres sous la surface du sol, nécessitant de lourds investissements miniers (GCL Développement Durable, 2010). Le problème de la ressource potassique réside dans sa répartition sur la surface de la terre et des actuelles capacités d'extractions, globalement insuffisantes pour une demande mondiale qui ne cesse d'augmenter depuis les années 1960 (http://www.fao.org). Le Canada est le premier producteur mondial de potasse. En 2015, 70% des ressources sont réparties entre le Canada, la Biélorussie, la Russie, l'Allemagne et les Etats-Unis (Fig. I.5). Dans l'Union européenne, seule l'Allemagne produit encore du K (entreprise K+S KALI GmbH).



Fig. I.5 Estimation des réserves de potasse en pourcentage (%) pour 2015. Source : U.S. Geological Survey, Mineral Commodity Summaries, January 2016

Le K est un nutriment essentiel pour les plantes qui intervient dans plusieurs processus physiologiques ou biochimiques, notamment impliqués dans la résistance aux stress biotiques et abiotiques (Wang et al., 2013).

2. Propriétés du potassium

Le K est disponible sous forme de cation monovalent K^+ dans la solution du sol. Ce cation est le plus concentré dans les plantes et possède une très grande mobilité, du fait de n'avoir aucune liaison avec le carbone. Cette mobilité lui permet d'être transporté facilement et

rapidement des racines vers les feuilles. La concentration dans les plantes est souvent en dessous de 2.5-3.5% (Öborn et al., 2005). Pour beaucoup de cultures, le seuil critique de concentration est en moyenne de 0.5 à 2% dans la matière sèche (Leigh and Jones, 1984). Les symptômes de carence se traduisent par une nécrose touchant en premier lieu les feuilles plus âgées. Elle apparaît en partant de l'extérieur vers l'intérieur des feuilles (Fig. I.6). Les tiges sont quant à elles moins rigides ce qui les fragilise et les expose plus aux phénomènes de verse.



Fig. I.6 Symptômes de carence en potassium (K) caractérisés par une nécrose des feuilles de leur extrémité vers la nervure centrale chez le maïs. Source: Martineau, 2015

3. Les effets du potassium...

a. ... Au niveau de la partie aérienne

Le K joue un rôle majeur sur le métabolisme, le développement, la croissance et le rendement des cultures (Oosterhuis et al., 2013). Des études montrent que la déficience en K accélère la sénescence foliaire sur le maïs (Armengaud et al., 2004; Battie-Laclau et al., 2013) et réduit la taille et le nombre de feuilles visibles (Jordan-Meille and Pellerin, 2004; Marschner, 1995; Oosterhuis et al., 2013).

Le K est responsable du changement de turgescence dans les cellules de garde durant les mouvements stomatiques. Le contenu en K des cellules de garde des stomates fermés est faible comparé aux concentrations dans les cellules annexes. Lors de l'ouverture stomatique, des quantités de K migrent vers les cellules de garde et augmentent leur potentiel osmotique. L'accumulation de K dans les cellules de garde constitue le mécanisme général d'ouverture et de fermeture stomatique (Humble and Raschke, 1971; Marschner, 1995). Le K, de ce fait,

améliore la conductance stomatique (Battie-Laclau et al., 2014a), et maintient la turgescence cellulaire des plantes. Le K participe donc activement à l'ajustement osmotique, et participe de ce fait à minimiser les pertes en eau et les effets négatifs du déficit hydrique (Premachandra et al., 1992; Wakeel et al., 2011). L'extension cellulaire, liée à l'élasticité des parois et le potentiel osmotique, est provoquée par l'accumulation de K dans les cellules qui diminue le potentiel osmotique. Cette accumulation de K est une condition préalable pour établir et maintenir un potentiel osmotique élevé, afin de permettre le transport des solutés (Gerardeaux et al., 2010; Marschner, 1995).

Le K agit aussi sur la retranslocation des assimilats des feuilles vers les racines *via* le phloème. Son transport à travers celui-ci est dirigé des feuilles les plus âgées aux plus jeunes pour se retrouver dans les tissus en croissance responsables du développement de celles-ci (Mengel et al., 2001).

Le transport des assimilats peut être altéré en cas de carence en K, ce qui provoque une accumulation de sucres dans les feuilles les plus déficientes (Zörb et al., 2014). La concentration en sucres dans les feuilles est une relation inverse à la concentration en K (Gerardeaux et al., 2009; Marschner, 1995). Cette accumulation de sucres serait induite par un défaut d'exportation du saccharose (Cakmak et al., 1994; Hermans et al., 2006). Une limitation de photosynthèse (A_n) peut être provoquée par cette accumulation, entrainant une formation de ROS (Cakmak, 2005). Cependant, les résultats des études comparant l'effet du K sur la photosynthèse sont controversés. Peaslee and Moss (1966) montrent que A_n est corrélée à la concentration du K dans les feuilles de maïs, tout comme Bednarz et al. (1998), Basile et al. (2003), Battie-Laclau et al. (2014a) chez d'autres espèces. Cette réduction du taux de photosynthèse est liée à la diminution de conductance stomatique (Peaslee and Moss, 1968a). En revanche, Sen Gupta et al. (1989) et Tsonev et al. (2011) n'observent un effet positif du K sur la photosynthèse que lorsque qu'il y a déficit hydrique.

b. ... Au niveau de la partie racinaire

La croissance racinaire est limitée par la déficience en K. Le rapport entre biomasse racinaire et biomasse aérienne (R/S) est réduit avec la diminution de K (Ericsson, 1995). Plusieurs résultats indiquent une cause métabolique plutôt qu'un problème de blocage physique au niveau des apex. L'exportation du saccharose vers les racines serait réduite chez les plantes déficientes en K, ce qui pourrait être attribué à une exigence en K lors du chargement du phloème (Deeken et al., 2002). De plus, les racines subissant une déficience en K ne montrent pas d'accumulation de sucres, mais plutôt des concentrations plus faibles en saccharose et en amidon que des plantes bien alimentées en K (Cakmak et al., 1994). Chez le maïs, les canaux spécifiques liés au K sont apparentés au chargement et déchargement des sucres (Philippar et al., 2003). Cette diminution de teneurs en sucres des racines, à l'origine de leur moindre croissance, est à mettre en relation avec l'augmentation des concentrations de saccharose dans les feuilles pour des plantes déficientes en K (Zörb et al., 2014). L'apport de K permet d'améliorer le développement et la distribution spatiale des racines, permettant ainsi d'augmenter le prélèvement des minéraux (Römheld and Kirkby, 2010). Pourtant, en cas de déficience en K, Hogh-Jensen and Pedersen (2003) ont montré sur plusieurs espèces, une augmentation de la densité et la longueur des poils absorbant, au détriment de la biomasse totale. L'effet du K sur la réponse racinaire reste donc encore à approfondir.

Les effets de la déficience en K, présentés précédemment sont résumés sous la forme d'un schéma (Fig. I.7).



Fig. I.7 Schéma récapitulatif de la réponse des plantes lors d'une déficience en potassium.

IV. <u>La fertilisation potassique pour pallier aux effets négatifs du stress</u> <u>hydrique</u>

Les cibles principales touchées, soit par un déficit hydrique, soit une déficience en K, ont été introduites. Il apparaît que beaucoup de réponses physiologiques sont communes à l'eau et au potassium (Fig. I.8). Le K est connu pour avoir un effet important sur le statut hydrique des plantes et leur meilleure capacité à résister au stress hydrique (Wang et al., 2013). Dans ce cas, pourquoi ne pas envisager le K comme levier pour limiter les effets négatifs du déficit hydrique sur les plantes ? Cette question pratique est à l'origine de nombreuses recherches récentes, lancées essentiellement en milieux contrôlés (Benlloch-González et al., 2008; Jákli et al., 2016; Wei et al., 2013).



Fig. I.8 Schéma représentant les réponses communes des plantes au déficit hydrique et potassique (en noir) et les réponses antagonistes entre le déficit hydrique et potassique (en rouge).

1. Atténuation du stress hydrique par l'effet du potassium sur les composantes du potentiel hydrique

Une forte accumulation de K par les cultures dans des conditions optimales de croissance pourrait être envisagée comme une stratégie d'assurance pour permettre à la plante de mieux survivre à un stress environnemental (Kafkafi, 1990; Zörb et al., 2014). Lorsqu'une plante est soumise à un déficit hydrique la nutrition potassique permet de maintenir la teneur en eau et le potentiel de turgescence à des valeurs relativement fortes en concentrant le K dans ses organes (Andersen et al., 1992a; Cakmak, 2005; Egilla et al., 2001; Sen Gupta et al., 1989). Cependant, cette concentration du K dans les organes de la plante n'est pas toujours observée, comme le montre Benlloch-González et al. sur le tournesol (2010a, 2010b).

Le K est connu pour améliorer la rétention de l'eau dans les tissus et donc de contribuer à un meilleur ajustement osmotique (Eakes et al., 1991; Lindhauer, 1985; Marschner, 1995; Mengel and Arneke, 1982; Römheld and Kirkby, 2010), qui a pour conséquence une

économie d'eau par la plante. Pour les cultures il s'agit d'un mécanisme très important quand un déficit hydrique se fait ressentir (Jones and Turner, 1978; Premachandra et al., 1992). Les concentrations en sucre et en K augmentent dans les tissus sous condition hydrique limitante en faveur d'une diminution du potentiel osmotique (Itoh and Kumura, 1987; Premachandra et al., 1992). Dans ces conditions stressantes le K apparaît donc comme un élément-clé qui permet de maintenir le potentiel osmotique et de turgescence (Ashraf et al., 2002; Lindhauer, 1985) dans un état favorable à la croissance racinaire et aérienne (Bahrani et al., 2012; Battie-Laclau et al., 2016; Egilla et al., 2001; Lindhauer, 1985).

2. Atténuation du stress hydrique par l'effet du potassium sur la régulation stomatique

De plus, la régulation stomatique est améliorée sous déficit hydrique par l'ajout de K (Arquero et al., 2006; Ashraf et al., 2001; Benlloch-González et al., 2008). Ce qui a comme conséquence directe, une transpiration réduite lors de stress hydrique (Ashraf et al., 2001; Teixeira and Dezordi, 2008) et une photosynthèse augmentée par la présence de K en concentration optimale (Egilla et al., 2005).

3. Atténuation du stress hydrique par l'effet du potassium sur la photosynthèse

En lien direct avec la photosynthèse, la biomasse et le rendement augmentent (Abdel-All and Seham, 2013; Andersen et al., 1992b; Battie-Laclau et al., 2016; Egilla et al., 2001). Comme soulevé précédemment, Tsonev et al. (2011) et Sen Gupta et al. (1989) ont montré des effets positifs de la nutrition K sur le taux de photosynthèse dans des cultures soumises à un déficit hydrique.

4. Atténuation du stress hydrique par l'effet du potassium sur l'exportation des sucres

Grâce au K, les produits de la photosynthèse sont mieux transportés, des feuilles vers les racines en condition hydrique limitante (Cakmak, 2005; Marschner, 1995; Wang et al., 2013). Ceci est d'autant plus important durant la période reproductive où la demande en

photoassimilat est augmentée pour le développement des fruits ou des grains (Römheld and Kirkby, 2010). La translocation des sucres des zones sources vers les zones de puits tel que les racines, permet une meilleure croissance (Römheld and Kirkby, 2010) et augmente la longévité racinaire (Egilla et al., 2001). Cependant, lorsque le déficit hydrique survient trop précocement dans le stade de développement des plantes, le prélèvement du K peut être empêché par l'inhibition de la croissance racinaire de la plante (Römheld and Kirkby, 2010). La nutrition potassique contribue à la détoxification des ROS en réduisant l'activité de NAD(P)H oxydases et en maintenant le transport des électrons photosynthétiques (Cakmak, 2005).

5. Atténuation du stress hydrique par l'effet global du potassium sur l'efficience d'utilisation de l'eau

A l'échelle des peuplements végétaux, les effets physiologiques du K sur les plantes stressées en eau se trouvent souvent traduite par une amélioration de l'efficience d'utilisation de l'eau (WUE), comme par exemple sur le riz (Mohd Zain and Ismail, 2016) ou encore sur le triticale et le maïs (Grzebisz et al., 2013). L'efficience d'utilisation de l'eau (WUE) est utilisée pour déterminer la performance des plantes à économiser l'eau. Elle est considérée comme une composante de la résistance à la sècheresse. Elle peut être définie soit à long terme (WUE) comme le rendement obtenu par rapport à la consommation d'eau utilisé par la culture (Hsiao and Acevedo, 1974) ou à court terme ou intrinsèque (WUE_i) comme l'assimilation net de carbone (A_n) sur la conductance stomatique (g_s), (Monclus et al., 2006). Des études ont montré l'augmentation du WUE par l'apport de K (Andersen et al., 1992b; Arquero et al., 2006; Bahrani et al., 2012; Battie-Laclau et al., 2016). Cependant, l'effet positif du K sur le WUE_i est mitigé (Battie-Laclau et al., 2016; Egilla et al., 2005; Jákli et al., 2016; Pervez et al., 2004).

Le potassium apparaît donc comme un levier à la tolérance des plantes à la sècheresse en leur permettant de mettre en place des processus favorables à la limitation des pertes en eau (ajustement osmotique, contrôle stomatique, croissance racinaire) et à l'amélioration de la croissance (A_n , translocation des photoassimilats...), se traduisant globalement par une meilleure efficience d'utilisation de l'eau (WUE).

V. Objectifs, hypothèses

1. Objectifs

L'objectif général du projet de thèse est d'étudier l'effet de la nutrition potassique sur la réponse physiologique du maïs, sous contrainte hydrique. En appui aux manipulations effectuées sous serre, la réponse intégrée de la plante en peuplement est testée en conditions agricoles de plein champ. Ce projet s'inscrit dans une démarche de maintien de la production agricole sous forte contrainte hydrique. Le changement climatique, qui est la principale cause de ces contraintes hydriques, pousse la recherche agronomique à trouver des alternatives et/ou de nouvelles stratégies culturales.

Les **objectifs cognitifs** consistent à i) hiérarchiser, parmi les réponses physiologiques des plantes à la nutrition potassique, celles qui expliquent le mieux leur tolérance vis-à-vis du déficit hydrique et ii) estimer le seuil de réponses physiologiques et de croissance des plantes à la déficience en K, selon leur alimentation en eau.

Les **objectifs opérationnels** possibles sont d'estimer le gain de rendement et la quantité d'eau économisée grâce au K, en cas de déficit hydrique. Ces objectifs consistent i) à faire évoluer le raisonnement de la fertilisation K en condition de déficit hydrique et ii) à adapter la fertilisation K selon la disponibilité en eau.

2. Hypothèses

* Le potassium agit sur le statut hydrique de la plante stressée en eau en :

- maintenant la vitesse d'élongation foliaire
- allongeant la durée de vie des feuilles

- diminuant les pertes en eau par unité de surface foliaire via une meilleure régulation stomatique

- améliorant l'efficience d'utilisation de l'eau intrinsèque et globale (WUEi, WUE)

* Le potassium agit sur le métabolisme de la plante stressée en eau en :

- améliorant la photosynthèse à l'échelle foliaire
- favorisant l'exportation des sucres vers les organes puits

* Le potassium permet au maïs stressé en eau d'améliorer son rendement

3. Choix méthodologiques

Diverses expérimentations (serre ou champ) et méthodes nous ont permis d'acquérir une base de données lors de cette thèse. Le travail de recherche s'est d'abord focalisé sur la réponse des plantes en conditions contrôlées, donnant lieu à deux expérimentations (Printemps 2013 et 2015). Dès le départ, le choix de travailler sur un substrat sableux s'est imposé afin de se rapprocher des conditions de culture de la zone des sables landais, mais aussi pour s'assurer d'une réponse rapide du maïs au déficit hydrique imposé. La durée des expérimentations en serre a permis d'analyser la réponse de plantes à un stade de développement avancé (préfloraison), ce qui a eu l'avantage de bénéficier de matériel végétal largement carencé en K et stressé en eau. La nécessité de tester les hypothèses en grandeur réelle nous a poussés à investir un essai de fertilisation K de longue durée, durant les saisons 2014 et 2015. Le dispositif d'irrigation présent sur site a été adapté de manière à créer deux modalités de teneurs en eau du sol.

Les résultats de cette thèse sont répartis en trois chapitres indépendants, présentés sous forme d'articles scientifiques. Le premier article étudie l'effet du K et de la teneur en eau du sol sur la vitesse d'exportation des sucres. Les deux articles suivants abordent la problématique du rôle du K en condition de déficit hydrique de l'échelle cellulaire à l'échelle du peuplement.

Chapitre II.

<u>Rôle du potassium dans l'exportation du</u> <u>carbone dans les feuilles de maïs en</u> <u>condition de déficit hydrique</u>

The role of potassium on maize leaf carbon exportation under drought condition

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<u>Abstract</u>

Climate changes are mainly characterized by an increase in air temperature and a decrease in rainfalls. Potassium (K) nutrition is generally considered to alleviate plants tolerance to water deficit, especially by improving photosynthesis and phloem transport of carbohydrates from leaves to roots. The main objective of this study was to measure the effect of K on sugar transport and allocation under water-stressed conditions on maize (Zea mays L.). Maize plants were grown in pots under different water and K treatments. We used ¹³CO₂ pulse-labelling to determine carbon exportation from leaves with $\delta 13C$ analysis, within one week. The diurnal sugar content in leaves was measured, and net carbon assimilation accessed. Water deficit strongly reduced plant growth, while K nutrition appeared to be efficient in attenuating these effects. K-deficiency significantly decreased starch content in leaves under well-watered but not under water-stressed treatment. A leaf carbon mass balance showed that K increased sugar export on a daily time scale, while instantaneous $\delta^{13}C$ measurements did not show any significant effect, partly because of the very rapid $\delta^{13}C$ decline after labelling. Our labelling chamber proved to be successful in monitoring diurnal changes in δ^{13} C for a C4 plant with high photosynthetic rates and fast carbon export, and also in determining the effect of a K deficiency on sugar export. Our results highlight a need for research into carbon export on leaves of different ages in fast-growing crops under the combined effect of water and nutrient stress.

Keywords: Potassium; Water deficit; Sugar transport; Carbon export; Pulse-labelling; Maize (*Zea mays* L.)
I. Introduction

Plant response to water limitation is very well documented and shows a sequence of physiological reactions, linked not only to cell turgor adjustment but also to metabolic activity (Hsiao 1973). The most classical physiological response of a water-stressed plant consists of stomatal closure and osmotic changes (Hsiao et al. 1976; Cochard 2002; Blum 2005). Water-stressed plants also reduce leaf growth (hormonal response), thereby favouring their root growth, thus increasing their root:shoot ratio (R/S) (Marschner et al. 1996). Moreover, plant metabolic functioning can be impacted, with a general decrease in photosynthetic activity, as well as sugar accumulation in photosynthetically active leaves (Hsiao and Acevedo 1974; Kim et al. 2000; Hoffmann 2010).

The role played by potassium (K) nutrition in mitigating plant water stress has been well documented (Cakmak 2005; Grzebisz et al. 2013; Wang et al. 2013). In response to the reduced quantities of rainfall predicted by climate models (IPCC 2014), some recent studies have been looking at the interactive effects of water stress and K nutrition on plant growth (Arquero et al. 2006; Battie-Laclau et al. 2014b; Christina et al. 2015; Jákli et al. 2016; Martineau et al. 2017). At plant scale, and under water stress, the main morphological results showed that K optimal nutrition promoted leaf longevity (Battie-Laclau et al. 2013), leaf area (Lindhauer 1985), root biomass (Arquero et al. 2006) and R/S ratio (Egilla et al. 2001). Moreover, all the results pointed towards a positive impact of optimal K supply on plant water use efficiency (Egilla et al. 2005; Bahrani et al. 2012; Battie-Laclau et al. 2016). At least four complementary physiological mechanisms can explain the potential positive role played by K on water stressed plants, as described below.

Historically, the scientific justification linking K nutrition to plant drought tolerance relied on the very strong solubility of K, which serves as the major inorganic osmoticum within plants (Leigh and Jones 1984). By enhancing tissue hydration (Carroll et al. 1994), K influences cell osmotic and turgor potentials (Van Volkenburgh and Boyer 1985), thereby favoring meristematic cell elongation and growth (Mengel and Arneke 1982; Marschner 1995; Triboulot et al. 1997; Benlloch-González et al. 2010). However, this simple model does not always account for the measurements obtained at cell scale, even on well-watered plants (Mengel and Arneke 1982; Itoh et al. 1997; Triboulot et al. 1997). Munns (1988) questioned the "osmotic adjustment theory", suggesting that the increase of osmotic potential could be the consequence of a growth limitation rather that an adaptation to drought.

The second means by which K could alleviate water stress would be by increasing stomatal sensitivity to soil water content (Benlloch-González et al. 2008). This phenomenon is closely bound up with maintaining the osmotic potential of guard cells (Fischer and Hsiao 1968; Cochrane and Cochrane 2009). The effect of K on stomatal control could explain the increase in plant water use efficiency (WUE) measured in water-stressed plants (Arquero et al. 2006; Battie-Laclau et al. 2016).

Thirdly, K nutrition could help plants overcome their water stress by increasing photosynthesis activity and carbon assimilation rate (A_n) . This role has clearly been demonstrated for well-watered plants (Huber 1985; Bednarz et al. 1998; Zhao et al. 2001; Gerardeaux et al. 2009), although it was not always clear whether this response was totally independent of g_s . As for the physiological effect of K limitation on A_n under water stress, this has been addressed, but has given somewhat divergent (Sen Gupta et al. 1989; Egilla et al. 2005; Battie-Laclau et al. 2014a).

Finally, K could be involved in the sugar reallocation that would have accumulated during water stress. This hypothesis is all the more credible as it has even been demonstrated under normal well-watered conditions (Marschner et al. 1996; Gerardeaux et al. 2009; Pastenes et al. 2014). According to Cakmak et al. (1994), the K effect on sucrose transport would account for most of the plant responses undergoing K stress: sugar accumulation, negative feed-back on photosynthesis (Ainsworth and Bush 2011), and lower growth rates. Those results were later confirmed by Armengaud et al. (2009) and Pettigrew (1999). Under water stress, optimal K nutrition could, accordingly, interact positively on metabolic reactions (Lemoine et al. 2013), although few studies have focused on this subject (Jákli et al. 2016). The present study aims at determining the role of K in the translocation rate of sugars from leaf to phloem, especially in water-stressed plants. Photosynthesis, leaf sugars concentrations and carbon (C) export rate using ¹³C labelling (Blessing et al. 2015; Epron et al. 2015) were measured, in order to disentangle the effect of K limitation and water stress on leaf photosynthetic and metabolic activity. We hypothesize that (i) K increases sugar export in maize grown under water deficit; and (ii) photosynthesis of water-stressed or K-deprived plants is reduced as a consequence of sugar accumulation in leaves.

II. Materials and methods

1. Experimental design

This study was conducted on maize (Zea may L. variety "DKC 5784") in greenhouse conditions at the French National Institute for Agricultural Research (INRA Bordeaux, France) during the spring of 2015. Thirty-two plants were grown in 14-litre pots filled with 10 kg of dry sandy soil free of any available nutrient. Photosynthetically active radiation (PAR) was measured with a LI-190R quantum sensor (LI-COR, Logan, UT, USA), and air temperature and relative humidity by means of an HMP 35C sensor (Vaissala, Finland). These greenhouse environmental conditions were recorded every 60 seconds and averaged every half-hour, using a CR1000 datalogger (Campbell Scientific, Logan, UT, USA). During the experiment, mean diurnal air temperature, relative humidity, and PAR were 22.9°C, 56.3% and 272.4 µmol m⁻² s⁻¹, respectively. Two weeks after the seeds were planted, two different nutritive solutions (modified-Hoagland type) were applied daily, so as to bring a total of either 400 mg of K (K+) or 50 mg of K (K-) per plant. For the first 26 days after sowing (DAS), when six leaves were visible, all plants were watered to prevent water deficit and to ensure that K uptake would not be limiting. The soil volumetric humidity corresponding to this wellwatered treatment (W+) was around 15% (m³_{water} by m³_{soil}), which corresponded to 80% of the field capacity for this type of soil. After this 26 day-period, irrigation was modified on half of the pots so as to to bring and maintain soil water content (SWC) to a volumetric humidity of 7%. These two contrasting water treatments (W+ and W-) lasted for one month before the plants were labelled with ¹³C. Each plant was therefore weighed daily and SWC was adjusted to either 15% or 7%. Fresh plant biomass was taken into account to calculate of the amount water to be added. Four plants per treatment (K+W+, K-W+, K+W- and K-W-) were then randomly selected for the labelling experiment, and 16 others in order to determine sugar concentrations.

2. Leaf δ^{13} C

a. Chamber system for labelling

A labelling chamber (length=1.8 m; height=1.2 m; width=0.7 m, for a total volume of 1.54 m³) was set up next to the greenhouse, in order to contain four maize plants (Fig. II.1a). This chamber was sealed hermetically with clear polyethylene film to prevent carbon dioxide (CO₂) leakage, and also to enable photosynthesis measurements during labelling. The chamber system consisted of three parts: 1) a system designed to rapidly remove the initial 12 CO₂ molecules, using a closed vacuum system connected to a sodalime filter; 2) a 13 CO₂ labelling injection system to set the initial ¹³CO₂ concentration at 400 ppm; 3) a combination of two infrared gas analyzers (a LI6262 LICOR Inc., NE, USA, and a Binos-100, ROSEMOUNT Analytical, Germany) comprising two spectral absorption ranges to differentiate ¹²CO₂ and ¹³CO₂. Just after closing the labelling chamber, air in the chamber was ventilated through sodalime to remove ¹²CO₂. When ¹²CO₂ concentration dropped below 40ppm, the filter system was disconnected and ¹³CO₂ (99atom%; Eurisotop; Cambridge Isotope Laboratory Inc., Andover, MA, USA) was injected in the chamber to reach a concentration of 400ppm. During plant labelling, concentrations of both ¹²CO₂ and ¹³CO₂ were monitored simultaneously. Each time ¹³CO₂ concentration decreased to 380 ppm due to CO₂ assimilation by plants, a new injection of ¹³CO₂ was made to bring back the concentration to 400 ppm. After 30 minutes of labelling, the chamber was opened to ensure a return to ambient atmospheric CO₂ concentration.

b. $\delta^{13}C$ labelling schedule

Four successive labellings were performed the same day in mid-May 2015, so that all sixteen plants had similar climatic conditions and were at the same development stage (14th visible leaf). Four plants of each K and water treatment were selected during each labelling period. Air temperature inside the labelling chamber was recorded and controlled, using a CR1000 (Campbell Scientific) to match the outside air temperature.

c. Sampling procedure and analysis

Leaf δ^{13} C was measured using 4 mm diameter punched disks collected on the same lower and upper leaves (respectively 8th-10th and 11th &12th leaf). The lower leaves had completed their growth, whereas the upper ones had not achieved their final size. The first six disks were collected immediately after the ¹³CO₂ labelling, and six additional disks two, four and seven days after labelling (Fig II.1b). Additional leaf samples were collected on four un-labelled plants in order to measure the initial leaf δ^{13} C (δ^{13} C_i). Leaf tissues were placed in a 1.5 ml Eppendorf tube before being dried for 48 hours in a kiln at 60°C, and then ground. Leaf extracts were transferred into small tin capsules (Elemental Microanalysis, Cambridge, UK; 6×4mm; ref. D1006, BN/139877), weighed and analyzed for carbon isotope composition and total carbon, using an elemental analyzer (EuroVektor, HEKAtech GmbH) coupled to a Delta-Plus XP isotopic ratio mass spectrometer (Finnigan MAT; Conflo III Thermo Electron Cooperation, Bremen, Germany). Leaf ¹³C/¹²C isotopic ratio (δ^{13} C) was expressed relative to the R_{VPDB} standard, as proposed by Smith and Epstein (1971):

 $\delta^{13}C(\%_0) = (R_{Plant}/R_{VPDB} - 1) \cdot 1000$

(Equation 1)



Fig. II.1 a) Schematic of labelling chamber during labelled time on four maize plants (K+W+, K+W-, K-W+, K-W-). Main parts of labelling system was composed to A: sodalime filter (A), ¹³CO₂ injection system (B), and infrared gas analyzers (C). b) Illustration of disk sampling (day 0 (D0), 1 (D1), 2 (D2) and 4 (D4)) in ¹³C in maize leaf labelled. For each sampling day (0, 1, 2, 4 and 7), we sampled on the same veins on all length of leaf.

d. Calculation of carbohydrate export capacity

To quantify the amount of ¹³C incorporated in the plant during labelling, the excess of leaf ¹³C ($^{13}C_{e}$) was calculated as follows:

$${}^{13}C_e = (\delta^{13}C - \delta^{13}C_i) \cdot R_{VPDB} \cdot C_{\%}/100$$
 (Equation 2)

with δ^{13} C for labelled samples, δ^{13} C_i for initial samples, and with C% referring to the concentration in the samples (Kaldy et al. 2013). Because labelling was made with a CO₂ concentration almost totally enriched in ¹³C, leaf ¹³C was taken as δ^{13} C. ¹³C_e was initially stored as non-structural (or soluble) carbohydrate compounds (${}^{13}C_{e,ns}$) only, since ${}^{13}C_e$ conversion to new tissues (new structural compounds, or ${}^{13}C_{e,s}$) had not been made yet (Epron et al. 2012). We assume that the evolution of ${}^{13}C_{e,ns}$ is due to its leaf exportation flux (${}^{13}E_e$), which corresponds to phloem loading, and also to its transformation flux into structural compounds (${}^{13}F_e$) according to:

$${}^{13}E_e(t) = \alpha \cdot {}^{13}C_{e,ns}(t)$$
(Equation 3)
$${}^{13}F_e(t) = \beta \cdot {}^{13}C_{e,ns}(t),$$
(Equation 4)

where α and β represent the carbohydrate export capacity factor and the fixation factor, respectively, and *t* represents time (s).

This simple model approach allows the evolution of the ¹³C pools to be expressed by the following equations:

$$\begin{cases} \frac{d^{13}C_{e,ns}}{dt}(t) = -(\alpha + \beta) \cdot {}^{13}C_{e,ns}(t) \\ \frac{d^{13}C_{e,s}}{dt}(t) = \beta \cdot {}^{13}C_{e,ns}(t) \\ {}^{13}C_{e}(t) = {}^{13}C_{e,ns}(t) + {}^{13}C_{e,s}(t) \end{cases}$$
(Equations 5, 6, 7)

The numerical solution of those differential equations expresses the evolution of the total leaf ${}^{13}C$, and is given by:

$${}^{13}C_e(t) = \left({}^{13}C_{e,0} - {}^{13}C_{e,\infty}\right) e^{-\alpha \left(\frac{{}^{13}C_{e,0}}{{}^{13}C_{e,0} - {}^{13}C_{e,\infty}}\right)t} + {}^{13}C_{e,\infty}$$
(Equation 8)

And where at t=0 and $t=\infty$, we have:

$${}^{13}C_{e,0} = {}^{13}C_e(0) = {}^{13}C_{e,ns}(0)$$
(Equation 9)
$${}^{13}C_{e,\infty} = {}^{13}C_e(+\infty) = {}^{13}C_{e,s}(+\infty) = \frac{\beta}{\alpha+\beta}{}^{13}C_{e,0}$$
(Equation 10)

Rearranging equation 10 at day=1 gives:

$$\alpha = \left(\frac{{}^{13}C_{e,\infty}}{{}^{13}C_{e,0}}\right) \ln \left(\frac{{}^{13}C_{e,1} - {}^{13}C_{e,\infty}}{{}^{13}C_{e,0} - {}^{13}C_{e,\infty}}\right)$$
(Equation 11)

For each treatment, α was determined using δ^{13} C values, following the assumptions that the measurements of leaf δ^{13} C just after labelling and one day after labelling reflected leaf ${}^{13}C_{e,0}$ and ${}^{13}C_{e,1}$, respectively. The value of δ^{13} C seven days after labelling was used as a proxy for ${}^{13}C_{e,\infty}$, since by the 4thday, δ^{13} C had already stabilized as a constant value. The standard error associated with α was estimated by differential propagation of the standard error of the treatment means for ${}^{13}C_{e,0}$, ${}^{13}C_{e,1}$ and ${}^{13}C_{e,\infty}$ in Equation 11.

3. Gas exchanges and biomass measurements

The last day of δ^{13} C sampling, leaf gas exchanges were measured on the leaf opposite the one from which δ^{13} C was measured. Net CO₂ assimilation rate (A_n), stomatal conductance (g_s) and transpiration rates (E) were measured at midday, using a portable photosynthesis system (LI-6400; LICOR Inc., NE, USA) set up at a constant ambient CO₂ concentration (400 ppm). Air temperature, relative humidity and PAR inside the leaf chamber were fixed at 23°C, 70% and 1400 µmol m⁻² s⁻¹, respectively.

4. Plant biomass and surface measurements

Just after gas exchanges measurements, all the leaves were harvested and whole-plant leaf area was measured, using a table top leaf-area-meter (LI-3100C; LI-COR Inc., NE, USA), and then weighed. Similarly, shoot dry matter was determined after drying leaves and stem at 80°C for 48h. Roots were separated from the stem and shoots, and were sorted out from the soil by dry-sieving samples over 2 mm wire mesh. The determination of their dry matter followed the same procedure as for shoot dry matter (DM). The root:shoot ratio (R/S) was calculated as the ratio of the dry matter for each plant. This value was used as an indicator of C partitioning, linked to the exportation rate.

5. Sugar concentration determination

Sugars (starch and hexoses) concentrations were measured on sixteen other plants (four plants from each of the four treatments), on the same day as the growth parameter and gas exchanges were performed. Samples were collected early in the morning (7 a.m.), at midday (1 p.m.) and in the evening (7 p.m.) on the same upper leaf.

a. Metabolite measurements

Metabolite measurements were performed enzymatically, as previously described in (Biais et al. 2014). Aliquots of about 20 mg of fresh frozen powder were weighed in 1.1 mL MicronicTM tubes (Lelystad, The Netherlands) and fractionated at 95°C for 15 minutes, twice with 80% (v/v) ethanol, respectively with 250 and 150 μ l, and once with 50% ethanol 250 μ l. Glucose, fructose and sucrose were determined in the ethanolic supernatant (Jelitto et al. 1992) and expressed respectively in μ mol per g DM or in glucose equivalents. Starch was determined on the pellet re-suspended in 100 mM NaOH and heated at 95°C for 30 minutes (Hendriks et al. 2003), and expressed in glucose equivalents. Extractions and assays were performed using a robotised Starlet platform (Hamilton, Villebon sur Yvette, France), and absorbencies were read at 340nm in MP96 readers (SAFAS, Monaco).

b. Chemicals

All chemicals and substrates were purchased from Sigma-Aldrich Ltd. (Gillingham, UK). All enzymes were purchased from Roche Applied Science (Meylan, FR)

6. Carbon balance

Carbon balance, which represents the net flux of carbon over a given period of time, was determined from leaf *C* net assimilation and the changes in sugar concentrations in leaves (source minus sink of *C*). The balance was performed on upper leaves that had almost completed their growth. Leaf sugar accumulation (S_a) was estimated by subtracting the maximum sugar concentration (usually at around 1 p.m.) to the initial sugar content measured in the morning before photosynthesis (at 7 a.m.). S_a was calculated for all 16 plants in µmol glucose g⁻¹ DM was converted to µg C cm⁻² (S_c) by using the leaf dry mass per area (data not shown). Because gas exchanges measurements were made at a PAR of 1400 µmol m⁻²s⁻¹, and labelling measurements at a PAR of around 660 µmol m⁻² s⁻¹, we corrected the A_n values (µmol CO₂ cm⁻² s⁻¹), using A-PAR response curves performed on maize plants at the same development stage, and under the same greenhouse conditions (Bornot et al. 2013). To compare A_n with the plant sugar accumulation (S_c), it was summed over the first course of the day and then expressed in µg C cm⁻² (A_c). The difference between S_c and A_c was considered to be the exported C into the phloem (at this stage, the newly structural C was neglected), and the percent C export (% E_c) was calculated as:

 $\% E_C = (A_C - S_C) / A_C$ (Equation 12)

7. Soil water and nutrient content

SWC was determined by weighing every plant the day of labelling and at repeated intervals until the final sampling for δ^{13} C was made. Nutrient concentrations were analyzed on labelled leaves after digestion in sulphuric acid and hydrogen peroxide. Potassium concentration was quantified using AAS technology (Varian Spectr-AA-20, Varian, Mulgrave, Australia).

8. Statistical analysis

Statistical analyses were performed using the R software (version 3.1.3) (R Development Core Team 2008) to calculate average values for n=4, \pm standard errors, and to test differences between potassium (K- and K+) and water (W- and W+) treatments. Because of the low number of replicates, data were analyzed using non-parametric test permutation (n=999). A two-way analysis of variance was performed to test the effect of potassium (K) and water (W) levels and their interaction (W x K).

III. <u>Results</u>

1. Effects of potassium and water treatments on plant chemical concentrations and growth

Reflecting our experimental design, SWC varied from an average of 19% in the W+ to 9% in W- treatment (Table II.1), with no effect of K fertilization within a W treatment. Regardless of the water treatment and the leaf position, K fertilization significantly increased (P<0.001) leaf K content (LKC). For example, for the W- plants, LKCs measured in the upper leaves were 17.0 and 5.7 mg K g DM⁻¹ for K+ and K-, respectively (Table 1). For the W+ plants, LKC varied between 10.6 and 3.0 mg K g DM⁻¹ for K+ and K-, respectively. However, within a treatment, LKC was lower (P<0.001) in the lower leaves than in the upper leaves. Conversely, water deficit induced a 38-47% increase (P<0.01) in LKC, and therefore the lowest and highest values of LKC were found in W+K- and W-K+ treatments, respectively. The other main solutes contents (N and P) were affected by water treatment (P<0.001, data not shown). However, mirroring the K effect, N and P concentrations were significantly higher (P<0.01 and P<0.001, respectively) in the upper leaves (14% and 21%, respectively) than in the lower ones.

Leaf area and dry matter were negatively affected by water deficit (P < 0.001) and K starvation (P < 0.01 and P < 0.001, respectively) (Table II.1). Leaf area of K- plants was 19% and 23% lower than K+ plants under W+ and W- treatments, respectively. The effect of K nutrition on biomass production was more pronounced, with a decrease of 42% and 31% for W+ and W- plants, respectively. The effect of drought on leaf area and biomass production was greater than the effect of K deficiency. Across K treatments, leaf area and plant production of the W- plants were 39% and 53% lower than those of the W+ plants, respectively. Similarly, the R/S ratio proved more sensitive to water (P < 0.001) than to K treatment (P < 0.05) (Table II.1), with a 100% relative increase of the root biomass on W- plants.

Table II.1 Leaf potassium content (LKC), soil water content (SWC), leaf area (LA), dry matter (DM), root:shoot ratio (R/S), carbohydrate export capacity factor (α), and fixation factor (β) under well-watered (W+) and water stressed (W-) plants and under high potassium supply (K+) and low potassium supply (K-) (mean ± se; n=4). Non-parametric two-way analysis of variance was used to test the effect of water (W), potassium (K) nutrition and their interaction (KxW) on each of the parameters.

		W+ K+	W+ K-	W- K+	W- K-	W _{effect}	K _{effect}	W x K
SWC (%)		18.7 ± 0.7	21.7 ± 0.3	8.5 ± 2.1	9.4 ± 2.0	*	ns	ns
LKC (mg g ⁻¹)	Upper leaf	10.6 ± 1.9	3.0 ± 0.4	17.0 ± 0.2	5.7 ± 1.8	*	*	ns
	Lower leaf	7.9 ± 0.9	1.7 ± 0.1	12.6 ± 0.7	2.1 ± 0.4	*	*	*
$LA(m^2)$		0.21 ± 0.01	0.17 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	*	*	ns
DM (g)		29.7 ± 0.9	17.2 ± 0.9	12.9 ± 1.2	8.9 ± 0.9	*	*	*
R/S		0.15 ± 0.02	0.16 ± 0.02	0.25 ± 0.01	0.36 ± 0.05	***	*	ns
α	Upper leaf	3.13 ± 0.50	2.86 ± 0.47	2.77 ± 0.44	2.91 ± 0.81	ns	ns	ns
	Lower leaf	2.64 ± 0.26	2.34 ± 0.39	2.57 ± 0.27	2.22 ± 0.35	ns	ns	ns
β	Upper leaf	0.24 ± 0.09	0.19 ± 0.06	0.22 ± 0.07	0.23 ± 0.08	ns	ns	ns
	Lower leaf	0.06 ± 0.03	0.10 ± 0.04	0.11 ± 0.02	0.13 ± 0.04	ns	ns	ns

* *P* < 0.05 ; ** *P* < 0.01 ; *** *P* < 0.001

2. Dynamics of δ^{13} C in leaves

Values of δ^{13} C decreased rapidly after labelling, reaching minimum values within two and four days in the upper and lower leaves, respectively (Fig. II.2a, b). The variability of δ^{13} C values between treatments stabilized after seven days. In the upper leaves, all the δ^{13} C(0) values were similar among treatments whereas, in the lower leaves, there was a difference (*P*<0.05) in δ^{13} C(0) between the K+W+ and K-W- plants. It is interesting to note that the last δ^{13} C values measured seven days after labelling had not completely reached the initial δ^{13} C in leaves. Despite these results on δ^{13} C changes, there was no treatment effect on the carbohydrate export capacity factor (parameter α) regardless of the leaf position, linked to the high standard errors (Table II.1). Conversely to α , β (fixation factor) showed a response to the leaf position (Table II.1), with the upper leaves still growing when labelling occurred, unlike the lower leaves whose C was mostly entirely exported. This logical result legitimates the model used. However, no treatment effect on carbohydrate fixation could be demonstrated.



Fig. II.2 Daily changes in δ¹³C of (a) upper and (b) lower maize leaves sampled from plants grown under well-watered (closed symbol: W+) and water stressed (open symbol: W-) conditions, and with high (circle: K+) and low potassium supply (triangle: K-)(mean ± se; n=4). Initial δ¹³C values are represented by black and white square symbols.

3. Composition and dynamics of total sugar content in leaves

Starch accounted for 46% of the total sugar concentration measured in the upper leaves. Leaf soluble sugar composition was composed of 32% sucrose, 13% glucose and 9% of fructose. Concentrations of fructose and sucrose were only influenced significantly (P<0.001 and P<0.05, respectively) by water treatment. Fructose concentrations did not vary during the day, and increased on average from 40.6 to 81.9 µmol g DW⁻¹ when undergoing water deficit. Diurnal change in sucrose concentration was highly variable, but average sucrose only decreased from 243 to 239 µmol g DW⁻¹ under water limitation. Glucose concentration was not influenced by K or water treatments (P=0.20 and P=0.13, respectively). In addition to its strong proportion in leaves, starch was the only sugar species to be significantly influenced by K (P<0.05) and water treatment (P<0.001).

Changes in total sugar concentration (Fig. II.3a) increased significantly (P<0.001) until midday and remained stable in the afternoon. At any sampling time, total sugar concentration was higher (P<0.01) for W+ plants than for W- plants. Morning (taken between 7 a.m. and 1 p.m.) sugar accumulation (S_a) was significantly greater (P<0.01) under K limitation (Fig. II.3a, Table II.1).

In each treatment, starch concentration increased continuously (P < 0.001) throughout the day (Fig. II.3b). A K effect was also observed with higher (P < 0.05) starch concentration in W+ plants than in W- plants throughout the whole day. K deficiency decreased significantly (P < 0.05) starch content in leaves under W+ treatment. In the W- treatment, there was no effect (P=0.35 and P=0.17 at 7 a.m. and 7 p.m.) of K on starch concentration.



Fig. II.3 Diurnal changes in starch (a) and total sugar (b) content in upper maize leaves sampled from plants grown under well-watered (closed symbol: W+) and water-stressed (open symbol: W-) conditions, and with high (circle: K+) and low potassium supply (triangle: K-) (mean ± se; n=4).

4. Gas exchanges

Gas exchange values were lower in the lower leaves than in the upper leaves for all treatments. In detail, stomatal conductance (g_s) and transpiration rate (E) were negatively affected (P < 0.05) by water limitation with a 25% reduction under the W- treatment on the upper leaves (Table II.2), and up to 66% reduction on lower leaves.

Table II.2 Net CO₂ assimilation (A_n), stomatal conductance (g_s) and transpiration (E) for well-watered plants (W+) and water-stressed (W-) leaf maize and under high (K+) and low (K-) potassium supply (mean ± se; n=2).

	Leaf	W+ K+	W+ K-	W- K+	W- K-
A_{n}	Upper	16.20 ± 0.20	16.90 ± 0.70	16.55 ± 0.20	17.10 ± 1.50
$(\mu mol m^{-2} s^{-1})$	Lower	13.20 ± 2.50	8.88 ± 1.52	6.65 ± 2.10	5.00 ± 1.88
$g_{ m s}$	Upper	11.30 ± 0.10	11.80 ± 1.20	8.76 ± 0.19	8.46 ± 0.96
$(\text{mmol } \text{m}^{-2} \text{ s}^{-1})$	Lower	10.27 ± 2.13	5.98 ± 0.81	2.74 ± 0.75	2.14 ± 1.38
E	Upper	1.02 ± 0.07	0.95 ± 0.06	0.70 ± 0.03	0.74 ± 0.12
$(\text{mmol } \text{m}^{-2} \text{ s}^{-1})$	Lower	0.93 ± 0.22	0.53 ± 0.08	0.25 ± 0.07	0.23 ± 0.15

Net CO₂ assimilation rate (A_n) was only reduced by water limitation on lower leaves (P<0.05). Conversely, K nutrition did not seem to influence gas exchanges (P=0.69, P=0.48, P=0.40 for A_n , g_s and E respectively), regardless of leaf position and water treatment. Across treatments, A_n was positively correlated (R^2 =0.68, P<0.01) to $\delta^{13}C_{DM}$ at t=0 (Fig. II.4).



Fig. II.4 Relationship between net assimilation rate (A_n) and the initial δ^{13} C, multiplied by the proportion of leaf carbon dry matter $(\delta^{13}C_{DM})$.

5. Carbon balance

Plant carbon balance was represented by both the *C* exported percentage (%*E*_C) and the α values. In the upper leaves, the amount of C exported via the phloem represented around 65% and 47% of the C produced by photosynthesis for K+ and K- plants, respectively (Table II.3). Water treatment had no influence on %*E*_C. However, these results were not similar to α , which did not show any difference between K and water treatments.

Table II.3 Sugar accumulation (S_a), carbohydrate export capacity factor (α) and percent carbon export (% E_c) in upper leaves for well-watered (W+) and water-stressed (W-) leaf maize, and under high (K+) and low (K-) potassium supply. Different letters within a row indicate a significant difference at

P=0.05

	S _a		$\%E_{\rm C}$	
	(µmol glucose $g^{-1}DM$)	α	(%)	
W+ K+	223.5 ± 26.0 ^a	2.6 ± 0.3 ^a	66.2 ± 6.7	
W- K+	$343.2\pm 76.2\ ^{ab}$	$2.6\pm0.3~^a$	64.5 ± 12.1	
W+ K-	$425.6\pm59.6\ ^{ab}$	$2.3\pm0.4~^{\rm a}$	43.5 ± 6.5	
W- K-	$513.6 \pm 45.7 \ ^{b}$	$2.2\pm0.4~^a$	52.0 ± 8.6	

IV. Discussion

1. ¹³C labelling for phloem loading analysis

Leaf δ^{13} C labelling is a common technique that has been used for decades in plant physiological studies to help understand carbohydrate movements in plants (Hofstra and Nelson 1969). The technique has been employed for crops and trees (Suwa et al. 2010; Epron et al. 2012). Most of the studies using ¹³C were designed to measure plant *C* allocation and partitioning between plant organs (Warren et al. 2012), or on the link between *C* source and sinks among species, including root exudates (Comeau et al.; Tomè et al. 2015). Fewer studies have looked at specific treatment-effects (water availability, fertilization, elevated atmospheric CO₂ concentration, pruning...) on C partitioning in plants (Gong et al. 2014). Like ¹³C/¹²C labelling chambers designed for C3 plants (Gamnitzer et al. 2009; Reinsch and Ambus 2013), our labelling chamber (Fig. II.1a) proved to be successful in monitoring ¹²C and ¹³C for a C4 plant with high photosynthetic rates and fast *C* export, and in determining the effect of K deficiency on sugar export.

2. Leaf potassium status and growth

K nutrition strongly influenced leaf K content (LKC) which was, however, under the influence of both water treatment and leaf position. LKC was greater in W- than in W+, in accordance with Lindhauer (1985). The K content followed a gradient from old leaves to young leaves as shown by Andersen et al. (1992). Leigh and Jones (1984) showed that the critical K content for photo- and bio-chemical mechanisms was in the range of 1 to 3 % of dry matter for maize. In our experiment, this threshold was only reached on lower leaves under the K- treatment. In upper leaves, K was above this 3% threshold, which means that K deficiency may have only influenced osmotic adjustment (Barraclough and Leigh 1993).

Under well-watered treatment, the effects of K deficiency on leaf area and dry matter were in accordance with recent studies (Pettigrew 2008; Jordan-Meille and Pellerin 2008; Römheld and Kirkby 2010). As expected, under water deficit, K nutrition improved these two parameters (Lindhauer 1985; Egilla et al. 2001; Battie-Laclau et al. 2016). For a given water treatment, the positive impact of K fertilization on leaf area induced slightly lower (although

not significant) values of soil water content (SWC) on K+ plants, due to higher transpiration rates. However, the level of SWC of W+ plants remained twice as high as in W- plants.

3. Photosynthesis results, alone, did not account for plant growth

The physiological mechanisms measured in this experiment (A_n, g_s, E, α , sugar concentration) have to be interpreted in function of the LKC, which may vary greatly according to leaf age. Under moderate K deficiency (upper leaves), no effects of K nutrition or of water stress have been observed on photosynthesis. For K-deprived plants, the LKC of upper leaves always remained above the threshold defined by Leigh and Jones (1984), as that affecting the biochemical processes. Several other studies have pointed out that K deficiency has a direct effect on A_n (Peaslee and Moss 1968; Egilla et al. 2005; Battie-Laclau et al. 2014a). Water stress reduced g_s and E, but not A_n , (Sen Gupta et al. 1989; Tsonev et al. 2011) which explains the higher WUE_i measured in those conditions (Battie-Laclau et al. 2016). Under severe K deficiency, A_n of lower leaves was reduced in the well-watered plants, as shown in the model of Leigh and Jones (Leigh and Jones 1984). However, on W- plants, there was no K effect, due to the negative impact of water deficit that inhibited any positive effect of K fertilization. The difference in A_n between the upper and lower leaves did not seem to depend only on LKC, especially for K+ plants, which showed a 25% reduction that was unexplained by water and K status. Therefore, we have to be cautious when interpreting the differences in metabolic functioning between lower and upper leaves, which is not only dependent on the control factors applied in this study (K and water).

Leaf area and dry matter (DM) production showed a strong response to both W and K treatments, decreasing in the following order: W+K+, W+K-, W-K+ and W-K-. The values of A_n , measured on upper leaves, alone, could not explain those changes. We hypothesize three reasons *(not mutually exclusive)* that could account for this response: a) upper leaves have high A_n but, because of their low LKC, C export rate is decreased, thus reducing plant growth potential; b) the A_n gradient of form upper to lower leaves explains the response to K and water; c) there are other limitations to plant growth, such as a lack in cell expansion due to insufficient turgor pressure (Mengel and Arneke 1982; Pettigrew 2008). Those limitations were not investigated in the present study.

4. Divergent results confirming the role of potassium in carbon transport

Sugar concentrations measured in the upper leaves were in the same range as those reported on maize by Setter and Meller (1984). Contrary to several studies (Cakmak et al. 1994; Gerardeaux et al. 2009), the sugar concentrations of the upper leaves were not K dependent, the higher values being only related to well-watered plants. Low starch is a common physiological response to water stress (Lemoine et al. 2013). This response is bound up with starch hydrolysis, which allows sucrose synthesis and phloem loading to roots (Pelleschi et al. 1997). The higher R/S ratio measured on water-stressed plants (Table 1) confirms this loading. Contrary to the findings of Pettigrew (1999), glucose and fructose did not accumulate in K- leaves at the expense of sucrose accumulation. However, when the increase in sugar concentrations was considered, only K- plants showed an accumulation of total sugars (S_a) . Sugar accumulation was mainly in the form of starch accumulation, which represented the major proportion of total sugars. Sucrose content, which was supposed to be K dependent (Cakmak et al. 1994) was not significantly different among K treatments. The increase in starch concentration under K deficiency has been described elsewhere (Marschner et al. 1996; Deeken et al. 2002; Hermans et al. 2006; Lemoine et al. 2013). The C mass balance performed from sunset to midday confirmed that K- plants exported 33% less sugar than K+ plants. In the upper leaves, the high values of sugar concentrations during daylight period did not inhibit photosynthetic activity.

Conversely, in upper leaves, the carbohydrate export capacity factor (α), based on δ^{13} C labelling, did not confirm that K-leaves had accumulated sugars (Table 3). This difference with the sugar accumulation rate could be due, in part, to the different time steps involved in both processes. In the case of δ^{13} C labelling, most of the δ^{13} C was exported at the end of the first day, indicative of a rapid sugar loading rate characterizing C4 plants, which contrasted with sugar loading in C3 plants, where the δ^{13} C signal remained high for several days after labelling (Blessing et al. 2015; Epron et al. 2015). The fact that most of the ¹³C was exported within one day (except for the structural C) demonstrated that the C efflux was fast, and that the K starvation did not interfere with the phloem loading. On lower leaves, α showed a slight tendency to be proportional to LKC, although this result was not significant. This absence of effect of K nutrition on C export from sources to sinks is coherent with the values of the R/S ratio, which did not depend on K treatment. This absence of K nutrition impact on C allocation rules is sometimes contradicted by the literature (Ericsson 1995; del Amor and

Marcelis 2004), although the most probable hypothesis is that allocation rules regarding mineral deficiencies may be plant-dependent (El Dessougi et al. 2002).

On lower leaves, the impact of K deficiency and water deficit on photosynthesis was more pronounced than on C export capacity. It appears that the cause of the lower photosynthesis activity was not linked to the increase in sugar concentrations, as is often suggested (Ainsworth and Bush 2011).

From a physiological point of view, we cannot definitively conclude about the significant role of K deficiency on sugar translocation in maize leaves. However, the lower values of A_n in the lower (older) leaves did not seem to totally account for the decrease in plant growth, because the reduction of dry matter was more pronounced than that in A_n . We therefore postulate that both A_n and α decreased under severe K deficiency, although not in the same proportions. Moreover, it is also not possible to conclude that leaf sugar content negatively influenced photosynthetic activity.

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Chapitre III.

Interaction du déficit potassique et hydrique sur la croissance et le développement du maïs en condition <u>contrôlée</u>

Interaction of potassium deficiency and water deficit on maize growth and development, under controlled conditions

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<u>Abstract</u>

Background and Aims

Global change leads to an increase in extreme climatic events such as severe droughts. Potassium (K) is a major element linked to water transport in plants.

The main objective of this study was to determine the interaction between the level of potassium nutrition and water stress in order to test if potassium nutrition can improve growth and development of maize (*Zea mays* L.) under drought conditions.

Methods

Plants were grown under three K treatments (K0, K1 and K2) and two water supply environments (drought vs well-watered) in greenhouse conditions during 2 months. This study focused on aboveground (leaf area, biomass, leaf senescence), belowground (root architecture and distribution) and whole-plant developmental, structural and morphological (Root/(Root+Shoot), Specific Leaf Area, Specific Root Area) responses of *Zea mays* L. to K and water supply.

Results

The main results show that aboveground biomass was more affected by K deficiency than by water deficit. At the root scale, no significant effect of K deficiency was measured. K and water seemed to share the same physiological roles.

Conclusion

The strong limiting effect of K on growth often hid the effect of water stress, but an overfertilization did not compensate for a lack of water.

Keywords: Drought; Potassium; Maize (Zea mays L.).; Growth; Interaction; Root morphology

I. <u>Introduction</u>

Global change is creating strong climatic disturbances, such as an increase in surface temperature, atmospheric CO₂ concentration and in some areas of the world, a decrease in precipitation (IPCC, 2014). Although annual precipitation is not necessarily predicted to decrease in southern France as a consequence of global warming, an increase in the frequency and duration of summer droughts and temperature-driven evaporative demand are expected (IPCC, 2014). The extreme drought events that occurred in southern France during the last decade has highlighted the need to understand the key processes that may allow plants to acclimatise to recurrent dry summers.

Maize (Zea mays L. subsp. mays) is one of the most cultivated crops worldwide, firstly for human consumption and secondly for cattle food. However, several factors can potentially limit maximum maize growth and yield: 1) poor soil fertility in particular low potassium (K) level (Pettigrew, 2008; Römheld and Kirkby, 2010); 2) soil water availability, and 3) the occurrence of frequent summer droughts. At present, the interaction between fertilizers and water availability in plant water use and K nutrition remains poorly understood. The few studies that exist highlight the importance of K nutrition on the plant's capacity to better tolerate water stress (Aslam et al., 2013; Fanaei et al., 2009). Water and K levels in plants are closely related since K is a very mobile element that is transported from roots to shoots via xylem water movement (Szczerba et al., 2009). There are numerous common well-known physiological and structural responses following K and water deficit (Hsiao et al., 1976). Mainly, former studies have already pointed out the close link of this entirely soluble element with water movement in plants (Leigh and Jones, 1984; Mengel et al., 2001), which influences turgor potential for its strong osmotic contribution (Carroll et al., 1994; Mengel and Arneke, 1982) and then leaf water content (Egilla et al., 2001). One major consequence of K deficiency is the increase of leaf senescence and mortality, which is also under the dependence of the accumulation of free radicals due to the lack of K (Cakmak, 2005). Another consequence of K deficiency is a decrease in plant shoot biomass through a slower leaf area development (Jordan-Meille and Pellerin, 2004). On the opposite, K nutrition has been shown to improve shoot biomass (Benlloch-González et al., 2008), and leaf area (Egilla et al., 2001). Another physiological important role shared with water is the influence on sugar translocation, from mature leaves to leaf and root meristems. A lack of water at the plant level

is known to favour root growth relatively to shoot growth. This would be linked to an increased proportion of glucose transformed into sucrose at the leaf level, which in turn favors the C translocation to roots (Xu et al., 2013). This carbohydrate translocation process is also known to be a key-process concerned by K nutrition in plants: conversely to water, a K deficiency lowers the proportion root dry biomass to total dry biomass (Alemán et al., 2011; Cakmak et al., 1994; Ericsson, 1995; Triboulot et al., 1997) This response is unusual compared to the other macronutrients effect (Hermans et al., 2006). Finally, besides these gross production functions, it has often been noticed that the root morphology was also controlled by water and nutrient (especially K) availability (Hogh-Jensen and Pedersen, 2003; Song et al., 2015). Altogether, the literature provide several arguments to link K physiology closer to plant water functioning but there are gaps, especially in maize, on their combined effects on plant growth, especially on the role of K in mitigating water stress.

The main objective of this study was to determine the interaction between potassium and water stress on maize architecture and growth. Specifically, to understand how K nutrition could reduce water stress, this study focused on 1) the morphological and structural response of maize aboveground (leaf area, biomass, leaf senescence); 2) its morphology such as R/(R+S), SLA, SRA; and 3) on belowground development (root architecture and root distribution). Three hypotheses were tested: 1) most of the physiological mechanisms impacted by K or water stress are the same, thus leading to comparable morphological impacts, at shoot or root scale. As a consequence, we can postulate that 2) plant growth will be limited either by K or by water stress, but not both (the expected cumulated effects of both stresses are actually attenuated, which is a mark of interaction) and that 3) an over nutrition of K on water-stressed plants partially offsets the deleterious effects of drought. The novelty of this study lies in the interactions between three levels of K and water (well-watered and water stressed), in order to determine if the K nutrition is able to minimize the negative effects of water stress on maize growth.

II. <u>Material and methods</u>

1. Plant preparation

The experiment was conducted at the Bordeaux French National Institute for Agronomy (INRA). Seeds of maize (*Zea mays* L. subsp. mays) were germinated under controlled greenhouse conditions in early March of 2013 in containers filled with distilled water at a constant temperature of 21°C in a controlled chamber. After emergence, plants without any visual growth defects were selected and transplanted into 10-liter pots filled with 14 kg of an organic podzol soil depleted in potassium. The other physico-chemical soil properties were as follows: 4.7% clay, 2.1% silt, 93.3% sand and 3.9% organic matter with CEC 5 cmol kg⁻¹ and a pH of 5.3. For two weeks after transplanting, the following 500 mL nutrient solution was added daily to each pot: (NH₄)₂SO₄ (72.6 mg L⁻¹), Ca(NO₃)₂,4H₂O (651.8 mg L⁻¹), Mg(NO₃)₂,6H₂O (87.18 mg L⁻¹), MgSO₄ (132 mg L⁻¹), NaH₂PO₄ (6.6 mg L⁻¹), Na₂HPO₄,12H₂O (19.7 mg L⁻¹), MnSO₄,H₂O (0.615 mg L⁻¹), ZnCl₂ (0.21 mg L⁻¹), CuSO₄,5H₂O (0.047 mg L⁻¹), H₃BO₃ (0.562 mg L⁻¹), for the high K treatment only.

2. Experimental design: water and K fertilization treatments

A complete randomized block design was set up in greenhouse conditions with three treatments of exchangeable K and two treatments of irrigation in order to assess the interaction of K and water stress on plant structural parameters. In the end, a total of 42 pots of maize were used (two water treatments, three K treatments and seven replicates). The three K treatments obtained as follows:

K0: no K added to field soil taken from a deep horizon (below 50 cm) with a K concentration of 3–5 ppm.

K1: no K added to field soil taken from a shallow horizon (0-2 cm) with a K concentration of 17 ppm.

K2: same soil as K1 but K concentration was increased via the nutrient solution with a final K concentration of 30 ppm.

On one half of the plants, the irrigation was continuously maintained at 20% of soil field capacity (W+). Deionized water was used for irrigation in order to not affect soil nutrient concentration. The drought treatment (W-) was imposed 37 days after emergence (DAE) on the other half of the pots. It consisted in maintaining the soil humidity equal to 5% of soil field capacity. The water stress was applied quite late after seedling in order to prevent any mineral deficiency. Therefore, at harvest (65 DAE), there was no difference in mineral content in plant when comparing irrigated and non-irrigated plants (3.9 vs 3.5 mg K g⁻¹ in K0 treatment for W- and W+ plants respectively and 10.2 vs 8.9 mg K g⁻¹ in K2 treatment for Wand W+ plants respectively). The expression of these values expressed towards the shoot tissue water are 29 vs 22 mM and 91 vs 75 mM respectively. The first measurements were performed at 22 DAE. Morphological characteristics (leaf biomass, stem biomass, root biomass, root architecture, length, width, number of leaves, leaf area) were measured continuously throughout the measuring period. The last day of measurements (destructive samplings) was done at 65 DAE. On all 42 plants, leaves were numbered from the first true leaf (not cotyledon) to the top of the plant to be compared between different treatments. Greenhouse environmental data (temperature, photosynthetic active radiation and soil moisture) were collected and controlled every 10 minute using a CR1000 datalogger (Campbell Scientific, Logan, USA).

Thermal time (in degree-days *i.e.* dd) was calculated as the sum of cumulative differences between daily mean temperature and a base temperature (taken at 10°C). Using this referential, water stress was then applied at 380 dd and the experiment lasted until 700 dd.

3. Leaf parameters

The leaf appearance rate (phyllochron) was determined by linear regression of visible leaf number on thermal time (TT) between 450 and 700 dd. The green surface area was deduced form three components: number of visible leaves, number of senescent leaves and individual final surface area, explained by the leaf elongation rate. Individual leaf length was measured every day during the whole experiment, on growing leaves. By plotting the cumulated length of leaf 10 (taken as a common reference for all plants of all treatments) in function of thermal time, we calculated its elongation rate (cm dd⁻¹), as the slope of the linear part of the curves. In addition to length, leaf width and leaf status (senescing, ligulate or not) were determined to

estimate leaf area. The determination of leaf area (LA) from length (L) and width (l) measurements were calculated according to Plénet et al. (2000) as:

LA (ligulate leaf) =
$$0.75*L*l$$

LA (not ligulate leaf) = $0.5 \times L^{1}$

The relative senescent leaf values were calculated by dividing the area of the senesced leaves by the total leaf area. Specific leaf area (SLA) was calculated as leaf area (m^2) divided by dry mass (kg).

4. Root measurements

The root system was separated by phytomer (McMaster, 2005) and sorted out from the soil by dry-sieving samples over 2 mm wire mesh. The cleaned roots were submerged in 10 % ethanol at 4°C before being scanned. Morphology of roots was investigated using the image evaluation software WinRhizo 2005a (Regent Instruments Inc., Canada). The images were analyzed to identify the morphological parameters of roots. Roots with a diameter greater than 5 mm were excluded from the analysis as only very few roots were above this value. Conversely, because of the cleaning method, root hairs could not be conserved, and the finest roots that could be measured had a diameter greater than 0.05 mm. The measured parameters were fine root length (cm), average diameter (mm), root volume (cm³), number of root tips and root surface area (cm²). Specific root area (SRA in m² kg⁻¹) was calculated for each sample after drying roots at 105°C for 48h as:

SRA $(m^2 kg^{-1})$ =Leaf Area (m^2) / Dry Matter (kg)

The root morphology was described using the distribution of root surface by diameter class for root diameters smaller than 5 mm, as only very few roots were above this value. The finest roots measured by WinRhizo had a diameter greater than 0.05 mm, which means that hair roots were not taken into account into this analysis. Root to leaf area index was then calculated as:

Root to leaf area index = Leaf Area
$$(cm^2)$$
 / Root Area (cm^2)

5. Analysis of water stress and potassium deficiency interactions

The effect of water stress and K deficiency and their combined effect on all parameters (X in the following formulas) were calculated using the method proposed by Luo et al. (2008) as:

Effect of W deficiency alone = $(X_{K2W} - X_{K2W+})/X_{K2W+}$ Effect of K stress alone = $(X_{K0W+} - X_{K2W+})/X_{K2W+}$ Effect of K + W stress = $(X_{K0W-} - X_{K2W+})/X_{K2W+}$

The interaction between water stress and K deficiency (I_{WK}) was calculated as the difference between the combined effect of W and K stresses and the sum of the effects of K and W individually as described in Christina et al. (2015).

6. Modeling the plant water losses

The effect of K nutrition on plant growth measured into this experiment (with or without water stress) was crossed with theoretical values of water use efficiency taken in literature in order to quantify its effect on water losses at the plant scale. We simulated first the green leaf area of every treatment, all along the leaf growing period, which will be used as a first support for water losses estimations. In a second step, we simulated the final biomass production that we multiplied by values of water use efficiencies (specific to every water and K treatment) to get modeled values of water losses. We aim at comparing both results, in order to see if K is enhancing (through surface area) or decreasing (through a higher stomatal control) the net water losses.

The evolution of green leaf area from seedling to the end of the leaf growth period uses a logistic growing model including the real leaf area measured at 700 dd (Fig. III.2a), with final leaf size equal to data already published (Jordan-Meille and Pellerin, 2004). Shoot biomass was calculated using SLA values for the K0 plants (Table III.2) because no stem biomass was produced due to K limitation. For the K2 plants, the evolution of the shoot biomass until the end of the leaf growth period mirrored the changes in leaf area final biomass values, with the constraint to get through the measured shoot biomass at 700 dd (Fig. III.2b).

A reference value of water use efficiency (WUE) of 4 g L⁻¹ was attributed to the well-watered and fertilized plants (Bahrani et al., 2012; Singh et al., 2014). The relative gaps between this reference value and those which have to take into account the effect of water and-or K deficiency were taken from Arquero, Barranco, and Benlloch (2006). Hence, the values taken by the WUE of K0W-, K0W+ and K2W- plants were 7.2, 3.5 and 8.8 g L⁻¹ respectively. The calculated plant water losses were obtained from the product of the biomass data with WUE.

7. Statistical analysis

Statistical analyses were performed in R (version 3.1.2; R Core Team 2014) to compute mean values and standard errors, and to test differences between treatments. Data were tested for normality (Shapiro's test) and homogeneity of variance (Levene's test). Post–hoc differences in means were tested using Tukey's test. Linear model was used to analyze differences between each treatment and two-way ANOVA was used to test the effect of potassium level (K) and water (W) and their interaction (W x K) on parameters based on a complete randomized system. Results were significant for P<0.05. We performed non-parametric test permutation (n=999) to compare the mean values with P<0.05 on root analysis (root area, specific root area, root to leaf area index) because this test was more appropriate for small sample sizes. Curve fits were carried out using Sigmaplot (version 12.0, San Jose, CA, USA).

III. <u>Results</u>

1. Plant development

Root development, characterized by the number of visible underground phytomers, was affected neither by water nor potassium with six growing phytomers for any treatment (P>0.05). However, aboveground plant development, which was characterized by the number of visible leaves as a function of thermal time, was affected by either K fertilization or water deficit. From the very first measurements, the increase in number of leaves with degree days was slower in plants grown with low K and under water stress than in plants grown with high K levels (Fig. III.1). Among water treatments there was a faster ($P \le 0.001$) leaf formation in K1 and K2 than in K0 treatments. In response to the imposed water-stress that started after 380 degree days, plants growing under low level of K (K0 W-) produced fewer leaves $(P \le 0.001)$ than well-watered plants (K0 W+). At the end of the experiment (700 dd), the difference in number of leave was around 2 to 4 according to the treatment. In K1 and K2 treatments, leaf production dynamics was a little bit different because water stress had only an effect just after the onset of the water stress (400 to 600 dd), and this effect was cancelled out after a prolonged drought (> 600 dd). Consequently, the phyllochrons, which correspond to the time between the sequential emergence of the leaves on the main stem, increased significantly (P<0.001) under water stress from 34 dd to 58 dd for K1 and K2, respectively.

For the K0 treatment, the phyllochrons were stable from 56 dd to 58 dd (P=0.79). If K1 and K2 W+ plants were taken as reference, the effect of water stress or K starvation had the same impact on the reduction of development, which was around 23 dd higher.



Fig. III.1 Number of leaves in relation to degree days for well-watered (W+, black symbols) and water-stressed (W-, white symbols) maize plants (n=7 plants; error bars are standard errors) for three potassium levels (K0=low, K1=normal, K2=high).

2. Leaf and root growth

At the end of the experimentation (29 days after the water stress was applied), green leaf area appeared to be very strongly influenced by K-fertilization (P<0.001, Table III.1), with values 4 to 5 times greater for K2 plants than for K0 plants (from 0.13 m² to 0.47 m² for W+ plants and from 0.07 m² to 0.37 m² for W- plants, Fig. III.2a). Water stress decreased leaf area (P<0.001) by a constant value of 0.1 m² per plant, which corresponded to a reduction of 50%, 30% and 25% for K0, K1 and K2 treatments, respectively. Differences in leaf biomass due to K-fertilization were even greater (P<0.001), showing an order of magnitude difference between K0 and K2 treatments (from 9 g to 86 g of leaf by plant for W+ treatment and from 5 g to 54 g for W- treatment, Fig. III.2b). At a given K-level, water stress decreased leaf biomass by 30–40% (Fig. III.2b).

Root area was similar between water treatments (P=0.83) but increased significantly (P<0.01) with increasing K-fertilization from 0.1 m² (K0) to 0.7 m² (K2) (Fig. III.2c). Similarly, the changes in root biomass due to K-fertilization (P<0.001) were not affected by soil water content and increased from 2 g in the K0 plants to 35–40 g in the K2 plants (Fig. III.2d). There were no significant differences (P=0.92) in root biomass between water treatments.



Fig. III.2 (a) Leaf area, (b) shoot biomass, (c) root area and (d) root biomass of well-watered (W+, filled symbols) and water-stressed maize plants (W-, open symbols) as a function of potassium levels (K0=low, K1=normal, K2=high) 29 days after the onset of the water stress (mean ± SE ; n=7). Statistics are summarized in Table III.2.

Leaf elongation rate (LER) for leaf 10 (leaf taken as reference) was at its maximum in K1W+ and K2W+ treatments with a similar value of 0.52 cm dd⁻¹ during the linear growing phase (Fig. III.3a). LER for K0W+ was 0.44 cm dd⁻¹, which represented a 15% decrease when compared to the maximum. This relative decrease was the same (P=0.29) between K0 and K2 treatments under water stress (0.31 cm dd⁻¹ vs. 0.36 cm dd⁻¹ respectively). Among all K treatments, water stress lowered significantly (P<0.001) LER by 30%.
Table III.1 Analysis of variance probability values for water (W) and potassium (K) fertilization

 treatments and their interactions on maize morphological characteristics. Number of leaves was taken

 at 700 degree days (dd). LER, SLA and (R/(R+S)) represent the leaf elongation rate, the specific leaf

 area and the root to root plus shoot ratio, respectively

		P values	
	W	Κ	W X K
Number of Leaves	*	***	*
Leaf Area (m ²)	***	***	*
Leaf Biomass (g)	***	***	***
Root Biomass (g)	ns	***	ns
LER (cm dd ⁻¹)	***	***	ns
Relative Senescent Leaf (%)	•	***	ns
Phyllochron (dd)	***	**	**
$SLA (m^2 kg^{-1})$	ns	ns	ns
R/(R+S)	**	•	ns

. P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001



Fig. III.3 (a) Leaf elongation rate for leaf number 10 and (b) percent relative senescent leaf at the same development stage (10 visible leaves) of well-watered (W+, black bars) and water stressed (W-, grey bars) of maize plants as a function of three potassium levels (K0=low, K1=normal, K2=high ; mean ± SE ; n=7). Senescence of K1W+ plants was equal to 0. Statistics are summarized in Table

3. Leaf senescence

At the end of the experiment, the proportion of senescent leaves in the K0 plants reached 29% and 34% for W+ and W- treatments, respectively, whereas for K1 and K2 plants it only represented a very small amount relative to well watered and fertilized plants (~5%). At that date (700 dd) plants at difference development rate. If we choose to calculate the proportion of senescent leaves (*i.e.* 10 visible leaves, which was the final stage for K0W- plants) the gap was even larger, as only the smaller bottom leaves began to dry at that stage (Fig. III.3b). Relative senescent leaf was mainly influenced by K starvation and not by water stress. Leaf senescence reached 31% for K0 versus 5% for K1 and K2 (P<0.001). There was no difference between K1 and K2 in leaf senescence (P=0.87 and P=1 for W+ and W-, respectively), and water stress only induced a slight increase that was in fact not significant (P=0.13). The effect of K nutrition on leaf development, LER and leaf senescence accounted for the observed differences in green leaf area (Fig. III.2a).

4. Morphology and architecture at organ and plant scales

Although not significantly (P=0.16), specific leaf area (SLA) was slightly higher in W+ plants than in W- plants (18 m² kg⁻¹ and 16.5 m² kg⁻¹, respectively; Table III.2, III.3). Moreover, there was no K effect on SLA (P=0.39). For the roots, the values of SRA showed no significant differences (P=0.21) between water treatments, with overall mean values of 93 m² kg⁻¹ and 70 m² kg⁻¹ for W- and W+ treatments, respectively. There was a marginal effect of K-fertilization on SRA (P=0.064) with means of 112, 64 to 66 m² kg⁻¹ for K0, K1 and K2 treatments, respectively. There was a strong effect of the K0 treatment on root architecture, as evidenced by the number of roots by phytomer (P<0.001, data not shown). Regardless of the water treatment, the younger the phytomer in the K1 and K2 plants, the higher the number of roots observed. On average, four roots were growing on phytomer number 3, whereas up to 10 where linked to phytomer number 6. However, the number of roots by phytomer stabilized at 4 for K0 plants, suggesting that there was a severe restriction of root emergence. This difference in root architecture by treatment can be seen Fig. III.4, which shows that the proportion of root surface carried by each phytomer increased from phytomer 3 to phytomer 5, with the exception of K0W- plants for which the older roots accounted for most of the surface. This difference can be explained by a lower root number, such as shown before, or by a difference in root morphology of the K-starved plants (P < 0.01).

Table III.2 Mean values (± SE, n=7) for specific leaf area (SLA), root to leaf area index (RAI/LAI) and ratio root to total plant biomass (R/(R+S)) for maize plants growing under three levels of potassium (K0, K1, K2) and two water regimes (W+ = well-watered, W- = water-stressed)

	K0		K	.1	K2		
	W+	W-	W+	W-	W+	W-	
SLA(m ² kg ⁻¹)	18.28 ± 1.42	15.98 ± 0.95	17.09 ± 0.67	16.07 ± 1.02	18.84 ± 1.87	17.75 ± 1.43	
RAI/LAI	0.50 ± 0.07	2.26 ± 1.02	0.99 ± 0.01	$1.26 \pm \mathrm{NA}$	1.66 ± 0.01	1.70 ± 0.17	
R/(R+S)	0.17 ± 0.02	0.31 ± 0.02	0.21 ± 0.03	0.34 ± 0.05	0.31 ± 0.04	0.34 ± 0.06	

Table III.3 Non-parametric analysis of variance probability values for water (W) and potassium (K) fertilization treatments on root area, specific root area and the ratio of root to leaf area (RAI/LAI).

	P va	P values		
	W	Κ		
Root Area (m ²)	ns	**		
Specific Root Area (m ² kg ⁻¹)	ns			
RAI/LAI $(m^2 m^{-2})$		ns		
P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001				

The analysis of root morphology globally showed that the finest roots (<1 mm) accounted for 80% of the total root surface (Fig. III.5a, b). Compared to K1 and K2 treatments, the proportion of K0 roots comprised between 1-2 mm was higher. Considered as a whole, the roots of the smallest class (< 1 mm) had a different pattern according to the water treatment. For W- plants, there was no effect of the K treatment on the proportion of roots into this class. Conversely for W+ plants, the proportion of roots increased with K nutrition from 65 to 80%. When looking at the 1 mm diameter class (see insert of Fig. III.5b), the higher proportion of roots occurred between 0.1 to 0.2 mm regardless of the water treatment. The K0 plants had a slightly higher proportion of roots in the very finest classes (0.1, 0.2 and 0.3 mm), at the expense of larger diameter (0.4–1 mm).



Fig. III.4 Distribution of root area (%) by phytomer for well-watered (W+, black symbols) and water stressed (W-, white symbols) maize plants grown at three different levels of potassium (K0=low, K1=normal, K2=high; mean; n=2)

At the whole-plant level, the ratio of root area to leaf area index was marginally higher (P=0.09) in W- plants than in W+ plants (Table III.2, 3), whatever the K treatments. This trend was confirmed by the ratio of root dry biomass to total dry biomass (R/(R+S), Table III.2, 3), which increased significantly (P<0.01) in the W- treatment indicating that relatively to leaf growth roots from the W- treatment grew more than the roots of the W+ plants. There was a small K effect on the R/(R+S) (P<0.1), the K starvation leading to a lower allocation of dry matter to roots (Table III.2, 3).



Fig. III.5 Distribution of relative root area (%) by root diameter classes (mm) for (a) well-watered (W+) and (b) water-stressed (W-) maize plants growing under low potassium (K0), normal potassium (K1) and high potassium (K2) levels (mean ± SE; n=2); insert represents the detailed distribution of relative root area between 0 to 1 mm.

5. Water and Potassium Interaction

Two types of strong interactions between water stress and K-fertilization were observed (Fig. III.6): amplification effect (for specific leaf area (6%) (SLA)) and attenuation effect (for phyllochron (53%), root morphology (18%), root biomass (23%), root area (18%) and leaf biomass (33%)). A weak attenuation effect was also observed on leaf area (8%) and on leaf elongation rate (6%) (LER). Amplification effect was defined as a synergy effect, *i.e.* when the combined effect (W-K0) was higher than the sum of K effect (K0) and water effect (W-). Attenuation effect consisted in a partial or total dissimulation effect of one factor by the other (W-K0 is lower than the sum of K0 and W-). An unusual compensation effect (W-K0 is null) was observed on the ratio of roots to total plant biomass (R/(R+S)).



Fig. III.6 Observed changes in maize main morphological traits relative to potassium (K) and water (W) levels, in response to water deficit (W- vs W+), K deficiency (K0 vs K2), the combined effects of K and W (K0W- vs K2W+) and the interaction between K and W (Interaction WK). Each effect is compared to the reference treatment consisting of no water stress and no K limitation (K2W+). When values of Interaction are positive, this means that the combined effects have a lesser impact than the addition of every single effect.

6. Effect of water and K treatments on whole-plant transpiration rate

By using a simple model of leaf area and dry matter production for our cultivated plants, and crossing with values of WUE taken in a similar literature dealing about water and K interaction, we aimed at evaluating the role of K in the plant water losses through two conflicting roles: increasing the losses related to the increase of transpiration surface, and decrease the water losses thanks to a better stomatal regulation. The evolution of green leaf area from seedling to the end of the leaf growth period is plotted on Fig. III.7a and ranges from 0.15 to 0.7 m² per plant according to the treatment. These predictions reflect the strong effect of K and water stress on the green leaf area, mainly due to a lower LER (water limitation), a delay in the leaf appearance (water limitation) and a higher proportion of senescence (K limitation).

The cumulated biomass values obtained were 126, 81, 13 and 9 g per plant for K2W+, K2W-, K0 W+ and K0W- treatments respectively (data not shown). The calculated plant water losses, deduced from the product of the biomass data with WUE are presented on Fig. III.7b.

Without any special water or K regulation on WUE, we would expect the losses to be exactly proportional to the surfaces. Taking these particularities into account, the level of losses on the K2W- plants were comparable to those expected for K0W+ plants. Thanks to the combined action of water stress and K fertilization (K2), half of the water was saved with only 25% of yield decline.



Fig. III.7 Model predictions of leaf area (a) and water consumption (b) during the vegetative period of a maize plant, according to K and water treatments. K0 and K2 represent a low and high potassium supply (3 and 30 ppm of exch. K respectively). W- and W+ represent water stressed and non-stressed plants. Leaf area data estimated at 700dd refer to measured values (Fig. III.2). The vertical arrow puts into relation the water lost by the K2W- plants which would correspond to K0W+ plants without any water use efficiency adaptation to drought and K nutrition.

IV. Discussion

1. Characterization of water and K limitations

The lack of water, potassium (K) and their interactions as growth limiting factors on maize plants were observed in this study. K starvation was applied to the seedlings by using soils with natural K concentrations was very low (4 ppm of exchangeable K, or 0.01 cmol kg⁻¹), as compared to other studies dealing with fertilization trials (Christina et al., 2015; Öborn et al., 2010; Zörb et al., 2014). The water stress was also quite severe, but its application was more progressive in order to prevent any mineral uptake disorders during early plant development (Ge et al., 2012). Thereafter, plants endured water stress for 29 days before being sampled with soil water content being maintained daily near the wilting point. On average, every well-watered plant used 20.0 L of water during the entire growth period, whereas non-watered plants only used 6.5 L (data not shown).

2. Do K-deficiency and water stress have the same impact on maize growth and development?

The effects of K deficiency and water stress on morphological and structural maize responses were compared independently and through their possible interactions. At the shoot scale, the components of green leaf area (plant development, LER, final leaf size, leaf senescence) were all impacted by water and K stress, but in different proportions. With the exception of LER, which was mostly limited by water availability, these factors were far more limited by K. Senescence was more sensitive to K stress than to water stress, when expressed at the same development stage (*i.e.* 10th visible leaf) although it has been widely established that limited water enhances leaf falls (Munné-Bosch and Alegre, 2004). As discussed by Cakmak (2005), the lack of K must have impaired the neutralization of reactive oxygen species, which in turn accelerate the leaf senescence. Finally, water stress impacted the same leaf growing parameters (as decomposed by leaf development, leaf elongation rate and leaf lifespan duration) as K deficiency. In detail, for water stressed plants, LER and phyllochron mainly accounted for the green leaf area decrease, whereas for K starved plants, senescence was a supplementary limiting factor.

At the root scale, our results showed that the relative root growth had significantly been enhanced under water deficit, and in a lesser extent by K fertilization (Table III.1). Under field conditions this limitation may reduce the amount of water transpired by plants, except if the root morphology induced by the K stress enhances the soil to root surface area. Regarding root morphology, our observations only concerned secondary and primary roots, and no clear pattern was discernable, even in water and K-stressed plants. The ratio of root to leaf area index (Table III.2) was not affected by water nor by K treatments. The distribution of laterals was different through the different phytomers comparing K0 and K2 treatments, but it was rather a question of number that of diameter distribution (Fig. III.4 and III.5).

3. K deficiency and water deficit interact themselves and occult their mutual effect

Related to the second hypothesis, for leaf-based morphological characteristics, such as LER and leaf area, our study revealed an absence of interaction between K and water stress (Fig. III.6). In other words, K and water supplies constitute additive limiting factors. This can be interpreted as if leaf growth was under the dependence of independent physiological mechanisms, which would be for a part a K dependant *i.e.* sugar translocation to leaf meristems for cell division, (Cakmak et al., 1994; Gerardeaux et al., 2010) and for another part would be water dependant turgor pressure for cell elongation (Hsiao et al., 1976). Inversely, at the whole-plant scale, there was an interaction between both factors with K deficiency explaining alone the results observed on shoot and root biomass for K and water stress. The expected damages on plant growth are attenuated, which constitutes a negative interaction. This result may be explained by the large effect of K on other growth parameters such as leaf senescence (Fig. III.3) or the physical and biochemical components of photosynthesis (Benlloch-González et al., 2008), to the point to dissimulate the effect of water on LER and phyllochron. From a physiological point of view, this dominance of one single limiting factor can be interpreted by the fact that K and water share some major physiological functions and influence growth and development through the same mechanisms: turgor and water movement (Fournier et al., 2005; Mengel et al., 2001), C allocation (Cakmak et al., 1994), and root architecture (Hogh-Jensen and Pedersen, 2003). Regarding R/(R+S), the response to K starvation (ratio decreased) evolved inversely to the effect of water stress. When combined together, there was a perfect compensation, which jeopardized the potential

positive effect of water stress on root growth. Finally, hypothesis 2 can partially be validated at the whole-plant level because K limitation occulted the effects of water stress.

4. Potassium fertilization mitigates the negative effect of drought

Since some key physiological mechanisms such as cell turgor pressure are very responsive to K and water limitations (Hsiao and Lauchli, 1986), it was of interest to see whether or not the lack of water was compensated by K addition. Our study revealed that there was no advantage of over-fertilizing water-stressed plants with K. The highest level of K fertilization (K2) applied to water-stressed (W-) plants did not allow some developmental parameters (LER, senescence, root morphology, plant development), and hence the main plant growth components (green leaf area, leaf lifespan, root biomass, shoot biomass) to be as high as the well-watered plants that were fertilized with a lesser quantity of K (K1). From a theoretical point of view, K nutrition is supposed to alleviate water stress through a better root prospection, a longer leaf lifespan, a better cell growing capacity thanks to the osmotic regulation, and in turn, through a better green leaf area and biomass (Grzebisz et al., 2013). Some plants experimented under controlled conditions give results showing such compensatory effects between K fertilization and water stress (Cakmak, 2005; Fournier et al., 2005; Oosterhuis et al., 2013; Zörb et al., 2014). However, the exact physiological mechanisms involved are still a matter of debate. Main benefits may rely on water regulation through stomata sensitivity to water stress, such as mentioned in the literature (Arquero et al., 2006), and whose effects have been applied to our plants, showing a theoretical economy of 25% of water losses. Undoubtedly, this represents another interesting aspect of the K and water interactions.

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Chapitre IV.

Effet de la nutrition potassique sur les pertes en eau et le rendement du maïs sous déficit hydrique

The effects of potassium nutrition on water use in field-grown maize (Zea mays L.)

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<u>Abstract</u>

Water is about to become increasingly limited for crop production, which jeopardizes the whole maize sector. Potassium (K) nutrition has been proposed to mitigate water deficit in plants, but field-scale studies involving grain yield components are scarce. In this study, we aimed at analyzing the effect of K nutrition on grain yield, vegetative growth and physiological parameters of maize subjected to water deficit. The effect of K nutrition on water use efficiency was also calculated at the whole-crop level (WUE) and through leaf gas exchange measurements (WUE_i). A large-scale field experiment was designed, combining three levels of K fertilization (low, normal and high K) and two water supply scenarios (normal and 30% deficit based on a water balance model). Water deficit induced a strong decrease in leaf area, which was essentially due to a lower leaf elongation rate. The grain yield of the water-stressed plants was 25% lower than that of the well-irrigated ones. Grain yield was even worse when K deficiency was superimposed on water deficit, with a specific effect of K on grain filling. The optimal K fertilization helped the plants mitigate the effect of water deficit, through a better WUE (+30%), which was related to lower leaf evapotranspiration (ET). Moreover, under water deficit, leaf rolling was more pronounced when K was added, which also prevented water losses. Leaf water potential measurements suggested that the isohydric behaviour (maintenance of close stomata during water stress) of the maize was made possible thanks to K fertilization. When calculated at field scale, ET was higher with K fertilization, due to its positive effect on leaf area, in spite of a better stomata control and better WUE. We concluded that K addition, in K deficient soils, can help maize to cope with droughts and could be used as a new management option.

Keywords: potassium, water deficit, maize crop, gas exchange, water use efficiency, yield

I. <u>Introduction</u>

Experts consider that the rates of warming and current climate changes are likely to increase in the coming years. According to a recent IPCC report (IPCC, 2014), scientists predict a rise in global temperature of several degrees Celsius, which will be correlated to a higher rate of summer drought. All regions will be impacted, including the temperate regions of Western Europe, where agriculture is likely to suffer major negative effects. Summer species such as maize (*Zea mays L.*) will be particularly impacted (IPCC, 2014). In the South West of France, a major maize production region, annual rainfall is expected to decrease by 2050 with, more importantly, a critical decrease in summer precipitation (IPCC, 2014; Lehner et al., 2006; Lobell et al., 2011). As maize is a high-value crop adapted to those sandy soils, farmers are looking for new growth strategies rather than for a new type of crop. Adapted potassium nutrition could constitute one of these new strategies.

The idea that potassium nutrition could play a role in plant resistance to water deficit has long been known (Blanchet et al., 1962; Hsiao and Lauchli, 1986). Nevertheless, it is only fairly recently that the topic has been discussed at length; since 2010, at least 40 published linking potassium (K) fertilization and water resources and their interactive effects on crop ecophysiology and yields have been published (Cakmak, 2005; Römheld and Kirkby, 2010; Sardans and Peñuelas, 2015; Zörb et al., 2014). Plants lacking K seem to develop an acute sensitivity to water deficit, while plants with sufficient K input do not (Aslam et al., 2013).

There are at least three reasons why plants without K would develop such acute sensitivity to water deficit (Wang et al., 2013): i) K, being the main cation in plants available in soluble form, is closely linked to the water potential (Mengel and Arneke, 1982). It therefore plays a major role in the turgor of growing cells, in maintaining their water content and in osmoregulation (Fournier et al., 2005) and stomatal regulation (Arquero et al., 2006; Benlloch-González et al., 2008; Hsiao et al., 1976). ii) Sufficient K supply promotes root growth, including lateral root growth (Armengaud et al., 2004; Cakmak et al., 1994; Claassen et al., 1986; Egilla et al., 2001; Ericsson, 1995), and could also increase root life-span (Egilla et al., 2001). These two aspects contribute to higher water uptake by improving deep root exploration. In addition, some recent results on soil properties show that K also enhances water retention in soils (Damm et al., 2013). iii) K lengthens leaf life-span by allowing the elimination of reactive oxygen species (ROS) which are often released following a water

deficit or thermal stress episode (Cakmak, 2005). Such elimination maintains a photosynthetically active leaf area under stress, one of the advantages mentioned by Christina et al., (2015) in a study on a Eucalyptus plantation, which shows that plant productivity benefits from an adequate supply of K.

Whole-plant water use efficiency (WUE) has been defined as the capacity for a plant to produce biomass as a function of crop water evapotranspiration (ET) (Hsiao, 1973). WUE is an integrative indicator for biomass production or grain yield (WUE). Intrinsic WUE (WUE_i) is measured on a short-term scale and defined as the ratio of net CO_2 assimilation (A) to stomatal conductance (g_s) determined from gas exchange measurements. In this study, both WUE and WUE_i were used to evaluate the impact of K nutrition on plants under water deficit (comparing different K supplies), in spite of methodological difficulties already pointed out by Andersen et al. (1992b) and Battie-Laclau et al. (2016). An alternative approach, using modeling studies, has been carried out to study the impact of K on WUE (Grzebisz et al., 2013; Yu et al., 2004), with results revealing a clear advantage for crops receiving sufficient inputs of K. The results for WUE_i are less clear-cut (Arquero et al., 2006; Egilla et al., 2005; Erel et al., 2015), probably due to the heterogeneity of the experimental parameters (age and K status of plant samples used...) and the diversity of tested species (Hsiao and Lauchli, 1986).

Most of the studies dealing with the interaction between water and K have focused on isolated mechanisms such as stomatal functioning (Arquero et al., 2006), or on the elimination of ROS (Cakmak, 2005). Crop responses to water limitation (WUE assessment, for example) are easier to set up in controlled conditions (Fournier et al., 2005) whereas large-scale field experiments are more subject to uncertain climate variations. In the field, different water regimes can be obtained either by rainfall exclusion (Battie-Laclau et al., 2014b) or by irrigation rate variation (Fanaei et al., 2009).

Faced with to this lack of field scale references, our objective was to test the physiological and growth effects (including yield) of three levels of K-fertilization on maize grown under two different water levels, using a long-term open-field experiment (Jordan-Meille and Pellerin, 2004). Several key variables related to plant development, growth and physiological attributes were measured. We hypothesize that i) K deficiency has the same impact on plant physiology as water deficit, thus leading to the same kind of plant symptoms. As a consequence of i), we assume that ii) K deficiency exacerbates the negative effect of water

deficit on plant leaf growth and grain yield and that iii) K nutrition mitigates the negative effects of water deficit through better water loss control.

II. <u>Materials and methods</u>

1. Experimental set-up

The study was conducted in 2015 at an existing, long-term experimental K-fertilization trial at INRA (French National Institute for Agricultural Research) located in Cestas-Pierroton, France (44°44'31.1"N and 0°47'01.1"W). The experiment started in 1996 with maize being planted every year since then. The soil is classified as an umbric ortsteinic podzol, composed for the first 75cm of 4.6% clay, 2.1% loam, 93.3% sand and 3.9% organic matter. Soil pH, measured just before maize was planted, was 6.2 ± 0.1 . The bulk densities measured at soil depths of 0-25, 25-50 and 50-75 cm were 1.36, 1.52 and 1.57 g cm⁻³, respectively. Volumetric moisture at field capacity and at wilting point was 19.7 and 4.4%, respectively (measured experimentally), leading to 15.3% available water for the plants.

Our experiment was separated into two experimental plots (W+ and W-) with three levels of K supply (K0.5, K1 and K2) provided in the form of KCl (60% of K₂O) and applied before sowing (in March) at three different K supply rates (0, 20 and 80 kg K ha⁻¹). Two months after K was applied, nine soil samples (0-50 cm depth) were collected in each plot and exchangeable K content was extracted using ammonium acetate (AFNOR, 1992). The average values of exchangeable K at those 0-50 cm depths were 22, 31 and 69 mg K kg⁻¹ for K0.5, K1 and K2, respectively. They reflected three different K statuses: low (K0.5), normal (K1) and high (K2).

Two water regimes were applied, using irrigation in addition to rainfall. W+ reflects an optimum water supply rate and W- a water deficit. A mobile irrigation system was used, covering the length of the field experiment. In each water regime, plots were chosen at random for K supply rates, but not for water supply rates. The irrigation for the plants of the W- treatment was initiated when soil volumetric moisture reached 5% (close to the wilting point) and remained at that level for a minimum of five consecutive days. Low soil water content (SWC) was confirmed by the leaf rolling observed each day in the late morning. Overall, single water application irrigation compensated for the cumulated four days of water deficit (20 mm). The irrigation system was programmable, and the amount of water applied

was exactly known and checked using rain gauges installed on each plot. On average, the irrigation system added 3.5 mm of water each day over the course of the experiment. The amount of rainfall between sowing and harvesting reached 236 mm, with irrigation supplementing this by 289 mm for W+ and 102 mm for W-. The quantity of water restriction between W+ and W- treatments represented 36% of the total water supply of the W+ plots. Maize (variety DKC5784, late hybrid variety) was sown on 6th May. The emergence rate was 81.800 plants per hectare. Nitrogen and phosphorus were supplied so as to be non-limiting. Nitrogen was applied at sowing (55 kg N ha⁻¹ as NH₄NO₃ 33.5 %) and at the beginning of June (195 kg N ha⁻¹ as NH₄NO₃ 33.5 %). Phosphorus was applied at the end of March (Ca(H₂PO₄)₂, 45 % P₂O₅); dolomite was spread out every three years to avoid any induced magnesium deficiency. The day before mechanical harvesting (30th Sept), all samplings were performed by hand for harvest characterizing at physiological maturity.

2. Measurements and characteristics related to the climate and water status

All climatic parameters (minimum and maximum temperatures, air humidity, net radiation, rainfall) were recorded by a weather station located next to the experimental plots. In each plot SWC was first measured on soil cores collected at three different horizons (0-25, 25-50 and 50-75 cm) throughout the whole growing cycle. The volumetric SWC was also continuously measured, using two types of probe, each of which was calibrated before being installed. One probe per treatment was installed in the top soil layer at 0-7 cm (ThetaProbe type ML2x, Delta-T Devices, UK) and three other probes at depths of 20, 40 and 60 cm (CS 616 Campbell Scientific, Logan, UT, USA). In sandy soils characterized by low soil moisture content values, it has been shown that TDR probes are not significantly influenced by variations in soil temperature (<0.25%) (Campbell Scientific, 2016). The summer of 2015 was a particularly dry one, with precipitations being 236 mm below the 20-year average. The cumulative water deficit between sowing and harvesting reached the 4th rank for the driest seasons in the past 20 years. No rainfall was recorded for six weeks after the early flowering stage (twelve visible leaves) was initiated (from 13th June to 27th July). Consequently, there was no rain during stem extension stages, male and female flowering stages, pollination and the beginning of grain filling. The hottest day was recorded on 30th June with a maximum temperature of 39.3°C. During this dry period, average daily temperatures ranged between 16°C and 20°C. The SWC in the W- plots was kept between 6 and 8% while the irrigation on the W+ plots maintained the volumetric soil moisture between 10 and 12% (Fig. A1.1). Soil water potential was obtained as a function of the soil saturation degree and from the water retention model formulated by Van Genuchten (1980) (Fig. A1.2). The three parameters of the Van Genuchten function were calculated using measured soil texture and soil bulk density.

3. Development, growth and grain yield measurements

Crop development variables were taken as the average number of visible leaves for the vegetative stage and the date at which 50% of the population reaches silk emergence for the flowering stage. In addition to these variables, the evolution of the number of dry leaves (dry on more than 50% of their surface) was monitored. For every treatment, this was carried out once a week on the same fourteen adjacent plants. The phyllochron, which represents the interval between the appearance of two successive leaves in degree days (dd, threshold temperature 6°C), was calculated from the relationship between the number of visible leaves and thermal time (Jordan-Meille and Pellerin, 2004).

Crop growth variables comprising total leaf area and crop height were measured at flowering (mid-July). Yield variables (grain yield, 1000-grain weight) were measured at harvest (end of September). Leaf area measurements were made on fourteen adjacent plants on each plot using a table top planimeter (LI-3100C; LI-COR Inc., NE, USA). Grain yield and 1000-grain weight measurements were made on two rows of maize of 8m each (which represents about 100 plants). The 1000-grain weight and the grain number per ear were attributed to grain filling and to pollination state respectively. Grain number per ear was calculated as the ratio of grain yield (kg m⁻²) to the 1000-grain weight (kg). The number of plants by m² and the ear number by plant were invariable between all treatments. The specific leaf area (SLA) was calculated as the leaf area divided by the dry matter.

In addition, leaf growth dynamics was measured every two days on leaf 14, from its emergence to the end of its growth (ligule visible). This parameter was determined on leaf 14 because its growth occured during the time when the water deficit was at its peak. Furthermore, leaf 14 was a fully developed leaf, which allowed easy and precise measurements. The leaf elongation rate (LER in cm dd⁻¹) of this leaf was calculated from the successive leaf length measurements during its quasi-linear elongation period (Plénet et al.,

2000). This was carried out on the same fourteen plants used for the measurements of development variables. Height measurements were performed on the same sampled plants.

4. Daily gas exchange dynamics measurements

Diurnal photosynthetic rate, transpiration rate and stomatal conductance (A in μ mol m⁻² s⁻¹, E in mmol $m^{-2} s^{-1}$ and g_s in mol $m^{-2} s^{-1}$ respectively) were measured with a gas analyzer (LI-6400; LICOR Inc., NE, USA). Diurnal measurements took place on 9th July in all treatments except for K1, which was not measured due to lack of time. Maximum values of A, E, g_s and WUE_i were calculated at midday, where photosynthetically active radiation (PAR) was around 1900 µmol m⁻² s⁻¹. Measurements were performed every 90 minutes from 3 a.m. to 8 a.m. on the upper fully expanded leaves of three representative plants. Time of day refers to International Solar Time (IST). Air temperature, relative humidity and PAR inside the leaf chamber were set to data recorded in the ambient air. Ambient PAR measured above the crop with the LI-6400 before each measurement was used in all gas exchange measurements, being progressively increased to 1900 μ mol m⁻² s⁻¹ at midday, and then gradually lowered to zero at night. The CO₂ partial pressure was maintained at 400 ppm. During those diurnals, measurements of soil moisture by gravimetric method indicated that SWC for W- values ranged between 5.8% and 4.9% in K0.5 and K2 treatments, respectively, just above the leaf wilting point. The SWC of the irrigated plots ranged between 13.2% and 9.5% in K0.5 and K2 treatments, respectively.

5. Whole-plant evapotranspiration measurements

The most robust calculation of evapotranspiration (ET, in mL m⁻² h⁻¹) over a given period is based on the sum of water supplies (rainfall + irrigation) while taking into account soil water content variation between the beginning and the end of the period after checking for the absence of drainage. On each plot, soil water reserve was measured to a depth of 75 cm, using the gravimetric method at the start and end of the cycle. The measuring period started at the beginning of June (1 month after sowing) and ended at the end of September (at harvest). The probes set up at 60 cm depth showed that SWC never exceeded 13% (*i.e.* 65% of field capacity), confirming that there was no drainage, even for the plots with the irrigated plants. The use of probes at 0-75 cm depths also allowed us to estimate the ET (in mL m⁻² h⁻¹), by adding the negative differences of all volumetric moisture for each horizon. Using this ET, night-time (calculated from 9 p.m. to 3 p.m.) and daytime (7 a.m. to 3 p.m.) water use were determined either on a ground (m^2 of soil) or leaf area (m^2 of leaves) basis. These were continuously calculated for four months, using time slots that excluded day transition periods.

6. Water use efficiency and plant water status measurements

On the one hand, plant water use efficiency (WUE in g DM L⁻¹) was calculated as the ratio of grain yield production to total crop water use (ET, June-September). On the other hand, intrinsic water use efficiency (WUE_i) was calculated by using daily gas exchange measurements (9th July). WUE_i was determined as the ratio of photosynthesis to stomatal conductance (A/g_s), which erased the influence of vapor pressure deficit (VPD) on plant transpiration (Medrano et al., 2015).

Gas exchange measurements were performed on 9th July, just before the flowering stage. At this date, leaf water status was also determined by measuring leaf water potentials on three random leaves per plant from each plot undergoing K0.5 and K2 treatments, combined with either W+ or W-. Water potentials (Ψ) were measured using a Scholander-type pressure chamber (SAMPrecis, Gradignan France). A first series of measurements was made at pre-dawn (4 a.m.), being followed by a second series carried out in daytime until 8 p.m. (after sunset).

Another variable taken into consideration for assessing water status is the leaf rolling mechanism, because it is a visual evaluation of plant water stress. This mechanism was measured on 9th July, on those plants whose development was measured. The apparent width of the median part of the rolled leaf was measured every two hours between 7 a.m. and 2 p.m. The leaf rolling degree is the percentage of width reduction (initial leaf width minus current width, divided by initial width).

7. Statistical analysis

Statistical analyses were performed using R software (version 3.1.3) (R Core Team, 2014) to calculate average values for n=14 (not for the daily dynamic for which n=3), \pm standard errors, and to test differences between treatments. Data were tested for normality (Shapiro's test) and homogeneity of variance (Levene's test). *Post-hoc* differences in means were tested

using Tukey's test. A linear model was used to analyze differences between each treatment and a two-way ANOVA was performed to test the effect of potassium (K) and water (W) and their interaction (W x K). Because of the low number of replicates (n=3), photosynthesis, transpiration, stomatal conductance and water potential were analyzed using non-parametric test permutation (n=450).

III. <u>Results</u>

1. Plant mineral nutrition

The concentrations in K at flowering stage (around 925 dd) measured in aboveground plant biomass grown under W- were 4.9, 16.2 and 26.3 mg K g DM⁻¹ for K0.5, K1 and K2, respectively (Table IV.1). For W+ plants, the concentrations were 7.0, 14.0 and 25.0 mg K g DM⁻¹ for K0.5, K1 and K2, respectively. Soil water availability was sufficiently high throughout the early growing stages (above 15% of SWC) to prevent any restriction in plant absorption of available K. The N and P average concentrations were 2.8, 3.2% and 0.3, 0.4% for W- and W+, respectively.

Table IV.1 Leaf potassium(K) content (LKC), phyllochron (P), number of final visible leaves (VL), leaf elongation rate (LER), leaf area (LA), plant height (H), leaf water content (LWC), specific leaf area (SLA), leaf rolling (LR) in maize at flowering stage grown under well-watered (W+) and water-stressed (W-) conditions and under three K levels (K0.5,K1, K2) (n=14,± standard errors). Effect of K-fertilization, water supply regime and their interaction (W x K) were tested using parametric two-way analysis of variance.

				analyse of variance (ANOVA)						
Wi	Ki	LKC (mg g ⁻¹)	P (dd)	VL	$\frac{\text{LER}}{(\text{cm dd}^{-1})}$	LA (m ²)	H (cm)	LWC (%)	$\frac{\text{SLA}}{(\text{m}^2 \text{ kg}^{-1})}$	LR (%)
W-	K0.5	4.9 ± 0.3	41.9 ± 1.0	19.1 ± 0.1	0.28 ± 0.01	0.45 ± 0.03	123 ± 4	79.6 ± 0.5	1.49 ± 0.02	-63.5 ± 2.9
W-	K1	16.2 ± 0.3	39.7 ± 0.7	19.2 ± 0.2	0.26 ± 0.00	0.57 ± 0.01	154 ± 2	82.2 ± 0.3	1.84 ± 0.02	NA
W-	K2	26.3 ± 0.3	39.8 ± 0.5	19.3 ± 0.2	0.25 ± 0.01	0.59 ± 0.01	149 ± 2	83.0 ± 0.4	1.87 ± 0.03	$\textbf{-75.9} \pm 1.8$
W+	K0.5	7.1 ± 0.2	39.5 ± 1.0	19.6 ± 0.3	0.58 ± 0.01	0.62 ± 0.02	231 ± 5	84.0 ± 0.1	1.69 ± 0.03	-1.6 ± 0.7
W+	K1	14.0 ± 0.4	37.3 ± 0.6	20.6 ± 0.1	0.60 ± 0.01	0.67 ± 0.02	253 ± 2	83.9 ± 0.2	1.77 ± 0.03	NA
W+	K2	25.0 ± 0.7	37.1 ± 0.8	20.5 ± 0.1	0.71 ± 0.01	0.70 ± 0.01	274 ± 3	85.0 ± 0.2	1.83 ± 0.03	-2.8 ± 2.3
We	effect	ns	***	***	***	***	***	***	ns	***
Кe	effect	***	**	**	***	***	***	***	***	**
W	x K	***	ns	*	***	ns	***	***	***	*
* D /	$* D < 0.05 \cdot ** D < 0.01 \cdot *** D < 0.001$									

* P < 0.05; ** P < 0.01; *** P < 0.001

2. The effect of water deficit on plants with sufficient K supply: W+K2 vs W-K2

a. An obvious effect of water deficit on growth and yield

The unusual dry weather conditions of the summer of 2015 had a marked impact on the experiment: the effect of water deficit was seen on all the variables. The interval between leaf appearances (phyllochron) measured on plants with sufficient K supply was significantly extended by water deficit (W-) (39.8 vs 37.1 dd, P<0.05), which led to a difference of about 1.5 visible leaves at the end of the vegetative period (Table IV.1). The flowering was delayed by 10 days for the W- plants as a consequence of deferred leaf emission. The final number of leaves decreased significantly (P<0.001) for the W- plants, from 20.5 visible leaves to 19.3 (Table IV.1). Nevertheless, considering the size of the leaves, these differences had minor impact on the leaf area.

The leaf area was 16% lower on average for the plants in W- K2 than for the well-irrigated ones (Table IV.1). Leaf area index of W+ and W- plants reached 5.7 and 4.8 m² m⁻², respectively. This was due to the leaf elongation rate (LER) being greatly slowed down during the linear phase of leaf emission, at the peak of the water deficit (from 24th June to 10th July). The LER decreased by 65% (P < 0.001) for W- (from 0.71 to 0.25 cm dd⁻¹) (Table IV.1). The number of dry leaves was not significantly different between W- and W+ treatments (P=0.06).

In K2 plants, grain yield for the W- treatment (Fig. IV.4) was 25% lower than for the W+ treatment (W+ K2 at 14.2 t ha⁻¹). Half of the grain yield reduction was due to poor grain filling (1000-grain weight) which decreased from 25.9 g to 22.7 g for W+ K2 and W- K2, respectively (data not shown). The other cause of those yield reductions was due to low pollination rates (grain number per ear) (data not shown).

b. Ecophysiology of a water deficit: fewer instantaneous gas exchanges, reduced ET, higher WUE, leaf rolling

For K2 plants, both drought and high VPD reduced maximum photosynthesis (A_m) (P <0.05) and stomatal conductance (g_{sm}) (P < 0.05) by 80% (Table IV.2 and Fig. IV.1a, b). The biggest difference between W+ and W- plants was measured around 11 a.m., when VPD reached 2 kPa. On 9th July, SWC under the W- treatment, as determined by direct SWC measurements from gravimetric method, reached very low values (5.8% of water for K0.5 plants and 4.9% for K2 plants), which translated into midday water potentials (Ψ_m) of around -1.5 MPa (Fig. IV.1c). The differences in water potentials measured at midday were coherent with the significant (P<0.001) lower leaf water content (LWC) measured under water deficit: 83% vs 85% (Table IV.1).

Table IV.2 Physiological parameters at midday such as maximum carbon assimilation (A_m) , transpiration ($E_{\rm m}$), stomatal conductance ($g_{\rm sm}$), intrinsic water use efficiency (WUE_i), leaf water potential (Ψ_m) (n=3,± standard errors) in maize at flowering stage grown under well-watered (W+) and water-stressed (W-) conditions and under three K levels (K0.5,K1, K2). Effect of K-fertilization, water supply regime and their interaction (W x K) were tested using non-parametric two-way analysis of

•		
varı	anc	e.

			non-parametric ANOVA				
wi	K;	A_{m}	$E_{\rm m}$	$g_{ m sm}$	WUE _i	W (MD _a)	
VV I	KI	$(\mu mol m^{-2} s^{-1})$	$(\text{mmol } \text{m}^{-2} \text{ s}^{-1})$	$(mol m^{-2} s^{-1})$	$(\mu mol CO_2 mol H_2O^{-1})$	$T_{\rm m}$ (IVII a)	
W-	K0.5	8.4 ± 3.1	1.24 ± 0.35	0.025 ± 0.008	317.5 ± 23.7	$\textbf{-1.30}\pm0.03$	
W-	K1	NA	NA	NA	NA	NA	
W-	K2	2.0 ± 0.3	0.41 ± 0.05	0.006 ± 0.001	332.5 ± 74.9	$\textbf{-1.41}\pm0.06$	
W+	K0.5	16.5 ± 1.9	1.45 ± 0.15	0.045 ± 0.008	374.4 ± 21.7	$\textbf{-0.82}\pm0.07$	
W+	K1	NA	NA	NA	NA	NA	
W+	K2	12.7 ± 2.0	1.38 ± 0.12	0.044 ± 0.005	288.8 ± 14.6	$\textbf{-0.78} \pm 0.11$	
Wε	effect	*	*	*	ns	*	
K e	ffect	*	ns	ns	ns	ns	
W	x K	ns	ns	ns	ns	ns	
* $D < 0.05 + ** D < 0.01 + *** D < 0.001$							

P < 0.05; ** P < 0.01; * * P < 0.001



Fig. IV.1 Diurnal changes in (a) photosynthesis (A); (b) stomatal conductance (g_s) ; (c) water potential (Ψ) ; (d) intrinsic water use efficiency (WUE_i) under well-watered (circles: W+) and water stressed (triangles: W-) plants under two potassium levels (open symbol: K0.5 and closed symbol: K2) (mean \pm se; n=3). Measurements were performed on 9th July.

The water deficit that caused leaf rolling (P<0.001), was only visible on W- plants (Fig. IV.2). Leaf rolling was at its peak around 1 p.m., with the width of the leaves becoming reduced to 70% of the usual width. At that time, water potential was around -1.5 MPa and VPD reached its maximum value at 2 kPa.



Fig. IV.2 Leaf rolling dynamic (expressed as % changes in leaf width) for well-watered (circles: W+) and water-stressed (triangles: W-) plants and for two potassium levels (open symbol: K0.5 and closed symbol: K2) (mean ± se; n=7). Measurements were performed on 9th July.

Over the long term, ET, calculated on water mass balance showed that W- plants consumed only 60% of the volume of water used by the W+ plants (321 vs 540 mm between June and September for K2 plants). A more precise approach, comparing ET rates between day and night, showed that the measured night-time ET was similar for W+ and W- plants. Consequently, the variation in ET only accounted for day time water use, as shown in Fig. IV.3a, b. The night-time ET reached 30% of the total ET for W+, and up to 40 % for W-, whether these proportions were calculated on soil or leaf area. The night water loss indicated a low plant water retention ability, despite water content being close to the wilting point. This result was confirmed by daily variations in stomatal conductance, which was of the same order of magnitude between W+ and W- plants before or after sunrise (Fig. IV.1b). This result may also be linked to the pre-dawn water potentials of W- plants, which were surprisingly close to zero, although more negative than those from W+ plants.



Fig. IV.3 Hourly average (from June to September) in growing season stand of evapotranspiration (ET) during daytime (7 a.m. - 3 p.m.) and night-time (9 p.m. - 3 a.m.) periods on a) soil area and b) leaf area basis.

The combined effects of water deficit on grain yield and ET indicated that WUE for grain production of K2 plants was higher for the W- plants by +0.6 g DM L⁻¹ on average (Fig. IV.4). However, the intrinsic WUE calculated at the leaf scale (WUE_i) did not reveal any difference between treatments (Fig. IV.1d).



Fig. IV.4 Effect of water supply (dark grey: W+; grey: W-) and potassium (K) treatment on water use efficiency (WUE). Numbers represent maize yields (t ha⁻¹).

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3. The impact of potassium addition on plants with sufficient water supply: W+ K2 vs W+ K0.5

a. K deficiency impacted less plant growth than water stress

Without water limitation K deficient plants had reduced growth and leaf area. Under W+, plant responded to K deficiency with a 6% average increase in the phyllochron (P < 0.001), from 37.1 dd for K2 plants to 39.5 dd for K0.5 plants. The final number of leaf therefore decreased, from 20.5 for K2 plants to 19.6 for K0.5 plants (P < 0.001). However, the leaf mortality did not vary between K0.5 plants and K2 plants (data not shown). For the leaves developing towards the end of the vegetative cycle (leaf 14), the LER was reduced by 18% (P < 0.001, Table IV.1). The result of these combined effects was a 11% reduction in leaf area (P < 0.001), from 0.70 m² per plant for W+ K2 to 0.62 m² per plant for W+ K0.5 (Table IV.1). Moreover, K nutrition impacted SLA by the same order of magnitude (Table IV.1), leading to no significant differences in leaf dry biomass (data not shown). K deficiency delayed the flowering dates by four days (data not shown). The relative grain yield of K deficient plants reached only 72% (K0.5/K2) under W+ treatment (Fig. IV.4). This decrease was mostly due to a lack of complete grain filling, which decreased from 25.9 to 19.2 g for 1000-grain weight for W+ K2 and W+ K0.5, respectively.

b. No K effect on gas exchanges in well-irrigated plants, but a better WUE for grain production

When water was non-limiting, K nutrition status did not impact gas exchanges. For the W+ plants, the photosynthetic and the stomatal conductance daily dynamics showed no variation from one K treatment to another (Fig. IV.1a, b). WUE_i was also independent of K treatments (Fig. IV.1d), whereas WUE increased with K addition (Fig. IV.4), rising from 1.57 to 2.24 g DM L⁻¹ (*i.e.* a 30% increase) on W+ plants.

4. Interaction between water addition and potassium fertilization

The effect of water deficit cumulated with the effect of K deficiency when plant developmental and growth variables were considered. The phyllochron of W- K0.5 plants reached 41.9 dd (Table IV.1), corresponding to a relative difference of 9% with the reference treatment (W+ K2 plants). The K deficiency on W- plants added an extra delay of five days to the flowering delay of ten days noticed on W- K2 plants. However, plants under a combination of both K and water deficits did not show any extra reduction in the number of final leaves.

Some growth variables showed no response to K deficiency, even under water deficit, which is indicative of marked interaction between water and K supplies (Table IV.1). Both the internode growth (measured through plant height) and the leaf growth (measured through the leaf elongation rate) seemed to be dependent on water status to such an extent that the impact of K deficiency was not observable. The addition of K effect to that of water could only be observed at plant level, as well as on integrative variables measured on a more long-term basis, such as leaf area (Table IV.1). K and water deficit effects on grain production were both cumulative: -3.9 t ha⁻¹ was the effect of K deficiency, -3.5 t ha⁻¹ was the effect of water deficit and -7.4 t ha⁻¹ the global effect. Yield reduction on K0.5W- plants (60%) was the consequence of poor pollination (data not shown). The impact could have been more negative, considering that leaf water content of the K0.5W- plants was very low (79%), even lower than what might have been expected from a combination of effects (K x W, Table IV.1).

On a daily basis, gas exchange measurements on W- plants showed lower values of A and g_s on K2 treatment, especially between 8 a.m. and 2 p.m. (Fig. IV.1b). Throughout the whole growing season, the same trend was observed on ET (Fig. IV.3b), especially during daytime, when K2 plants lost 17% less water per hour than K0.5 plants.

IV. <u>4. Discussion</u>

1. Mineral and water treatments: questioning the experimental design

There have been no large-scale and open-field studies on the combined effects of K-limitation and water stress on maize leaf physiology, and their downstream impacts on growth and yield. The field experiment was designed so as to combine varied quantities of water and K resources. The range of K concentrations measured on plants was independent of water treatments, and equivalent to that found in other studies dedicated to plant response to K nutrition for maize (Jordan-Meille and Pellerin, 2004), for sunflowers (Benlloch-González et al., 2008), bean field (Cakmak et al., 1994) and for cotton crops (Gerardeaux et al., 2009). Although K is known to interfere with N (nitrate is conveyed by K⁺ during its acropetal transport, (Koch and Mengel, 1974) and/or P uptakes (through its impact on root growth), concentrations measured at flowering showed no values below critical thresholds (Plénet et al., 2000).

Water treatments were more difficult to cope with: it was not completely possible to avoid certain bias due to different ET within the same water treatment, because of the differences in leaf area index (LAI) for plants receiving greater or lesser amounts of K. This particular problem has already been encountered in field conditions (Battie-Laclau et al., 2014b). It is also this difficulty which could explain the limited numbers of studies based on in situ experimentations. In order to overcome this constraint, we ensured that the W- plants both reached and remained at a low SWC level (close to wilting point) between two irrigations. However, certain differences may have persisted, as in the case of the SWC data for the particular day on which gas exchange dynamics were performed (W-K2 SWC was 0.9 % lower than W-K0.5). Such a gap was small, however, compared to the overall difference measured in the irrigated plots (6 % during dry periods). For irrigated plots, this bias was easier to handle.

2. Water stress and K deficiency: same impact on leaf growth, complementary response on grain yield, opposite responses on gas exchanges

At flowering, water deficit and K deficiency decreased the green leaf area, with the same relative effect. This decrease was not related to the number of leaves and/or senescence, but only to the leaf area. The detrimental effects of water deficit on leaf area is very well documented, and represents an important, precocious symptom (Hsiao et al., 1976) which allows the plant to be less exposed to water loss. Reduced leaf area has already been described for K-deficient maize (Jordan-Meille and Pellerin, 2004) and, more generally, for many other plants (Arquero et al., 2006; Battie-Laclau et al., 2013; Lindhauer, 1985). In effect, K is known to play a major role in cell growth via turgor pressure maintenance (Mengel and Arneke, 1982; Premachandra et al., 1993) and cell-wall elasticity (Triboulot et al., 1997). It is also suspected of influencing the number of cells in developing primordial organs by disrupting sugar distribution in plants (Gerardeaux et al., 2010). Leaf senescence, which is generally a key development parameter, did not emerge as a sensitive factor in the present experiment despite what might have been expected regarding the roles of K in cell water content (Battie-Laclau et al., 2013). The crop was probably not old enough to develop this symptom, even if no significant differences were measured just before harvesting.

Although water deficit and K deficiency reduced grain production, they did so in different ways. Most of the yield reduction caused by the lack of K was due to a default of grain filling and was probably linked to K's role in sugar translocation toward growing areas (Tohidloo et al., 2012). In the case of water deficit, a default in both grain filling and pollination contributed to the lower yield, as is often reported (Andersen et al., 1992b; Fanaei et al., 2009). Even though two complementary mechanisms were involved, the yield reduction due to water was not higher than with K stress.

Photosynthesis measurements, unlike growth symptoms, showed a very different pattern in response to water and K deficiencies. It is well known that plants under water deficit experience a lower photosynthetic activity per leaf area unit (Damour et al., 2009; Gallé and Feller, 2007). In the present study, the reduction in photosynthesis was mostly due to the decrease in stomatal conductance (g_s). The altered photo-chemical processes did not seem to worsen the water deficit symptoms. The decrease in g_s globally accounted for the decrease in A. For K, the absence of response to the different levels of K nutrition has already been

observed in maize (Peaslee and Moss, 1966), but most studies have shown a reduction in photosynthesis under K deficiency (Egilla et al., 2005; Pervez et al., 2004). Measurements were taken from the upper parts of the plants, where K is known to mitigate stress in case of K deficiency at plant level (Andersen et al., 1992a). The K concentrations in upper leaves were possibly above the threshold concentration for photosynthetic activity (Leigh and Jones, 1984) but below the value needed to maintain normal growth as determined from results obtained on LER on upper leaves under K deficiency.

The observed positive response of the WUE in the case of water deficit, or/and that obtained by adding K to K deficient crops (suffering from drought or not), was similar to the literature (Davies and Zhang, 1991; Jones, 1992). As evaporation was taken into account at the beginning of the cycle (June), this could explain why WUE values (between 1.57 and 2.84 g DM L⁻¹) were fairly low compared to those measured on maize in non-limiting conditions (around 4 g DM L⁻¹, Bahrani et al., 2012; Singh et al., 2014). Equally, it should also be noted that WUE calculation referred exclusively to grain production. WUE_i behaved differently, showing no clear effect, either with water or with K. This may be due to the fact that WUE_i did not include night or root processes (respiration, exudation), and nor did it concern the whole plant leaves. As pointed out very recently by Battie-Laclau et al. (2016), the positive effect of K nutrition on WUE cannot be linked to WUE_i, which is measured on upper leaves.

3. K deficiency exacerbates the negative effect of water deficit on plant leaf growth and grain yield

In certain circumstances, K and water can be simultaneously involved in the same physiological mechanisms, maintaining turgor pressure in either growing or guard cells. This leads to the hypothesis that the resulting response of the organ/plant will be restrained by the effect of the stronger limiting factor, which masks the effect of the weaker limiting factor. This has typically been the case with internode growth (measured in terms of plant height) and leaf growth (measured in terms of leaf elongation rate), which are both known to be very sensitive to water deficit (Aslam et al., 2013; Hsiao et al., 1976). Moreover, stomatal conductance and photosynthesis also showed greater sensitivity to water deficit than to K deficiency, under our experimental conditions. However, in most circumstances, and especially when phenomena are examined on a long-term basis (integrative variables such as leaf area and grain yield, WUE), the lack of K added its negative effects to those of water

deficit. This cumulative effect could be due to the fact that the different symptoms were spread out over time during the cycle. At the early stages, K was involved in the leaf area until the emergence of the 10th leaf, whereas the water deficit impact seems to have started from the emergence of the 12th leaf. At that stage, the water deficiency effect overtook that of K, thereby explaining why the effect of the K deficiency disappeared, as shown by the result on the LER of leaf 14. The yield variable had different components (flowering initiation, flowering, pollination, grain filling) over time. In conclusion, it appears that when water becomes a highly limiting factor (less than 30% of soil water availability, for example), a concomitant reduction in K would further exacerbate the negative effect of water plant stress on maize productivity.

4. K nutrition mitigates the negative effects of water deficit through better water loss control

Following our third hypothesis, the leaf level, the physiological role of K on leaf maize water use showed that the maximum dose used in our experiment (K2) reduced the impact of water deficit (W- plants). These plants responded to a K input by reducing their stomatal conductance (Fig. IV.1b). For example, the hourly mean ET calculated at the leaf level showed a 17% reduction on K2 plants for the whole cycle, but during the daytime only. This reduction was accompanied by a proportionally similar reduction in photosynthesis activity. There did not seem either to be any additional reducing element, like ROS, or an excess of sugars, which would have impacted the photosynthesis via retroactive mechanisms (Cakmak, 2005). A study on sunflowers (Arquero et al., 2006) showed similar results on stomatal conductance. In addition to this osmoregulation process, K2 plants showed a significantly greater leaf rolling response (P < 0.01), which reduced water losses (Kadioglu and Terzi, 2007) and photosynthesis. Although a 10% variation in leaf width was measured during the eight hours of testing (Fig. IV.3), other results from Premachandra et al., (1993) on maize, indicated a different pattern: the rolling was greater on plants under K deficiency, due to the lower turgor pressure. This apparent disparity could be explained by differences both in experimental protocols and in the cultivars considered.

At the plot level, the hourly mean ET (mL m^{-2} soil) was slightly higher, due to higher LAI for K2 treatments. This increase would have been even worse without the positive effects of K fertilization in mitigating water deficit. The large proportion of transpiration during the night

period seem surprising, but this phenomenon has already been observed (Rogiers et al., 2009). Unfortunately, in maize, there are no such studies on night-time transpiration in field conditions. The high level of water loss, which is even stronger on W- plants, may be explained by cuticular evaporation, and/or by a difficulty to close stomata, as shown during pre-dawn gas exchange measurements. K had no effect on night losses at the plot level, and only reduced by a small percentage the night losses measured at the leaf scale.

More generally, everything happened as though the presence of K held the key to the isohydric regulation of the plants. Isohydric plants maintain leaf water potential at a constant level regardless of soil moisture by decreasing stomatal conductance, thus limiting transpiration under drought. The water deficit was simply a constraint for the K2 plants (*i.e.* minor decrease of water content), but represented a real stress for K0.5 plants (reduced water content, yield, pollination and grain filling). So far, we do not have sufficient results allowing us to determine optimum K nutrition input. Greater fertilization (K2 *vs* K1) did not significantly increase either leaf area, or the WUE above 30 mg kg⁻¹ of exchangeable K (K1 level). However, regardless of water conditions, K fertilization increased crop yield, leading to a more efficient use of the other resources (Christina et al., 2015; Rutkowska et al., 2014).

5. Working pattern of a plant under water deficit according to the potassium nutrition it receives

The classical response of plants to water deficit (Hsiao et al., 1976) is to reduce water loss, by reducing leaf area, stomatal conductance and plant height (Allen et al., 1998), as well as nocturnal transpiration, by enhancing root prospection and by avoiding dehydration from osmotic adjustment. This general pattern constituted a good framework to illustrate the effect of K nutrition on water-stressed plants (Fig. IV.5). However, although K nutrition can be used to tackle water deficit symptoms, it should be remembered that whereas it allows better growth (greater leaf area), it also regulates gas exchanges. The maize crop under water deficit benefited from K input as it reduced stomatal conductance, increased leaf rolling, development speed and osmotic adjustment (linked to the water content). However, K fertilization also allowed better leaf growth, which can potentially lead to emptying the soil water reserve more rapidly. The K effect on all other parameters influencing water loss (plant height, final leaf number and life-time), was only slight (Table IV.1). On the whole, these results were in favor of K inputs in order to mitigate the effect of summer drought on maize
yield, with the advantages outweighing any possible drawbacks. If an experiment had been conducted without irrigation, the bigger evapotranspiration surface would have been lethal to maize, especially because of nocturnal and uncontrollable water loss. Could summer crops be grown, when half the water they need is lacking? In such a case, even, mineral nutrition would prove quite inadequate.



Fig. IV.5 Conceptual model of plants response to potassium nutrition under water deficit. Dark and white arrows represent the effects of water deficit and optimal potassium nutrition, respectively. Up arrows mean that the factor is enhanced, and vice versa. Crossed arrows mean that the potassium effect has not been clearly established. The well-known but non-measured effects of potassium are represented in the dotted box.

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Chapitre V.

Synthèse et Conclusion

I. <u>Contextualisation</u>

L'objectif général de la thèse a été d'étudier les effets croisés de la nutrition potassique et du déficit hydrique sur le maïs. Sur le plan expérimental, les résultats présentés dans cette thèse ont été obtenus à travers plusieurs expérimentations. L'approche en serre, destinée à acquérir des données sur des mécanismes ou des variables nécessitant un contrôle climatique et/ou relevant de méthodes difficilement applicables au champ. Les variables classiques de développement et de croissance ont été mesurées, ainsi que celles de l'architecture racinaire, de même pour des mesures d'échanges gazeux et des variables liées au métabolisme carboné (teneurs en sucres, exportation des sucres). L'approche en conditions réelles, au champ, a eu pour objectif de valider les résultats obtenus en serre et d'étudier la réponse physiologique de cette même variété de maïs sur un cycle complet du semis jusqu'à la mesure d'un rendement-grain. Les mesures au champ ont été effectuées en 2014 et 2015. Compte tenu de l'absence d'épisode de déficit hydrique en 2014, seuls les résultats de 2015 ont été présentés.

II. Questionnements méthodologiques

1. Influence du traitement K sur la teneur en eau du sol

La conséquence directe d'une carence en K est la diminution de la surface foliaire. Comme attendu, dans chacune des expérimentations, les plantes carencées en K présentaient donc une plus petite surface évaporante. De ce fait, la baisse de transpiration a ralenti la diminution de la teneur en eau du sol, induisant un déficit hydrique moins élevé, en terme d'intensité, mais aussi moins long, en terme de durée, comparé aux plantes bien alimentées en K. Ce biais a été rencontré au champ, où les conditions d'irrigation ne tenaient pas compte des traitements K (Fig. A1.1, A1.2). Il a aussi été observé en serre où l'ajustement quotidien de l'arrosage, en fonction des traitements, n'était pas assez fréquent, surtout aux stades avancés, pour éviter des différences de teneurs en eau pour une même modalité hydrique. Il aurait été possible de palier ce biais en ayant recours à une culture hydroponique. Avec cette technique le stress hydrique aurait été induit par l'ajout de PEG, maintenant ainsi le potentiel hydrique constant, quelles que soient les surfaces foliaires. Cette méthode a été utilisée avec succès par Jákli et al. (2016).

2. Risques de confinement racinaire, en pot, et de réactions induites incontrôlées

Lors des expérimentations en serre, la taille des pots dans lesquels ont poussé les plants de maïs, ont entraîné un confinement racinaire dont les conséquences sur la croissance n'ont pas été évaluées. Il est cependant possible qu'un tel confinement racinaire modifie la croissance de la plante (Inglese and Pace, 2000), par l'induction d'un signal de stress, analogue à celui d'un stress hydrique (Hurley and Rowarth, 1999; Ternesi et al., 1994). C'est une des raisons pour laquelle la plupart des mesures qui ont été effectuées lors des expérimentations en serre se sont concentrées sur des stades jeunes (entre feuilles 10 et 12, sur un total de 20 feuilles).

3. Non représentativité des feuilles du haut, en terme de fonctionnement photosynthétique, par rapport au fonctionnement moyen de la plante

La plupart des mesures d'échanges gazeux a été réalisé sur de jeunes feuilles, situées, pour le maïs, au sommet de la plante. En général, la dernière feuille ligulée (croissance juste terminée) était désignée pour la mesure des échanges gazeux. De cette manière, les feuilles mesurées avaient toutes le même statut, quel que soit le stade de développement de la plante. Le choix d'un rang de feuilles fixes aurait provoqué des mesures sur des feuilles d'âges différents, donc peu comparables. La position haute nous a semblé également plus pertinente du fait que le rayonnement solaire intercepté y est maximum. De plus, des mesures sur des feuilles présentant des symptômes de carences voulaient être évitées. Il s'est cependant avéré que l'étage foliaire supérieur n'était pas représentatif de la teneur moyenne en K dans les plantes non ou peu fertilisées en K (Fig. A2). Dans les traitements carencés en K, la teneur en celui-ci a été plus élevée dans les feuilles du haut, en raison de sa forte mobilité. Ce phénomène, bien connu (Marschner, 1995; Mengel and Arneke, 1982), ne permet toutefois pas aux feuilles du haut des plantes carencées, d'avoir les mêmes concentrations en K que leurs homologues non carencées. Mais, si l'on raisonne en termes de seuils de réponse à la carence en K (Leigh and Jones, 1984), force est d'admettre que ces feuilles ne seront pas affectées de la même manière que celles présentes sur des étages foliaires inférieurs.

III. <u>Synthèse et conclusion : Impacts du potassium sur le fonctionnement</u> <u>du maïs soumis à un déficit hydrique</u>

1. Impacts du potassium sur le métabolisme du carbone

a. Impact du potassium sur les échanges gazeux

Les résultats issus d'une dynamique journalière d'échanges gazeux ont permis de mettre en évidence une tendance positive du K dans la fermeture stomatique lors d'un stress hydrique (Fig. IV.1), permettant au maïs de mieux réguler ses pertes en eau. Un résultat similaire a été montré par Arquero et al. (2006) sur l'olivier. Dans ces conditions, le K serait en mesure d'influencer la stratégie fonctionnelle du maïs adoptée face au déficit hydrique. En effet, le maïs est connu pour être une espèce isohydrique (maintien du potentiel hydrique proche de zéro en réduisant la transpiration et la conductance stomatique); or en condition de déficience en K, son comportement s'apparente à une espèce anisohydrique, puisqu'il n'y a pas de déclin de la conductance stomatique et de la transpiration. Cependant, même dans ces conditions, le potentiel hydrique ne semblait pas être affecté par l'effet de la limitation en K. La maîtrise des pertes en eau (par unité de surface), grâce au K, peut aussi être appuyée par un bilan réalisé à partir de sondes d'humidité (Fig. IV.3).

L'influence du K sur la fermeture stomatique (dynamiques journalières) n'a cependant jamais été re-confirmée lors de mesures instantanées réalisées avec des paramètres fixés (sous fort éclairement et faible VPD). Dans cette série de résultats basés sur des mesures en serre et au champ (>100 mesures), la photosynthèse ne montrait pas de sensibilité à la nutrition K, ni aux teneurs en eau du sol (Fig. A3.1b). Pourtant, les études traitant de l'impact d'un stress potassique font état d'une diminution de photosynthèse (Battie-Laclau et al., 2014a; Egilla et al., 2005), y compris sur maïs (Peaslee and Moss, 1968b). A l'inverse, d'autres études ne montrent un effet positif du K sur la photosynthèse, qu'en conditions hydriques limitantes (Sen Gupta et al., 1989; Tsonev et al., 2011).

Pour l'ensemble des mesures réalisées en serre comme au champ, en conditions fixes ou suivant le climat ambiant, aucune relation n'a jamais été démontrée entre la nutrition en K et la valeur de l'efficience d'utilisation de l'eau intrinsèque (WUE_i) (Fig. IV.1d, Fig. A3.1a et Fig. A3.3). L'effet de la teneur en eau sur cet indicateur est difficilement mis en évidence. On pourrait conclure de cette non-réponse qu'il n'y a pas d'effet du K sur la fermeture stomatique,

au moins dans les gammes des teneurs en K des feuilles mesurées [2-27 mg g⁻¹]. Des diminutions parallèles de g_s et de A_n , entraînant une constance de réponse du WUE_i, ne pourraient s'expliquer que par l'existence d'un autre facteur de contrôle commun, par exemple lié à une modification de morphologie des feuilles (Battie-Laclau et al., 2014a; Zhao et al., 2001) ou de teneurs en sucres totaux (Cakmak et al., 1994), entraînant une conductivité non pas stomatique mais du mésophile différente. Sur maïs, une telle hypothèse reste à valider. L'absence de réponse de la photosynthèse au statut K sur les feuilles du haut peut s'expliquer

par la non-représentativité des feuilles par rapport au statut moyen de K dans plante (Fig. A2).

Un moyen de s'affranchir de cette non-représentativité a consisté à utiliser un drone muni d'une caméra infra-rouge au-dessus du couvert végétal, pendant une période de stress hydrique modéré, à trois moments de la journée. Des mesures d'imagerie thermique ont été effectuées dans le but de comparer des températures de feuilles entre les différents traitements (Fig. A4 et Table A4). Ces mesures ont été utilisées pour évaluer la conductance stomatique, *via* la transpiration du couvert. Les résultats montrent, sur les parcelles irriguées, des températures en moyenne inférieures de 1°C, par rapport aux parcelles non irriguées. L'effet K se manifeste surtout au midi-solaire, avec une température foliaire plus élevée chez les plantes déficientes en K, ce qui peut être traduit par une meilleure fermeture stomatique.

b. Impact du potassium sur la mobilité des sucres

La carence en K a provoqué une accumulation de sucres totaux, au champ (Fig. A5) comme en serre (Fig. II.3). Ce constat a été observé à maintes reprises (Battie-Laclau et al., 2014a; Gerardeaux et al., 2009; Lemoine et al., 2013; Pettigrew, 1999), y compris sur le maïs (Pretorius et al., 1999). Le saccharose est présent en grande quantité dans nos études et le rôle joué par le K sur le chargement du phloème en saccharose porte une responsabilité majeure dans la croissance des plantes (Cakmak, 2005). Dans des conditions de stress hydrique, l'accumulation des sucres au niveau des feuilles est une signature connue (Hoffmann, 2010), et a bien été mesurée au cours des expérimentations au champ (Fig. A5) et en serre (Table II.3). Dès lors, le rôle du K montre son importance sur des plantes stressées en eau, celui-ci permettant aux sucres accumulés de rejoindre les zones puits, en limitant ainsi les risques d'accumulation de ROS et de rétro-inhibition de la photosynthèse. Toutefois, les résultats obtenus à partir des mesures de δ^{13} C, ne montrent globalement aucun effet ni du K, ni de l'eau sur la capacité d'exportation du carbone (α) (Fig. A3.2). La mise en évidence de cette contradiction des résultats, peut être liée à des fréquences d'échantillonnages non concordantes entre les mesures de sucres et celles du δ^{13} C. La périodicité de mesures du δ^{13} C dans les feuilles (inter-journalière) n'apparaissait pas discriminante, à l'inverse de celle des sucres (infra-journalière).

2. Impacts du potassium sur la croissance et le développement de l'échelle de la feuille à celle du peuplement

a. Impact du potassium sur la turgescence et la croissance des cellules

A l'échelle de la feuille, l'effet de la nutrition K s'est fait ressentir sur la vitesse d'allongement foliaire (LER), en conditions contrôlées (Chapitre III) comme au champ (Chapitre IV). La forte concentration en K dans les vacuoles explique son rôle *a priori* primordial dans le maintien du potentiel osmotique des cellules, et donc dans l'allongement foliaire (Mengel and Arneke, 1982). La nutrition K tient donc une place particulièrement importante dans la croissance des plantes placées en conditions de déficit hydrique, qui ont du mal à maintenir leur pression de turgescence (Hsiao et al., 1976). Cette compensation du déficit hydrique par le K a été observée en serre (Fig. III.3a). En revanche, au champ, l'effet positif du K sur le LER ne s'est exprimé qu'en condition hydrique non limitante (Table IV.1).

L'effet du K sur le LER, quel que soit le statut hydrique de la plante, n'est pas expliqué par une accumulation des sucres, ni par une diminution de la photosynthèse. La diminution de la croissance foliaire peut, dès lors, être expliquée par la différence de teneur en eau des feuilles (LWC), directement corrélée à la teneur en K des feuilles (LKC) (Fig. A3.1c,d) La diminution du LWC, lié à la contrainte hydrique, peut être maintenue à des valeurs suffisantes et compatibles avec la croissance foliaire à l'aide du K. Des mesures portant sur les différentes composantes du potentiel hydrique ont été réalisées, dans le but de quantifier la part du K dans le maintien ou non de la pression de turgescence. Au champ, des mesures de potentiels hydriques ont été réalisées en parallèle à des mesures de potentiels osmotiques (Fig. A6), à l'instar de résultats probants publiés par Van Volkenburgh et Boyer (1985), les résultats de pressions de turgescence ont donné des valeurs aberrantes (négatives) non exploitables.

b. Impact du potassium sur la surface foliaire

L'effet positif du K sur les surfaces foliaires, bien connu en conditions hydriques non limitantes (Andersen et al., 1992b; Jordan-Meille and Pellerin, 2008; Lindhauer, 1985) s'est également bien exprimé dans les conditions de déficit hydrique (Chapitre II, III, IV), conformément à ce qui a été mesuré dans des recherches analogues (Battie-Laclau et al., 2016; Eakes et al., 1991; Egilla et al., 2001). Dans le détail, l'absence d'interactions entre les effets K et ceux de l'eau sur les surfaces foliaires (Table II.1, Fig. III.2, Table IV.1) signifie que les effets positifs du K compensent les effets négatifs du déficit hydrique (Fig. III.6). C'est dans cette situation que le rôle du K dans la tolérance au stress hydrique prend toute son importance. En d'autres termes, la croissance foliaire apparaît comme l'étape qui bénéficie le plus de la présence de K en conditions de stress hydrique. Concernant la sénescence des feuilles, l'effet attendu du K sur une meilleure longévité (Battie-Laclau et al., 2013) a été minime (Fig. III.3b), du fait d'une mortalité globalement faible des feuilles les plus âgées, en serre comme au champ, y compris en conditions de stress hydrique. Ce résultat peut être mis en relation avec le faible taux de symptômes visuels de carences sur les feuilles âgées, malgré des teneurs en K très faibles (Fig. A2). Cette "résistance" au vieillissement des feuilles carencées en K peut aussi être mise en parallèle avec le faible impact de la carence en K sur les accumulations de sucres, et par déduction, des radicaux libres (ROS). Un des rôles connu du K est d'éliminer l'énergie en excès qui s'accumule au niveau des feuilles (Cakmak, 2005). C'est une piste qui n'a pas été explorée pendant la thèse.

c. Impact du potassium sur la biomasse des plantes et le rendement à l'échelle du peuplement

A l'échelle de la plante, les biomasses répondent aux modalités K et eau selon le même gradient que les surfaces foliaires, avec comme facteur limitant principal l'eau, et comme facteur limitant secondaire le K (Chapitre II, III, IV). Ces réponses concordent avec d'autres études croisant ces deux types de stress (Andersen et al., 1992b; Teixeira and Dezordi, 2008). Le résultat d'une moindre biomasse en cas de carence K s'explique *a priori* plus par la diminution de surface foliaire que du fait d'une diminution de photosynthèse.

L'impact de la nutrition en K sur la croissance racinaire, estimé à travers le ratio "R/S", n'a pas montré de tendance nette (Table III.2, 3 et Table A7), alors qu'une réponse positive du K était

attendue (Egilla et al., 2001; Ericsson, 1995). Concernant un éventuel impact de la nutrition en K sur l'architecture et la morphologie racinaire (densité de racines latérales, diamètres moyens, nombre de racines par phytomères), il a été assez difficile de conclure à un effet significatif du K (Jordan-Meille and Pellerin, 2008), et à un schéma simple de fonctionnement (Fig. III.4, 5). Nos outils de mesure n'avaient pas la précision suffisante pour travailler à l'échelle des poils absorbants, et les résultats de Hogh-Jensen and Pedersen (2003) n'ont pu être reproduits.

A l'échelle du peuplement, les rendements-grains obtenus lors de l'expérimentation au champ confirment l'effet positif de la nutrition K sur la production de biomasse, en particulier même lorsque l'apport en eau est diminué de 30% (Fig. IV.4 et Fig. A3.3). La diminution globale de rendement sous stress croisés K et eau est imputable, pour 50%, à un défaut de fécondation, et pour 50% au taux de remplissage des grains. Le déficit hydrique a touché relativement plus l'étape de fécondation, alors que la carence en K s'est plus exprimée sur l'étape de remplissage des grains.

d. Impact du potassium sur l'efficience d'utilisation de l'eau à l'échelle du peuplement

L'étude au champ a montré que l'efficience d'utilisation de l'eau pour le rendement-grains (WUE) tend à augmenter avec la nutrition K et sous contrainte hydrique (Fig. IV.4 et Fig. A3.3). Cette augmentation résulte presque exclusivement de la production de biomasse en grains, les quantités d'eau consommées entre les différents traitements K étant sensiblement identiques. Or, les plantes bien alimentées en K ont une surface foliaire supérieure qui induit des pertes en eau potentiellement plus importantes. La meilleure efficience d'utilisation de l'eau en condition hydrique limitante peut donc être expliquée par les deux mécanismes suivants (non exclusifs) :

 a) L'augmentation de surface foliaire entraîne une consommation plus rapide du stock d'eau présent (Fig. A1.1, A1.2), et laisse les plantes en situation de stress hydrique sur une période plus longue. Or un stress hydrique renforcé entraîne une augmentation du WUE (Boyer, 1982). Cette hypothèse n'est cependant pas soutenue par les résultats de potentiels hydriques mesurés au cours de la dynamique journalière (Fig. IV.1c). b) La présence de K permet une meilleure régulation stomatique (Fischer and Hsiao, 1968). Cette hypothèse a été confirmée par la dynamique d'échanges gazeux (Fig. IV.1b), contrairement aux mesures instantanées (conditions fixées) qui ne montrent aucune influence du K sur g_s (données non montrées).

Les résultats obtenus sur le WUE ne convergent pas avec ceux issus des mesures d'efficience d'utilisation intrinsèque (WUE_i). Cette différence a été soulevée récemment et expliquée notamment par une conductance stomatique nocturne qui serait plus faible en présence de K (Battie-Laclau et al., 2016; Jákli et al., 2016). Une autre cause possible réside dans la mauvaise représentativité des feuilles sur lesquelles ont été effectuées les mesures d'échanges gazeux. A l'automne 2015, une expérimentation a donc été mise en place, au sein de l' "Institute of Applied Plant Nutrition" (Université de Göttingen), portant sur "l'influence du potassium sur l'efficience d'utilisation de l'eau journalier et de la respiration nocturne du tournesol". Les données de cette expérimentation sont en cours d'analyse.

IV. Conclusion

* Le K agit sur le statut hydrique de la plante stressée en eau en maintenant une vitesse d'élongation foliaire, mais cet effet n'allonge pas la durée de vie des feuilles.

* Le K permet aussi de diminuer les pertes en eau par unité de surface, mais ce phénomène n'est pas seulement expliqué par une meilleure régulation stomatique.

* La nutrition potassique n'a montré aucune implication dans l'augmentation du WUE_i malgré un effet positif observé sur le WUE.

* Le K augmente le WUE malgré une absence d'effet au niveau du WUE_i.

* Le K n'a montré aucune action améliorant le métabolisme photosynthétique de la plante stressée en eau.

*En condition hydrique limitante, l'effet du K favorisant l'exportation des sucres vers les organes puits n'a pas été montré.

* Le K permet au maïs stressé en eau d'améliorer son rendement, grâce à son effet positif sur le poids de 1000 grains.

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Fig. A1.1 Changes in soil water content (SWC) in K0.5 and K2 treatments based on CS616 probes measurements on the whole layer (20-60cm).



Fig. A1.2 Estimation of soil water potential (MPa) in K0.5 and K2 treatments according to a function of the soil saturation degree and from the water retention model formulated by Van Genuchten (1980).



Fig. A2. Leaf potassium content (LKC) in maize according to leaf position (upper leaves (U), middle leaves (M) and lower leaves (L) for well-watered (W+) and water-stressed (W-) under three potassium levels (low (K0.5), normal (K1) and high (K2) a) at six visible leaves stage and b) at flowering stage.





Fig. A3. 1 a) Intrinsic water use efficiency (WUE_i, μ mol CO₂ mmol H₂O m⁻² s⁻¹), b) net carbon assimilation (A_n , μ mol CO₂ m⁻² s⁻¹) c) leaf elongation rate (LER, cm dd⁻¹) d) leaf water content (LWC, %) in function of leaf potassium content (LKC) in mg g⁻¹ for maize in greenhouse and field experiment



Fig. A3. 2 Leaf export carbon coefficient (α) in function of leaf potassium content (LKC) in mg g⁻¹ for maize in greenhouse experiment



Fig. A3. 3 Maize yield (t ha⁻¹) and water use efficiency (WUE, $g_{DM} \Gamma^1$)in function of leaf potassium content (LKC) in mg g⁻¹ in field experiment



Fig. A4. Infrared picture of field experiment using drone.

Table A4. Means of leaf temperature (°C) in each plot using infrared picture.

		K0.5	K0.75	K1	K2	K4	Weffect	Keffect	WxK
8:46a.m.	W+	21.35 ± 0.04	21.43 ± 0.08	21.52 ± 0.08	21.69 ± 0.06	$21.52\pm\!\!0.05$	***	0.059	0.062
	W-	$21.82{\pm}0.06$	21.78 ± 0.15	22.10 ± 0.03	21.63 ± 0.04	$21.90\pm\!\!0.12$			
1:49p.m.	W +	26.70 ± 0.05	26.33±0.12	26.30 ± 0.07	26.25 ± 0.09	26.09 ± 0.06	***	**	0.735
	W-	27.76 ± 0.12	27.31 ± 0.10	27.29 ± 0.08	27.48 ± 0.14	27.14 ± 0.09			
3:09 p.m.	W +	26.80 ± 0.08	26.60 ± 0.05	26.22 ± 0.07	26.36±0.11	$26.40\pm\!\!0.05$	***	*	0.123
	W-	$27.50{\pm}0.14$	27.17 ± 0.14	27.18 ± 0.09	27.48 ± 0.10	27.05 ± 0.05			



Fig. A5. Daily evolution of total sugar concentration in maize leaf under two water supplies (wellwatered=W+, water-stressed=W-) and two potassium nutrition (optimal=K+, low=K-). The shades area represent the night period. Values are means and their standard errors for three plants per treatment.





Utilisation de l'osmomètre Vapro® pour la mesure du potentiel osmotique de plantes au champ. Ordres de grandeurs, cohérence.

L. JORDAN-MEILLE - E. MARTINEAU – JC DOMEC

1. Problématique de la recherche

Nous nos intéressons à l'impact de la nutrition en potassium sur la résistance des plantes au stress hydrique. Le potassium est connu pour être le cation le plus concentré dans l'eau des tissus des plantes. A ce titre, il participe massivement au potentiel osmotique des cellules, aux côtés des sucres et protéines solubles. Ce potentiel osmotique est notamment impliqué dans le régulation des échanges d'eau par les stomates, et dans la croissance des cellules, via la pression de turgescence exercée sur leurs parois. La littérature suppose l'existence d'une interaction entre les niveaux de nutrition en K et d'alimentation en eau. Nous formulons l'hypothèse que les plantes bien nourries en potassium ont une meilleure pression de turgescence que les plantes carencées en K, ce qui permet de maintenir de la croissance cellulaire lors de stress hydriques modérés, par rapport à des plantes placées en confort hydrique.

2. Dispositif expérimental et mesures physiologiques

La parcelle expérimentale de maïs comporte deux modalités hydriques (W+ : irrigation non limitante, W- : stress hydrique) et trois modalités d'alimentation en potassium (K0,5, K1 et K4). 6 plantes sont prises au hasard dans chaque modalité. Des portions de la dernière feuille ligulée de chaque plante sont prélevées, pressés et directement congelées. Des disques
foliaires sont également prélevés, et subissent la même conservation. Simultanément, des mesures de potentiels hydriques sont réalisées à l'aide d'une chambre à pression de type Scholander. Les mesures sont réalisées le 31 juillet 2014 autour du midi solaire.

Les concentrations en K dans les feuilles sont mesurées par absorption atomique suite à une extraction à l'acide nitrique. L'expression en mM repose sur la teneur en eau des plantes mesurée une semaine auparavant sur des plantes au stade floraison.

Les teneurs moyennes du sol en K_{échangeable} dans le sol et en eau sont récapitulées dans les tableaux 1&2. Les mesures de teneurs en eau ont été effectuées le 31/07 par la méthode par pesées. Les mesures en K_{ech} ont été réalisées juste avant le semis, en avril, sur l'horizon 0-75 cm.

	K0,5	K1	K4
W+	18+/- 1.28	13.8 +/- 0.49	12.3 +/- 2.28
W-	7.8+/- 1.51	5.9 +/- 0.13	7.0 +/- 0.16

<u>Tableau 1</u> : Mesures des teneurs en eau volumiques (+/- écart-type) dans le sol

	K0,5	K1	K4
W+	13.3 +/- 5.0	25.3 +/- 5.0	56.0 +/- 21.5
W-	17.3 +/- 3.8	29.3 +/- 5.0	60.0 +/- 22.9

<u>Tableau 2</u> : Mesures des teneurs en K_{éch} (+/- écart-type) en mg K. kg⁻¹ de sol selon les modalités W et K

3. Mesures des potentiels osmotiques par l'utilisation du Vapro®

Les échantillons de jus de feuilles congelés ont été mis à température ambiante 4 heures avant les mesures. Des volumes d'environ 10 μ L ont été pipetés sur les "disques échantillons" de l'osmomètre. Des mesures directes sur disques foliaires n'ayant pas donné une aussi bonne discrimination et surtout répétabilité que les mesures sur jus, nous les avons abandonnées. Les 36 mesures ont été achevées au bout d'une 1.5 heures seulement.

Les données d'osmolarité, exprimées en mmol.kg⁻¹ sont directement traduites en mmol.L⁻¹.

4. Equations utilisées pour les calculs

L'équation (1), analogue à la loi des gaz parfaits, permet de calculer le potentiel osmotique à partir de la concentration d'un soluté.

Potentiel Osmotique (MPa) = - R T (n/v) <u>équation 1</u> avec R = 0.00831, T = 273 + 25 = 298 °K et (n/v) la molarité mesurée en Mol.L⁻¹

L'équation (2) permet de relier le potentiel de turgescence aux mesures de potentiels hydriques et osmotiques.

$$\Psi_{\text{hyd}} = \Psi_{\text{P}} + \Psi_{\text{s}} \qquad \underline{\acute{equation 2}}$$

$$(<0) \quad (>0) \quad (<0)$$

Avec Ψ_{hyd} le potentiel hydrique, Ψ_P le potentiel de pression et Ψ_s le potentiel osmotique.

5. Résultats – Discussion

5.1. Mesures d'osmolarité brutes et calcul des potentiels osmotiques

Les résultats bruts d'osmolarité montrent des valeurs comprises entre 100 et 200 mM (Figure 1). Ces données sont globalement du même ordre de grandeur sur les plantes irriguées et stressées en eau. En revanche, la nutrition potassique influence significativement l'osmolarité, qui lui est proportionnelle, sur les traitements hydriquement stressés. La forte variabilité des données sur les plantes irrigués ne permet pas de mettre en valeur un effet de la nutrition en K. Cette fourchette de valeur est plutôt faible, par rapport à d'autres références connues, plus proches de 400 (Itoh *et al.* 2007) à 600 mM (Mengel et Arneke 1982).



<u>Figure 1</u> : Osmolarité (mmol / L) en fonction des traitements en potassium (K0.5 – K1 – K4) et du régime hydrique (irrigué à gauche, stressé à droite). 6 répétitions.

Les potentiels osmotiques calculés à partir de la concentration en osmoles (équation 1) évoluent de -2.5 à -5 bars (Figure 2). Une fois de plus, ces valeurs apparaissent bien moins négatives par rapport à d'autres valeurs référencées dans la littérature (Egilla *et al* 2005) qui s'abaissent en deçà de -20 bars.



<u>Figure 2</u> : Potentiel osmotique (bars) en fonction des traitements en potassium (K0.5 – K1 – K4) et du régime hydrique (irrigué à gauche, stressé à droite). 6 répétitions.

5.2. Contribution du K à l'osmolarité des feuilles de maïs

La proportionnalité entre la nutrition potassique et le potentiel hydrique est bien marquée (Figure 3) et cohérente : la nature minérale et entièrement soluble du K lui confère un rôle bien connu dans sa contribution à l'osmolarité (Hsiao et Laüchli 1986). La contribution du K à l'osmolarité totale s'élève à environ 60% pour les traitements hydriquement stressés (W-) et à 45 % pour les traitements correctement irrigués (W+) (Figure 3). Le fait que l'osmolarité augmente plus vite que la teneur en K (pente > 4) pour les traitements W- est difficile à expliquer. La relation est plus logique pour les plantes du traitement W+. Il est possible que la carence en K s'accompagne aussi d'une carence en protéines solubles, aggravant ainsi l'effet sur l'osmolarité totale.



<u>Figure 3</u> : Relation entre la concentration en K mesurée dans les feuilles et l'osmolarité mesurée avec le Vapro®. Les mesures portent sur la dernière feuille ligulée. Le trait central représente la bissectrice sur laquelle se trouveraient les

La part du K à l'osmolarité totale est un résultat assez rarement publié; la détermination exhaustive des espèces chimiques en solution est contraignante. Dans un expérience sur haricot, Mengel et Arneke (1982) ont mesurés la somme des solutés (tableau 3). La proportion de K au sein des espèces contribuant au potentiel osmotique varie de 5 à 16 % selon la richesse en K du milieu.

Solute	Older leaves		Younger leaves	
	К,	K 2	Κ1	K 2
Organic anions	129	94	116	106
Organic N	290	335	433	408
CI-	33	32	27	21***
K+	25	101***	50	135***
Mg ²⁺	21	12***	24	12***
Ca ²⁺	66	51***	47	21***
Calculated osmotic potential, M Pa	-1.27	-1.42	-1.60	1.60

<u>Tableau 3</u> : Effet d'un apport insuffisant (K1) et suffisant (K2) en potassium sur le contenu en espèces solubles (en mM)

Les proportions de K que nous obtenons semblent beaucoup trop élevées, confirmant le chiffre probablement sous estimé de l'osmolarité totale.

5.3. Le potentiel hydrique et ses composantes

Le calcul de la pression de turgescence, déduit de l'équation 2, aboutit à des valeurs négatives, du fait de valeurs de potentiels hydriques plus basses que les valeurs de potentiel osmotique (Figure 4). Ceci traduirait un état physiologique des feuilles proche du flétrissement permanant, ce qui n'était pas le cas. Une sous estimation de la grandeur absolue du potentiel osmotique est à l'origine de ce biais.



<u>Figure 4</u> : Représentation des composantes du potentiel hydrique, déduits de mesures directes (ψ h), indirectes (ψ s) ou déduites (ψ p), en fonction des traitements K et des régimes hydriques

Une décomposition des différentes composantes du potentiel hydrique est fournie par Egilla *et al.* (2005). Les valeurs de potentiel hydrique y apparaissent deux fois moins négatives que celles du potentiel osmotique, permettant à la pression de turgescence calculée de prendre des valeurs positives (tableau 4).

Tableau 4 : Effets du niveau d'alimentation en K et en eau sur les relations hydriques à midi sur Hibiscus rosa-sinensis L. sur une période de stress hydrique de 21 jours. Moyennes \pm erreur standard. $\Psi I =$ leaf water potential, $\Psi \pi =$ leaf osmotic potential, $\Psi P =$ leaf pressure potential [MPa]. K0 = 0.0 mM K, K2.5 = 2.5 mM K, K10 = 10 mM K.

Time	[d] K supply	Ψ_1	Ψπ	Ψ_{p}
0	K ₀	-0.62±0.08	-1.99±0.04	1.37±0.09
	K2.5	-0.43±0.04	-1.87 ± 0.09	1.44±0.10
	K10	-0.48±0.05	-2.18 ± 0.22	1.70±0.22
21	K ₀	-1.50±0.04	-2.10 ± 0.14	0.59±0.13
	K2.5	-1.52 ± 0.02	-2.23±0.08	0.71±0.09
	K10	-1.61 ± 0.04	-2.37±0.11	0.76±0.13

6. Conclusions sur la fiabilité des mesures d'osmolarité

Les valeurs d'osmolarité mesurés par l'osmomètre à tension de vapeur Vapro® donnent des valeurs cohérentes, relativement aux traitements en potassium, mais trop faibles en absolu, que ce soit par rapport à la littérature ou aux mesures de potentiels hydriques réalisées sur les mêmes plantes. Les valeurs auraient dû être au moins 4 fois plus élevées. L'équation utilisée pour le calcul du potentiel osmotique, à partir de l'osmolarité, n'est pas en cause, puisque ce sont les valeurs brutes d'osmolarité qui sont la cause du décalage avec la littérature. Les causes de ce décalage sont multiples, et il ne sera pas possible de les hiérarchiser, le protocole expérimental ne l'ayant pas prévu en amont : problème de précipitation lors de la congélation d'espèces chimiques qui n'auraient pas eu le temps de se rediluer au moment de la mesure, problème de conservation d'espèces ioniques peu stables telles que nitrates et ammonium, peut être congelées trop tard, problème de représentativité de l'échantillon (un seul segment de feuille), mauvaise utilisation de l'instrument (?) ... Un retour sur l'utilisation d'autres chercheurs dans le domaine de l'écophysiologie pourrait nous aider à progresser sur ces incompréhensions.

7. Références

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Annexe 7

Table A7. Means of root and shoot ratio (R/S) (n=5, ± se). Effects of water treatment (water-stressed=W-, well-watered=W+), potassium levels (low=K-, optimal=K+) and their interaction (WxK)were tested using non-parametric two-way analysis of variance (P-values are shown).

	W+		W-	
	K+	К-	K+	К-
R/S	$0.15 \pm 0.02^{\ a}$	0.16 ± 0.02^{-a}	0.25 ± 0.01 ab	0.36 ± 0.05^{b}
K effect	0.03 *			
W effect	0.00 ***			
WxK		0.	.10	

Résumé

Le potassium (K) est un élément majeur connu pour contribuer à la résistance des plantes à la sècheresse. L'étude de son influence sur la réponse physiologique du maïs (Zea mays L.) sous contrainte hydrique est essentielle pour prédire la future productivité dans un contexte de changements climatiques, en particulier de la diminution des précipitations. Des modalités d'apports en K et en eau ont été croisées et soumises à des plants de maïs, élevés en condition contrôlées ou cultivés au champ. La croissance (biomasses aériennes et racinaires, rendements en grain) ainsi que les mécanismes écophysiologiques du métabolisme carboné (photosynthèse, transport des sucres) et du statut hydrique (transpiration, conductance stomatique, potentiels hydriques) ont été étudiés. L'apport de K a contribué à l'augmentation de la croissance, le développement et le rendement grain quel que soit le régime hydrique imposé au maïs et les conditions d'expérimentation. Les résultats attendus sur la meilleure régulation stomatique en cas de déficit hydrique sont moins évidents. L'effet du stress hydrique ou de la déficience en K tendent à diminuer la photosynthèse. Cependant, ces effets ressortent plus sur les feuilles âgées que sur les feuilles jeunes. Dans ces mêmes conditions, le transport des sucres ne semble pas être un élément limitant de la croissance. Plusieurs résultats convergent pour attribuer au K un rôle dans la maîtrise des pertes en eau (par unité de surface foliaire) et sur la meilleure efficience d'utilisation de l'eau. Néanmoins, cette efficience est imputée à des meilleurs rendements, liés à une surface foliaire plus importante, et non pas à une moindre consommation de l'eau.

Mots clés : Potassium, déficit hydrique, maïs (*Zea mays* L.), croissance, conductance stomatique, transport des sucres, rendement, efficience d'utilisation de l'eau

Abstract

Potassium (K) is a major nutrient known to help plants resist drought. In the context of climate change, quantifying the role of K on maize physiological acclimation to reduced precipitations is essential to better predict future productivity. Maize (*Zea mays* L.) plants grown under controlled or field conditions were submitted to different K and water levels. Plant growth (shoot and root biomass, grain yield) as well as plant water status (transpiration, stomatal conductance, water potential) and ecophysiological mechanisms of Carbon metabolism (photosynthesis, sugar transport) were studied. Regardless of the water regime and experimental conditions, K nutrition increased growth and whole-plant development and improved grain yield. The effect of water stress on stomatal regulation was not straightforward and depended on the level of K fertilization. The effects of water or K deficit tend to decrease photosynthesis. Drought or K nutrition affected more leaf photosynthesis in old than in young leaves, and sugar transport did not seem to be a growth limiting factor. Our results demonstrated a strong effect of K on biomass production and a higher water use efficiency with less of an impact on leaf-level physiology. This better water use was mainly the consequence of the positive effect of leaf area on yield, and not due to a reduce water use.

Keywords: Potassium, drought, maize (Zea mays L.), growth, stomatal conductance, sugar transport, yield, water use efficiency

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