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Ricardo Andrés Alvarez Flores

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SCIENCES ET TECHNIQUES DU LANGUEDOC

THÈSE

Pour obtenir le grade de Doctorat

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*Ecole Doctorale :* SIBAGHE – Systèmes Intégrés en Biologie, Agronomie, Géosciences,  
Hydrosciences et Environnement

**Réponses morphologiques et architecturales du système racinaire au déficit hydrique chez des *Chenopodium* cultivés et sauvages d'Amérique andine**

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## **RÉSUMÉ : Réponses morphologiques et architecturales du système racinaire au déficit hydrique chez des *Chenopodium* cultivés et sauvages d'Amérique andine**

Le genre *Chenopodium* comprend environ 150 espèces réparties sur l'ensemble du globe et établies dans une large gamme de milieux. En Amérique du Sud, différentes espèces, cultivées comme *C. quinoa* Willd. et *C. pallidicaule* Aellen, ou sauvages comme *C. hircinum* Schrader, sont distribuées sur des gradients pédoclimatiques allant du niveau de la mer au Chili, jusqu'à plus de 4000 m d'altitude sur l'altiplano boliviano-péruvien, sur des sols plus ou moins profonds et riches en nutriments, et sous des climats allant du tropical humide jusqu'au froid aride. Ces espèces sont phylogénétiquement apparentées, et on admet généralement que *C. quinoa* a été domestiqué à partir de *C. hircinum* et qu'une partie de son génome proviendrait de *C. pallidicaule*. Leur large distribution dans des écosystèmes naturels ou agricoles et leur plus ou moins grande tolérance aux contraintes du milieu, font de ce groupe d'espèces un modèle intéressant pour examiner la diversité des réponses des plantes, notamment face à la faible disponibilité en eau dans le sol. La totalité de l'eau nécessaire à la vie de ces plantes passant par le système racinaire, nous nous sommes intéressés aux variations intra- et interspécifiques de l'architecture et de la croissance des racines et à leurs réponses au déficit hydrique, en faisant l'hypothèse que les plantes provenant d'un milieu aride ou d'un système de culture à faible usage d'intrants, ont développé des traits racinaires qui leur permettent d'accroître l'acquisition des ressources en eau du sol. Pour tester cette hypothèse nous avons comparé la croissance et le développement racinaire de plantes de deux écotypes de *C. quinoa* de régions plus ou moins arides, et de populations de *C. pallidicaule* et de *C. hircinum*, placées dans des conditions de culture contrôlées non limitantes ou déficitaires en eau, en pots ou en rhizotrons. Les principaux résultats de ce travail de thèse montrent que, malgré de grandes différences dans la production de biomasse et la morphologie aérienne, les populations étudiées présentent toutes la même typologie racinaire. Elles diffèrent entre elles par plusieurs traits d'architecture et de morphologie racinaire qui déterminent la capacité d'exploration et d'exploitation des ressources du sol. Certains de ces traits, comme la vitesse d'élongation de la racine principale, présentent une grande plasticité de réponse au déficit hydrique. D'autres traits, comme la longueur spécifique des racines, sont moins plastiques mais présentent des différences interspécifiques importantes. Ces variations de l'architecture des plantes forment des syndromes adaptatifs favorisant la survie des plantes dans les milieux les plus contraignants.

**Mots clés :** *Chenopodium quinoa*, *Chenopodium hircinum*, *Chenopodium pallidicaule*, système racinaire, architecture racinaire, topologie racinaire, ontogénie, rhizotron, élongation racinaire, analyses de croissance, espèces cultivées, espèces sauvages, croissance racinaire, morphologie racinaire.



**ABSTRACT: Morphological and architectural responses of the root system to water deficit in cultivated and wild *Chenopodium* cultivés from Andean America**

The genus *Chenopodium* comprises about 150 species distributed all around the world and over a wide range of environments. In South America, different species, either cultivated as *C. quinoa* Willd. and *C. pallidicaule* Aellen, or wild as *C. hircinum* Schrader, are distributed over pedoclimatic gradients from the sea level in Chile, up to an altitude of 4000 m in the altiplano of Bolivia and Peru, on soils more or less thick and rich in nutrients, and under climates from tropical humid to arid and cold. These species are phylogenetically related, and it is generally admitted that *C. quinoa* was domesticated from *C. hircinum* and that part of its genome comes from *C. pallidicaule*. Their wide distribution in natural and crop ecosystems and their more or less high tolerance to environmental constraints, make this group of species an interesting model for examining the diversity of responses of the plants, in particular facing a low availability in soil resources. As all the water necessary for plants' life passes through the root system, we focused our interest on the intra- and interspecific variations in root growth and architecture, and their responses to the water deficit, with the hypothesis that plants from arid habitats or from low-input agrosystems, developed root traits that allowed them to increase the acquisition of resources from the soil. To test this hypothesis we compared the root growth and development in plants of two ecotypes of *C. quinoa* from more or less arid regions, and of populations of *C. pallidicaule* and *C. hircinum*, placed under non-limiting or water deficit growth conditions, in pots or in rhizotrons. The main results of this research show that, despite large differences in biomass production and morphology of the aerial plant part, the studied populations showed the same root typology. They differed by several traits of root architecture and morphology which control the capacity of the plant to explore and exploit the soil resources. Some of these traits, such as the taproot elongation rate, showed a high plasticity in response to the water deficit. Other traits, like the specific root length, were less plastic but showed large interspecific differences. These variations in plant root architecture conforms adaptive syndromes that favor the plant survival in the most limiting environments.

**Key words :** *Chenopodium quinoa*, *Chenopodium hircinum*, *Chenopodium pallidicaule*, root system, root architecture, topological index, ontogeny, rhizotron, root elongation, plant growth analysis, cultivated species, wild species, root growth, root morphology.



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# INTRODUCTION GÉNÉRALE

## 1. DIVERSITÉ DES CHENOPODIUM DANS LES ANDES

### *1.1 Diversité taxonomique et domestication des chenopodium*

Le genre *Chenopodium sensu lato* comprend environ 150 espèces réparties sur l'ensemble du globe et établies dans une large gamme de milieux (Kühn 1993, Fuentes-Bazan et al. 2012). La majorité des espèces sont annuelles, se trouvent dans des environnements arides et semi-arides et supportent des sols à teneur élevée en sel. Cependant, à l'inverse de nombreuses espèces fréquentes dans les milieux arides et salés, ce genre ne comporte pas d'adaptations physiologiques (e.g. photosynthèse de type C4) ou morphologiques (e.g. anatomie foliaire de type Kranz) particulières si ce n'est la présence de vésicules sur les feuilles dont le rôle physiologique n'est pas entièrement élucidé (voir Bois et al. 2006). Plusieurs espèces sont cultivées et présentent un intérêt économique, il s'agit de *Chenopodium quinoa* Willd. (quinoa), *C. pallidicaule* Aellen (*cañahua* ou *kañiwa*) en Amérique du Sud, *C. berlandieri* Moq. (appelé *huauzontle* au Mexique) en Amérique du Nord (Simmonds, 1976). D'autres espèces ont un intérêt médicinal comme par exemple *C. ambrosioides* L. (*païco* en Bolivie et en Argentine) (MacDonald 2004).

Concernant la quinoa, sa domestication remonterait à 7000 ans environ, à partir d'un centre d'origine supposé être la région du Lac Titicaca (National Research Council 1989 ; Bruno and Whitehead 2003). Elle a ensuite été cultivée pendant des siècles par les populations indigènes du Pérou, de Bolivie, Colombie, Équateur, Argentine et Chili (Bruno and Whitehead 2003).

L'étude récente de Fuentes-Bazan et al. (2012) s'est attachée à reconstruire une phylogénie moléculaire du genre *Chenopodium*. Ces auteurs proposent *in fine* de regrouper les différentes espèces de *Chenopodium* en cinq clades différents. Les espèces du complexe phylogénétique qui serait à l'origine des populations actuelles de quinoa cultivée sont *C. hircinum*, *C. petiolare*, *C. pallidicaule* et *C. carnosolum* (Mujica et al. 2001). Depuis longtemps des hypothèses ont été avancées concernant l'origine des caractères adaptatifs de la quinoa. Wilson (1990) et Mujica et al. (2001) considèrent ainsi que *C. carnosolum* aurait donné à la quinoa cultivée sa résistance à la salinité, *C. pallidicaule* sa résistance au froid et *C. petiolare* sa morphologie générale. L'espèce la plus apparentée à la quinoa serait *C.*

*hircinum*, allotétraploïde comme elle, et possiblement formée à partir des autres espèces apparentées diploïdes (Mujica, Izquierdo et al. 2001).

### 1.2 La culture des *Chenopodium* dans les Andes

La quinoa est cultivée traditionnellement sur une large aire de distribution depuis 2° lat. N en Colombie jusqu'à 42° lat. S. au sud du Chili (Fig.1), et du niveau de la mer au Chili, jusqu'à plus de 4000 m d'altitude sur l'altiplano boliviano-péruvien sous des climats allant du froid aride jusqu'au tropical humide (Risi and Galway 1989a, Montes de Oca 1997). L'aire de répartition des cultures de *cañahua* (*C. pallidicaule*) est plus restreinte, principalement située dans l'altiplano central dans les régions périphériques du Lac Titicaca (Gade 1970, Izquierdo Fernández et al. 2001).



Fig. 1. Carte de la distribution des populations de *Chenopodium quinoa* d'Amérique andine. Modifié d'après Montes de Oca 1997.

Depuis les années 1970, une forte extension de la culture de quinoa s'est produite, notamment dans la partie sud de l'altiplano bolivien autour du Salar d'Uyuni (e.g. Vassas et al. 2008, Winkel 2011, Winkel et al. 2012). Deux caractéristiques essentielles de la quinoa ont joué un rôle primordial dans cette dynamique : sa capacité à croître dans des environnements fortement contraints (limitations climatique et édaphique) et la grande qualité nutritionnelle de ses grains ayant permis l'émergence d'un marché à l'exportation (pour une analyse détaillée de ce processus se reporter à Winkel 2011). En effet, les grains de quinoa ont une teneur élevée en protéines (entre 14 et 20% de la matière sèche) - teneur souvent supérieure à celle de la plupart de céréales -, une composition équilibrée en acides aminés avec en particulier une teneur importante en acides aminés soufrés (Ruales and Nair, 1992) et nulle présence de gluten.

La diversité des populations de quinoa cultivées ne reflèterait pas nécessairement une adaptation étroite aux conditions locales de milieu mais traduirait plutôt une certaine polyvalence écologique permettant à ces populations de rester viables malgré l'imprévisibilité spatiale et temporelle du climat andin (Del Castillo et al. 2007, Winkel et al. 2009, Garcia et al. 2007). Néanmoins, il est reconnu que les populations du nord, du centre et du sud de l'altiplano ainsi que les populations chiliennes diffèrent sur le plan génétique (Wilson 1990, Del Castillo et al. 2007) ainsi que par certains traits morphologiques tels que la forme des feuilles, la hauteur des plantes, ou le degré de ramification. A partir d'une étude détaillée de la morphologie de 295 accessions de quinoa, Risi and Galwey (1989ab) ont ainsi identifié cinq grands types de quinoas correspondant à autant de secteurs écologiques contrastés : l'écotype « *subtropical* » correspond aux vallées humides amazoniennes, à une altitude allant de 1500 à 2000 m avec une pluviométrie de 1000 à 2000 mm/an ; l'écotype de « *vallées andines* », à une altitude de 2000 à 3500 m avec des précipitations relativement modérées entre 500 à 1500 mm/an ; l'écotype « *altiplanique* » correspond aux secteurs nord (alentours du lac Titicaca) et centre de l'altiplano, entre 3800 et 4200 m d'altitude avec un niveau de précipitations variant de 400 à 800 mm/an ; l'écotype « *Salar* », appelé également de la « zone Inter-Salar » ou de l'Altiplano Sud, caractérisé par un climat aride avec des précipitations inférieures à 250 mm/an et une altitude supérieure à 3600 m ; et enfin l'écotype « *littoral* » proche du niveau de la mer avec un climat tempéré et des précipitations qui fluctuent entre 1500 et 2000 mm/an (Tapia et al. 1979, Lebonvallet 2008) (Fig. 2)

Parmi ces types, nous avons choisi d'étudier des quinoas provenant de deux groupes très distincts : le premier correspond à l'écotype « *Salar* » du sud de la Bolivie, le second à

l'écotype « littoral » du sud du Chili. Ces deux écotypes diffèrent par certaines caractéristiques morphologiques (Risi and Galwey 1989a) mais leurs différences de *traits fonctionnels* n'ont pas été examinées et leur rôle éventuel dans l'adaptation aux conditions contrastées de milieu n'est pas connu.

Les caractéristiques pédoclimatiques des régions d'origine de ces deux groupes diffèrent notablement. Ainsi au sud de l'altiplano bolivien, à près de 4000 m d'altitude, le climat est froid (plus de 250 jours de gel/an dans les zones les plus extrêmes), aride (150 à 250 mm de pluies/an en moyenne) et les sols ont généralement une texture sableuse. Les faibles intensités et fréquences des précipitations, le taux d'évapotranspiration élevé et la faible capacité de rétention en eau des sols sont des facteurs extrêmement contraignants pour le développement des cultures (Garcia et al. 2007).

Deux mille cinq cents kilomètres plus au sud, sous les hautes latitudes pluvieuses du littoral pacifique du Chili, les conditions de culture sont totalement différentes. Un climat tempéré humide (plus de 2000 mm/an de précipitations réparties tout au long de l'année), et des sols avec une teneur en matière organique et une capacité de rétention en eau élevées offrent des conditions beaucoup plus favorables à l'agriculture (Tosso 1985, Anónimo 2000).

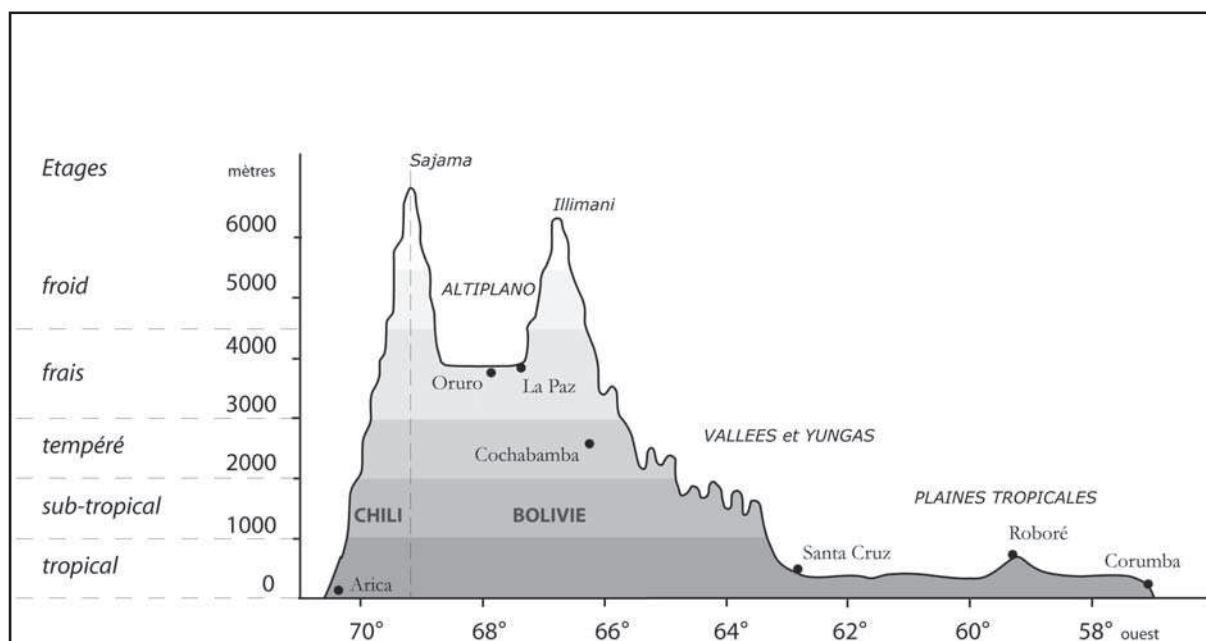


Fig. 2. Profil physiographique de la Bolivie montrant trois grandes zones écologiques : altiplano, vallées andines et littoral (d'après Montes de Oca 1997).

La diversité des écotypes de quinoa fait de cette espèce un modèle végétal de plus en plus étudié, notamment en ce qui concerne le fonctionnement de la partie aérienne dans les aspects photosynthétiques (Bertero 2001, Winkel et al. 2002, Jacobsen et al. 2005, Ruiz and Bertero 2008), hormonaux (Jacobsen et al. 2009, Gómez et al. 2011), les interactions plante-insecte (Sigsgaard et al. 2008), les réponses aux faibles températures et au gel (Monteros 2000, Bois et al. 2006, Winkel et al. 2009), l'absorption des nutriments (Razzaghi et al. 2012), et la réponse à l'irrigation temporaire (Geerts et al. 2008a,b,c).

Toutefois la connaissance du système racinaire se limite à des descriptions botaniques sommaires (Mujica et al. 2001), et à une étude sur les signaux hormonaux racinaires contrôlant le fonctionnement des stomates (Jacobsen et al. 2009), sans aucune étude de la plasticité morphologique ou architecturale des racines.

## 2. LE PARCOURS D'UN SYSTÈME RACINAIRE

### 2.1. *Système racinaire, définition et implications*

Le système racinaire est un assemblage de structures tissulaires qui, par cycles successifs de division et élongation cellulaire, forment un réseau souterrain, capable d'effectuer de multiples fonctions fondamentales (p.ex. absorption, transport, stockage, support, ancrage, exsudation). La réalisation et l'efficacité de chacune de ces fonctions sont directement liées à des facteurs *intrinsèques*, par exemple à l'espèce et/ou l'écotype, au degré de maturation des tissus (lignification ou subérisation des parois de l'endoderme) (Peterson and Enstone 1996), à l'ontogénie et au développement de ces tissus, à la formation de structures spécialisées, et à des facteurs *extrinsèques* biotiques (symbiontes fongiques et bactériens, prédation, parasitisme) et abiotiques (p.ex. structure, température, humidité et salinité du sol).

Les états initiaux du développement racinaire détermineront la formation des *primordia*, puis la formation et la croissance des racines latérales qui donneront au système racinaire la configuration spatiale lui permettant d'*explorer* et *exploiter* le sol (Malamy 2005; Hodge 2009). L'architecture et la distribution optimale de la longueur des racines sont déterminées par la pression de sélection des interactions compétitives entre les espèces et l'influence du milieu (Casper and Jackson 1997, Hill et al. 2006). Fitter (1991) souligne que toutes les composantes de l'architecture des systèmes racinaires peuvent être sensibles aux modifications de l'environnement, bien que leur topologie (disposition des racines) paraisse

moins variable que leur géométrie (longueur et diamètre des racines). Les différences de structure entre systèmes racinaires peuvent être expliquées par l'emplacement et la taille des méristèmes, ainsi que par l'âge des racines. La taille des méristèmes a une grande influence sur le contrôle postérieur de la croissance et du fonctionnement des racines adventives (Yoav and Amram 1991, Kerk et Feldman 1994, Shishkova et al. 2007). La formation de certaines structures comme les *clusters* racinaires contribuent à optimiser l'absorption de phosphore dans les sols acides (Kerley 2000, Wissuwa and Ae 2001, Neumann and Martinoia 2003).

La capacité des plantes à acquérir des ressources dans les différents horizons du sol est corrélée à la quantité et la qualité des racines produites (Hutchings and John 2004, Reader et al. 1993, Ritchie and Tanaka 1993) et varie donc selon le stade de développement et les conditions de croissance (Nicotra et al. 2002). Les espèces avec une forte capacité d'expansion racinaire peuvent accéder à des zones riches en ressources plus rapidement que les espèces avec une expansion racinaire lente (Hutchings and John 2004). Signalons que dans le système racinaire des plantes herbacées, les racines latérales contribuent à la plus grande partie de la longueur racinaire totale (p.ex. 99% in *Secale cereale*, 96% in *Oryza sativa* and 95% in *Triticum aestivum*) (Ito et al. 2006, Yamauchi et al. 1987).

La variation de la fréquence linéaire (nombre de racines latérales par unité de longueur de l'axe racinaire principal), ainsi que les différences dans la taille et l'ordre des racines, modifient également certains aspects fonctionnels, comme l'absorption des nutriments et de l'eau, et leur transport vers d'autres tissus (Pregitzer et al. 1996, Ito et al. 2006). En général, les racines de petit diamètre (< 0.5 mm) contiennent l'ensemble des segments de racines terminales qui permettront la prochaine *poussée* racinaire. Ces jeunes racines non subérisées supportent l'activité d'élongation des méristèmes.

Deux caractéristiques propres aux racines profondes, la grande proportion de tissus structuraux et conducteurs et une faible teneur en azote, reflètent leur rôle fonctionnel lié à la captation de l'eau plus qu'à l'absorption de nutriments (Pregitzer et al. 1998). L'hétérogénéité des systèmes racinaires (*heterorhizy*) est un élément important pour évaluer la capacité de réponse des systèmes racinaires à l'hétérogénéité du milieu (Pagès 1999). En effet, l'activité métabolique peut varier de manière significative entre une partie du système racinaire et une autre, certaines *classes* de racines pouvant jouer un rôle plus sensible dans la détection du déficit hydrique que d'autres (David and Zhang 1991).

### 2.2. *Expansion racinaire*

A l'intérieur du processus de croissance racinaire on distingue deux types de croissance : la croissance axiale qui est le facteur le plus important de la production biomasse du système racinaire et la croissance longitudinale associée à la capacité d'exploration. Cette croissance en longueur dépend directement de la division cellulaire et de l'extension des cellules dans le méristème apical (Pritchard 1994). Ces deux types de croissance sont étroitement associés à la taille des méristèmes apicaux qui est un élément important de la reconstitution de l'architecture des systèmes racinaires (Thaler et Pagès 1998, Pagès et al 2010).

### 2.3 *Ajustements et plasticité racinaire*

La capacité du système racinaire à réagir et/ou s'ajuster aux changements du milieu peut être reconnue comme *plasticité phénotypique* (Fitter 1991), une caractéristique étroitement liée au concept d'optimisation. Kroon et al. (2005) suggèrent que la plasticité phénotypique, en tant que réponse des méristèmes des feuilles, des branches et des racines, est déclenchée par des conditions locales de l'environnement. Un aspect essentiel de la plasticité racinaire est en effet de contribuer à la capacité de réaction face au caractère éphémère, tant spatial que temporel, des ressources hydriques et minérales du sol (Reader et al. 1993).

Plusieurs attributs et/ou traits racinaires ont été utilisés pour évaluer la plasticité racinaire : la longueur spécifique de racines (SRL) associée à une plus grande capacité d'exploration (Eissenstat 1992, Ryser 1996 ; Roumet et al 2006), ou le ratio racines/tiges (*root shoot R/S*) qui représente le rapport de biomasse dans les deux compartiments, restent les plus largement employés. Ainsi, par exemple, les espèces de milieux arides ont tendance à avoir des vaisseaux de xylème larges, un diamètre racinaire important et un faible SRL (Nicotra et al. 2002). Cette combinaison de traits (ou syndrome) associée à d'autres modifications anatomiques permettrait d'augmenter la capacité de transport ou la capacité de pénétration dans les horizons secs du sol (Nicotra et al. 2002). Cependant, l'augmentation du diamètre des vaisseaux du xylème entraîne aussi une plus grande sensibilité à la cavitation et à l'embolie pendant le stress hydrique (Pockman and Sperry 2000). Ces réponses ont un coût carboné élevé (Yoav and Amram 1991) et peuvent parfois retarder le développement des autres types de racines, comme les racines basales ou adventives (Walk et al. 2006).



### 3. ARCHITECTURE RACINAIRE

L'analyse de l'architecture racinaire s'intéresse à la configuration spatiale du système racinaire considéré comme un assemblage de sous-unités, cette information exprime les notions de hiérarchie entre les composantes de l'embranchement racinaire (Godin 2000). Les typologies racinaires ont permis dans le passé diverses interprétations géométriques, formalisées dans des modèles généraux d'architecture racinaire : le *developmental model* de Rose (1983), le *morphometric model* de Fitter (1982), le *topological model* de Fitter (1986). Les modèles architecturaux de Fitter (1987) utilisés pour décrire l'efficacité d'absorption des éléments très mobiles (azote), moyennement mobiles (potassium) et faiblement mobiles (phosphore) ont permis de montrer que l'efficacité d'acquisition des ressources minérales était influencée par l'architecture racinaire. Fitter (1991) décrit l'architecture racinaire comme un ensemble de segments (*links* dans sa description originale) et d'intersections, les segments étant eux-mêmes différenciés en segments externes et internes.

#### ***Définitions des composantes de l'architecture racinaire selon (Fitter 1991) et Fitter et al. (1991ab)***

Un segment est une section de racine entre deux noeuds ou entre un noeud et l'extrémité de la racine. On peut donc définir plusieurs types de segments selon leur position dans l'ensemble du système racinaire.

➤	<i>Type de segment</i>	Externe-Externe (EE), Externe-Interne (EI), Interne-Interne (II)
➤	<i>Taille ou magnitude</i>	Nombre total de segments externes
➤	<i>Topologie</i>	Distribution de l'ensemble des branches à l'intérieur du système racinaire
➤	<i>Longueur du segment</i>	Distance entre les points de l'embranchement
➤	<i>Angle</i>	Inclinaison entre un segment et le segment d'origine
➤	<i>Diamètre des segments</i>	

Cette description a permis de dégager deux typologies racinaires extrêmes pour le fonctionnement hydrique des plantes : le type *dichotomique* et le type *herringbone* (en arête de hareng). Théoriquement, sur un sol humide, un système racinaire de type dichotomique sera plus efficace en termes d'absorption de l'eau et de coût carboné. Au contraire, en cas de déficit hydrique prolongé, une structure de type *herringbone* permettrait d'augmenter l'efficacité du système racinaire pour extraire de l'eau en profondeur (Berntson, 1994). Mais cette typologie serait moins efficace en termes de coût de transport et de construction (Eissenstat 1997). Lynch (1995) signale qu'un système racinaire du type *herringbone*, plus efficace dans l'acquisition de ressources, serait également plus vulnérable à une attaque de prédateurs. Nielsen et al. (1994) en utilisant le modèle SimRoot ont constaté que pendant les premières 50 heures de modélisation les typologies *herringbone* et *dichotomique* avaient des réponses similaires en termes de compétition inter-racinaire.

Les plantes qui se développent dans des conditions de déficit hydrique intense ont une plus grande proportion de segments Externes-Internes, ce qui peut être quantifié au moyen de divers indices topologiques (Fig. 5). A partir de paramètres descriptifs du système racinaire tels que l'altitude  $\alpha$  (i.e nombre de segments du chemin le plus complet depuis le collet jusqu'à l'extrémité de la racine) et la magnitude  $\mu$  (nombre total d'extrémités racinaires), des indices topologiques ont été construits, généralement par régression linéaire (Fitter 1986). Ces indices permettent de différencier les typologies racinaires *idéales* de type *dichotomique* ou *herringbone*. Cependant, la sensibilité à la taille des plantes de ces indices basés sur des régressions linéaires rend leur interprétation difficile, notamment aux stades juvéniles lorsque la racine principale domine la structure d'ensemble du système racinaire (Glimskär 2000, Paula and Pausas 2011). Glimskär (2000) propose un indice topologique calculé sur le quotient de  $\alpha$  et  $\mu$ , moins sensible à l'effet de taille des plantes.

Actuellement, de nombreux travaux continuent à appliquer les indices proposés par Fitter (1986) pour différencier les typologies racinaires et caractériser les stratégies fonctionnelles d'utilisation des ressources en conditions de sécheresse (Abd Allah et al. 2010). Ces typologies servent aussi à comparer les stratégies de conservation des ressources d'espèces annuelles et pérennes (Roumet et al. 2006), en fonction des vitesses d'élongation des racines (Eissenstat 1991), ou encore pour tester l'effet de l'hétérogénéité de la distribution des racines sur l'absorption des nutriments (Johnson and Biondini 2001, Zhuang, Yu et al. 2001).

Il est intéressant de souligner que des programmes de sélection basés sur des traits racinaires ont été initiés dès les années 1990 et sont un axe privilégié des recherches menées dans le cadre de la seconde révolution verte (voir encadré).

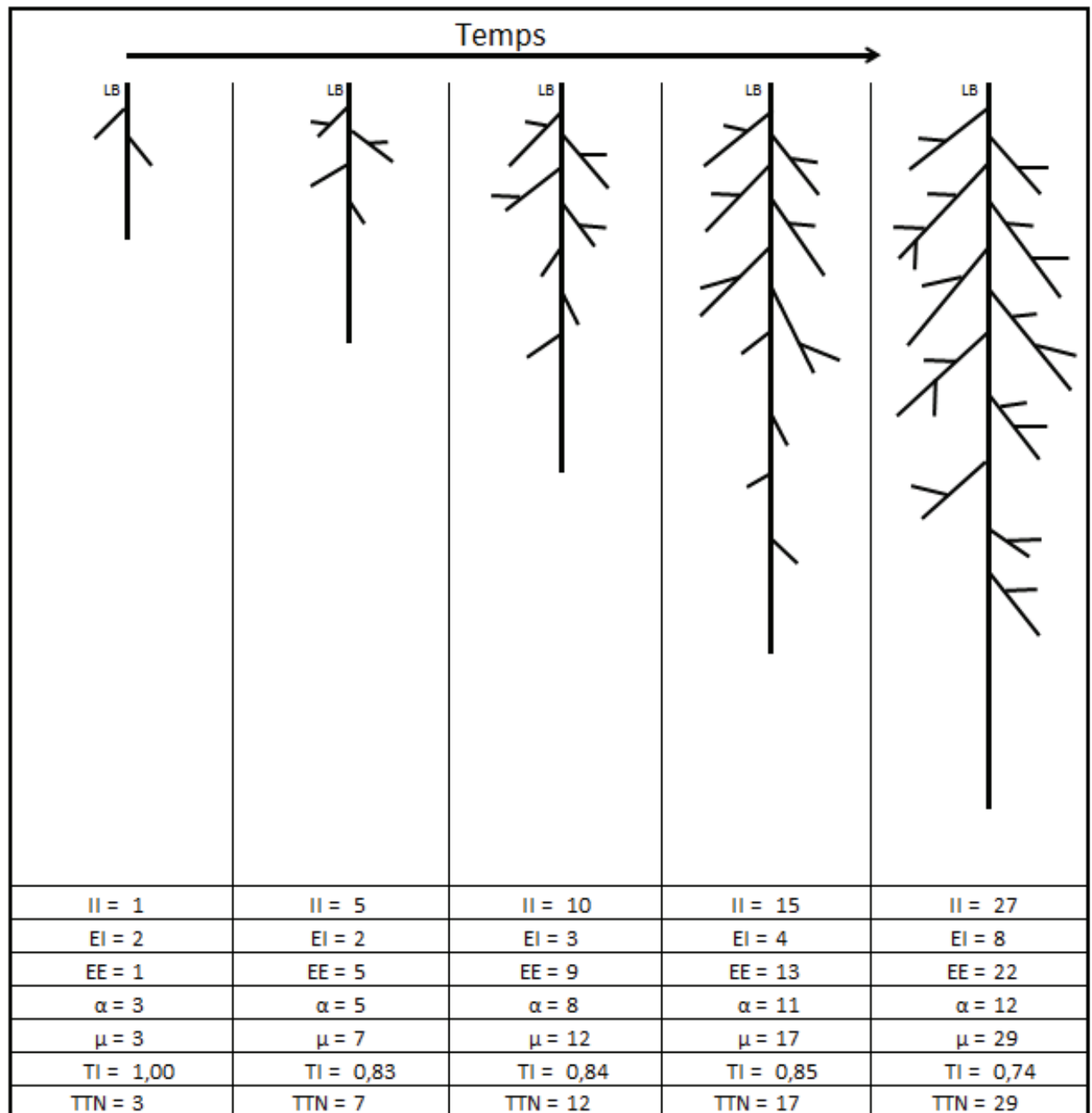


Fig. 5. Exemple de variation des paramètres architecturaux au cours du développement racinaire d'une dicotylédone. II = nombre de segments Interne-Interne; EI = nombre de segments Externe-Interne; EE = nombre de segments Externe-Externe ;  $\alpha$  = altitude;  $\mu$  = magnitude; TI (Topological Index) =  $\log\alpha/\log\mu$ ; TTN = nombre total de segments externes =  $\mu$ .

#### 4. DYSTRESS ET EUTRESS DANS LES RACINES

Très souvent dans la littérature les concepts de stress hydrique et déficit hydrique sont employés comme synonymes. Avant de commencer cette deuxième partie de l'introduction et sans entrer en détail dans la sémantique du concept du stress, il est nécessaire de clarifier ces deux concepts. Le terme de *stress* ou *contrainte* a été introduit dans la théorie de l'élasticité par Cauchy (1821), puis adopté pour la biologie par Levitt (1972) et par la suite reconceptualisé par Gordon (1992), Lichtenthaler (1996) et Kranner et al. (2010). Selon Levitt (1972), un stress biologique peut être défini comme tout facteur de l'environnement capable d'induire un préjudice potentiel pour la vie d'un organisme. Lichtenthaler (1996) le définit comme une condition défavorable, l'effet d'une substance ou encore comme un blocage du métabolisme, qui empêcherait la croissance ou le développement de la plante.

Les facteurs qui provoquent le stress peuvent être de nature biotique, comme l'attaque d'un champignon ou d'un insecte, ou abiotique, résultant de facteurs comme un déficit hydrique, des températures extrêmes, la salinité du sol ou encore la présence de polluants.

##### ***La seconde révolution verte***

*Des programmes de sélection variétale des traits racinaires ont débuté en Australie dans les années 1990 dans le but de réduire les besoins en eau des cultures et d'améliorer ainsi la durabilité agricole. Richards et Passioura (1989) ont travaillé sur les effets de la modification anatomique chez le blé en diminuant la conductance axiale des racines pour l'eau par la sélection de variétés possédant des vaisseaux du xylème de petit diamètre dans les racines séminales.*

*Actuellement, plusieurs programmes mettent en place la sélection variétale de traits racinaires pour aborder le problème « souterrain » de la durabilité de l'agriculture avec des approches multiples de modélisation, transgénèse, et hybridation.*

*La capacité d'exploration en profondeur, la longueur racinaire, ainsi que la taille ou le nombre de vaisseaux du xylème sont déterminants pour faire face aux événements de sécheresse et sont pris en compte depuis peu dans les recherches devant guider la « seconde révolution verte » (Lynch 2007).*

*Cette nouvelle approche basée sur l'efficacité d'utilisation des ressources hydriques a pour objectif de limiter l'impact de la sécheresse sur les cultures et d'atténuer l'effet des changements climatiques sur la production agricole (Ho et al. 2004, Passioura et al. 1983, Palta et al. 2011, Kondo et al. 2003, De Smet et al. 2012, Wasson et al. 2012).*

Le stress hydrique subi par les végétaux peut être aussi bien causé par un excès que par un manque d'eau. L'excès d'eau entraîne généralement une réduction de l'apport d'oxygène aux racines provoquant une diminution de la respiration, de l'absorption de nutriments et des autres fonctions racinaires importantes. Le déficit hydrique, par contre, correspond à une carence d'eau affectant le développement normal de la plante et pouvant entraîner la réduction des taux de croissance et également diminuer de manière concomitante l'activité respiratoire.

Le déficit hydrique se produit globalement quand l'absorption de l'eau par les racines est insuffisante pour satisfaire la demande évaporatrice de la plante. Les mécanismes de réponse des racines sont complexes (Kranter et al. 2010) (Fig. 3).

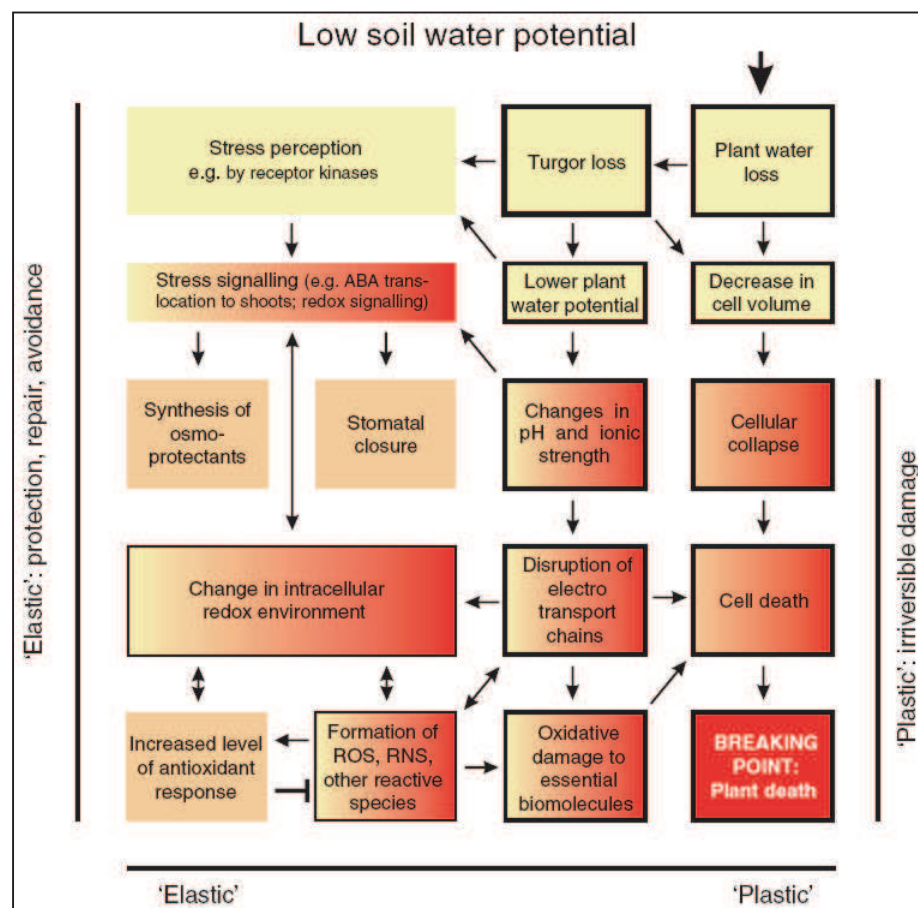


Fig. 3. The flowchart is an extremely simplified example of the intricately linked effects of a 'stress', water deprivation, to give examples of strains (bold lines around boxes) that evoke responses of the plant (no lines) and intermediate processes that have elements of strain and response (thin lines). The responses of the plant can feed back downstream and upstream into the system, leading to resistance based on protection and repair. The individual processes are also assigned the colours yellow, orange and red according to 'alarm', 'resistance' and 'exhaustion'. Two or three colours within one box indicate that the process corresponds to than one of the phases in Selye's stress concept (Kranter et al. 2010)

Le bilan tolérance / sensibilité au stress permet de déterminer si le facteur de stress a eu un effet positif (eustress) ou négatif (dystress) sur la plante (Lichtenthaler, 1996, Kranner et al. 2010). Ainsi, un déficit hydrique modéré peut entraîner une augmentation du taux d'élongation racinaire (Pritchard 1994) (situation d'eustress), mais l'aggravation de l'intensité du déficit hydrique peut ensuite ralentir ce taux d'élongation (Sharp et al. 1985), créant alors une situation de dystress dans laquelle les tissus racinaires peuvent commencer à subir des dommages à l'approche du point de flétrissement (Pritchard 1994). En-dessous de ce point, l'effet du stress devient irréversible, au point de provoquer la mort des tissus et de l'organisme. Au-dessus d'un seuil létal, l'effet du stress sera fortement dépendant de sa durée (Lichtenthaler 1996).

### *4.1 Les systèmes racinaires face au déficit hydrique*

La complexité des relations entre régime des précipitations et dynamique de l'eau dans les sols entraîne une grande variabilité de la disponibilité de la ressource hydrique disponible pour les végétaux dans les différents horizons (Fitter 1991). La saisonnalité des pluies alliée à une forte demande évaporative contribue à l'irrégularité temporelle du déficit hydrique (Vacher et al. 1994, Schwinning and Ehleringer 2001) entraînant chez certaines plantes des changements adaptatifs importants, notamment une modification de la distribution verticale des racines dans les différents horizons ou même avoir des effets délétères aux différents stades du développement de la plante (Chaves et al. 2002).

Cependant, les réponses au stress hydrique ne sont pas toujours faciles à évaluer puisque d'autres contraintes, venant se superposer, peuvent avoir des effets synergiques ou antagonistes (Chaves et al. 2002, Bengough et al. 2011). Entre ces interactions complexes, le système racinaire contrôle l'acquisition des ressources en eau et en nutriments et joue donc un rôle essentiel dans l'adaptation et la réponse des végétaux à des conditions de milieu limitantes.

Les différences architecturales et morphologiques des systèmes racinaires, génétiquement définie dans les différentes populations et espèces de plantes, contrôlent en grande partie la capacité d'acquisition des ressources du sol (Igram and Malamy 2010, Berntson 1994). Mais les systèmes racinaires peuvent aussi répondre de manière plastique et rapide à l'hétérogénéité spatiale et à l'imprévisibilité temporelle du milieu en ajustant leur capacité à coloniser le sol, leur architecture, ou encore leur vitesse de croissance. Gang et al.

(2012) ont montré que les modifications de croissance et de distribution de biomasse chez *Artemisia halodendron* dans des habitats différents ont un impact significatif sur la dynamique et la longévité racinaire. Des tentatives de modélisation destinées à reproduire la dynamique des systèmes racinaires dans les régions arides et semi arides ont montré qu'un ensemble d'ajustements de paramètres physiologiques et morphologiques des systèmes racinaires établissaient *in fine* une stratégie permettant la survie de ces végétaux (Schwinning and Ehleringer 2001).

Les stratégies employées par les plantes pour faire face au déficit hydrique font appel à des réponses d'évitement (*avoidance*) et/ou de tolérance qui varient avec les génotypes. La capacité de réponse de ces relations, en particulier pour les herbacées, est fortement liée à la rapidité d'ajustement des mécanismes d'acquisition de ressources du sol et de carbone, et ce dès les premiers signes perceptibles de stress (Chaves et al. 2002). En se fondant sur les principes physiques d'*élasticité* et de *plasticité* des parois cellulaires, Levitt (1972) a proposé quatre types de réponses possibles face aux différents stress, incluant le stress hydrique et en tout point compatibles avec les notions de dystress et eustress de Lichtenthaler (1996,1998) (Fig. 4). Dans le cas de *Chenopodium quinoa* et à partir de ce cadre conceptuel, nous pouvons définir deux types de réponses au déficit hydrique faisant appel aux mécanismes suivants :

- Tolérance : par l'élasticité des tissus, la sensibilité à la fermeture stomatique et des ajustements osmotiques, ou par la prolongation du cycle de croissance dans les stades végétatifs initiaux ou encore l'accélération de la maturation des graines dans les stades reproductifs (synchronie de la floraison) (Jacobsen et al. 2009).
- Evitement : la plante est capable de s'affranchir d'un déficit hydrique dû à une limitation des ressources hydriques des horizons superficiels par un développement racinaire profond (Geerts 2008).

### *4.2 Contraintes du milieu et réponses de la quinoa au déficit hydrique*

L'étude approfondie des régions arides et des espèces adaptées à de fortes contraintes environnementales devient un défi majeur pour la compréhension des mécanismes de réponse à la sécheresse du point de vue biologique et écologique (Wasson et al. 2012). Des observations anciennes effectuées dans des écosystèmes arides et semi-arides ont montré que certaines plantes modifiaient leur patron de colonisation des profils du sol avec des pivots pouvant pénétrer jusque 8 m de profondeur dans le cas de *Prosopis velutina* (Canon 1911).

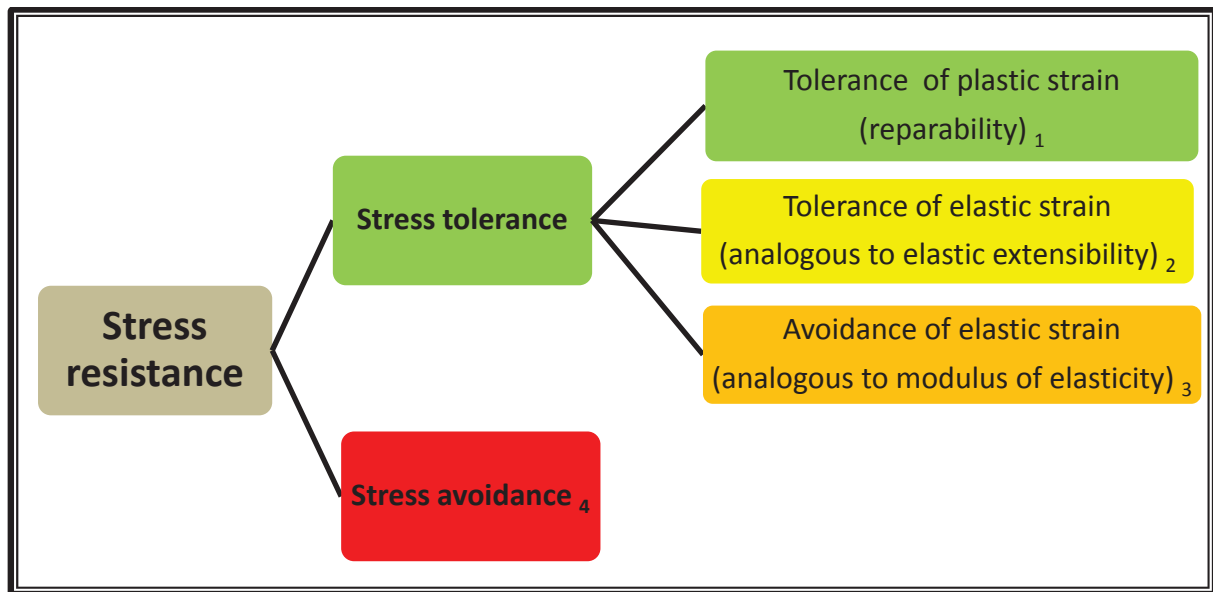


Fig. 4. Cadre conceptuel des deux composantes de la résistance au stress hydrique selon Levitt (1972) tolérance et évitement (les couleurs vert, jaune, orange, et rouge et les nombres représentent l'intensité croissante du stress hydrique)

Comme nous l'avons vu dans la première partie de l'introduction, la culture de quinoa doit affronter de fortes contraintes climatiques dans l'altiplano bolivien : faible niveau de précipitations et forte irrégularité du régime des pluies entraînant au final un nombre élevé d'événements de sécheresse (Geerts et al. 2006, Vacher 2008), forte probabilité de températures fortement négatives ( $< -5\text{ °C}$ ) en début et en fin du cycle de culture (Winkel et al. 2009), forte demande évaporative et radiation solaire extrême du fait de l'altitude (Vacher et al. 1994, Bosque et al. 2003). Les réponses physiologiques des quinoas à ces contraintes montrent une grande variabilité intra-spécifique (Geerts 2008). La relation entre conductance stomatique et potentiel hydrique des feuilles n'est pas linéaire pour les quinoas du nord de l'altiplano comme elle peut l'être pour la pomme de terre amère (*Solanum juzepczukii*) (Vacher 1998). Enfin, Bosque et al. (2003) ont noté une grande efficacité d'utilisation de l'eau et une augmentation de la relation racines / partie aérienne dans des conditions de déficit hydrique.

#### 4.3 Autres mécanismes de tolérance au stress hydrique chez la quinoa

La régulation stomatique et l'ajustement osmotique qui permet de maintenir la turgescence des tissus (Jensen et al. 2000) sont parmi les mécanismes physiologiques les plus courants de réponse au stress hydrique chez la quinoa. Le métabolisme de la proline joue un rôle



particulier : dans les tissus turgescents et en l'absence de stress, la proline s'oxyde rapidement, alors que dans le cas contraire cette oxydation est inhibée (Aguilar et al 2008) et les concentrations en proline peuvent augmenter d'un facteur 10 à 100 chez certaines plantes (Hopkins 2003). Connecté à la synthèse de sucres, ce métabolisme particulier contribue à constituer aussi une réserve d'azote pendant la récupération du stress (Bates et al. 1973, Bosque et al. 2003, Aguilar et al. 2003a,b). Aguilar et al. (2003b) ont montré que les contenus de proline peuvent être plus élevés chez les écotypes de quinoa de zones arides que chez les écotypes de zones humides.

Les vésicules accumulatrices de  $\text{Ca}^+$  et d'oxalates, appelées *bladder cells*, situées sur les deux faces des feuilles de quinoa, ont selon certains auteurs (Bosque et al 2003, Jacobsen et al. 2003) un rôle important dans la tolérance au déficit hydrique et à la salinité. Cependant, à notre connaissance, ce rôle n'a pas été validé expérimentalement. La présence de ces vésicules accumulatrices chez nombre d'espèces de chénopodes dont *C. pallidicaule* et *C. hircinum* remet en perspective l'affirmation par ces auteurs d'une adaptation spécifique de la quinoa au déficit hydrique (voir Bois et al 2006 pour une discussion sur le rôle de ces vésicules).

## 5 OBJECTIFS, HYPOTHÈSES ET PLAN DE L'ÉTUDE

Le thème central de ce travail est d'explorer la plasticité phénotypique des systèmes racinaires de quatre populations de *Chenopodium* afin d'apporter des éléments de réponse aux questions suivantes : (i) la dynamique de mise en place du système racinaire est-elle la même chez les *Chenopodium* cultivés et sauvages apparentés ? (ii) y a-t-il des différences morphologiques du système racinaire entre les populations de milieux plus ou moins arides ? (iii) quelle est la plasticité de ces systèmes racinaires en réponse à la variabilité des ressources hydriques ?

La comparaison de *Chenopodium* sauvage et cultivés provenant d'écosystèmes fortement contrastés doit permettre de tester l'hypothèse selon laquelle les plantes provenant d'un habitat à faible disponibilité de ressources dans le sol ou d'un système de culture à faible usage d'intrants ont développé des traits racinaires qui leur permettent d'augmenter l'acquisition de ressources du sol.

Ces questions ont été abordées par l'étude comparative de quatre populations de *Chenopodium*. Il s'agit de deux populations cultivées de quinoa (*C. quinoa*) de milieux contrastés de l'Altiplano Sud de Bolivie et du sud du Chili, d'une population de l'espèce

cultivée cañahua (*C. pallidicaule*) et d'une population sauvage de *C. hircinum* provenant toutes les deux de l'altiplano bolivien.

Le premier chapitre présente les résultats provenant d'une expérimentation en serre en conditions non limitantes avec une humidité édaphique maintenue pendant neuf semaines proche de la capacité au champ. Cette expérimentation a eu pour objectif de comparer les patrons de croissance, l'allocation de biomasse et la morphologie racinaire chez les quatre populations décrites.

Le deuxième chapitre traite de l'analyse ontogénétique comparative de ces quatre *Chenopodium*. Pour cela, les espèces ont été cultivées dans des rhizotrons en conditions non limitantes afin d'observer leur croissance racinaire pendant six semaines. Il s'agissait de caractériser les différences phénotypiques entre les populations étudiées en caractérisant la dynamique temporelle d'allocation de biomasse et le patron architectural de leurs systèmes racinaires.

Le troisième chapitre analyse les résultats d'une expérimentation ayant pour but de comparer la plasticité de ces systèmes racinaires en réponse à la variabilité des ressources hydriques. Il s'agissait en particulier de tester l'hypothèse selon laquelle les populations de *C. quinoa* provenant d'une région à climat très aride de l'altiplano bolivien avaient une plus grande plasticité que celles provenant d'une région tempérée humide de sud du Chili et de comparer populations cultivées et sauvages.

En conclusion, les principaux acquis de ce travail sur la réponse au déficit hydrique des systèmes racinaires des *Chenopodium* étudiés seront synthétisés en revisitant les hypothèses initiales et en ouvrant des perspectives sur l'intégration de ces acquis dans des modèles plus généraux de fonctionnement des plantes.



## CHAPITRE 1

# PLANT GROWTH DYNAMICS AND ROOT MORPHOLOGY IN WILD AND CULTIVATED *CHENOPODIUM* SPECIES FROM CONTRASTING ECOLOGICAL ORIGINS.

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### ABSTRACT

In low-resource habitats or low-input agriculture, plant morphology affects the ability to capture limited resources, a feature crucial for early growth in annual species. We compared plant growth and rooting patterns in cultivated and wild Andean chenopods to identify different plant traits and their ecological significance and human selection. Plants of two ecotypes of *Chenopodium quinoa*, one population of *C. pallidicaule*, and one population of their wild relative *C. hircinum*, grown in pots under non-limiting conditions, were used to compare biomass allocation, and shoot and root traits over nine weeks of early vegetative growth. All populations followed the same sequence of biomass allocation, whatever their geographic origin or domestication status. Shoot and root RGR showed similar maximal values in the four populations, but with high root RGR values maintained for a longer time in *C. quinoa*. Differences in plant biomass, net assimilation rate, total root length and specific root length were associated to seed mass ranking, the small-seeded *C. pallidicaule* being less productive than its relatives with larger seeds. Stem branching was at the cost at the root allocation in *C. hircinum* and *C. pallidicaule*. *C. quinoa* produced less branched stems, and main root growth was faster in the ecotype from low-resource habitat. Plant morphology and growth dynamics were different among the studied species, showing an improved capacity of exploration of deep soil layers at early growth stages in the cultivated populations from low-resource habitats.

Keywords : Biomass allocation – Cultivated species – Phenotypic variation – Plant growth analysis – Root growth – Root morphology – Wild species.

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## 1 INTRODUCTION

In the Andes, under contrasted and often extreme environmental conditions (Brush 1982), a diversity of *Chenopodium* species has flourished, some of them as spontaneous chenopods (e.g. *C. hircinum* Schrad., *C. petiolare* Kunth, *C. carnosolum* Moq.), others as domesticated crop species like quinoa (*C. quinoa* Willd.) and cañahua (*C. pallidicaule* Aellen) (Wilson 1990, Bonifacio et al. 2001, Bruno 2006). *C. hircinum* grows today in lowlands as well as highlands of Bolivia and Argentina and might be the wild progenitor of cultivated quinoa, both being tetraploid species (Mujica et al. 2001, Bonifacio 2003, Mujica and Jacobsen 2006, Pearsall 2008). Quinoa is supposed to have been domesticated in the semi-arid and cold highlands of Peru and Bolivia (ibid.). Today, this grain crop shows an ample niche diversification from cold highland deserts down to tropical intermontane valleys and temperate ocean littoral. Such diversification at a continental scale is coupled with a multiplicity of local uses for food and medicine, producing several tenths of local quinoa varieties and landraces (Bonifacio et al. 2001). As regards cañahua, it may have originated as a grain and forage crop in dry areas of extreme elevations (Gade 1970, Bonifacio 2003, Bruno 2006, Pearsall 2008). Some authors consider cañahua as a possible ancestor of *C. hircinum*, its diploid genome combining with that of *C. petiolare* or *C. carnosolum* to produce the allotetraploid progenitor of quinoa (Wilson 1990, Mujica et al. 2001). Comparative studies of plant morphology and growth dynamics can bring some insights regarding different plant traits developed under natural and human selection among these Andean chenopods. In quinoa, genetic differentiation corresponding to broad-scale ecogeographic patterns has been inferred from molecular markers (Del Castillo et al. 2007, Costa Tártara et al. 2012) as well as from morphological or agronomic traits (Rojas 2003, Bertero et al. 2004, Bhargava et al. 2007). But this information is still lacking for its relatives and, in all cases, the root traits of these Andean chenopods remain poorly known despite their crucial role for resource capture under nutrient-poor conditions and low-input agriculture.

In wild as well as in cultivated plant species, the ability to capture resources from the environment is largely controlled by the individual plant morphology (Garnier 1991, Jackson and Koch 1997, Reich et al. 1998). Both natural and human selection have produced a range of plant forms and organ allometry which result in plant growth, biomass allocation and morphological traits varying systematically among species originating from contrasted habitats (Reich et al. 1998, Ross-Ibarra et al. 2007). In seed crop species, selection for grain production has deliberately modified the aerial plant parts but also, in an unconscious way,

the root systems as found in *Oryza sativa*, *Triticum aestivum*, or *Glycine max* (Siddique et al. 1990, Gersani et al. 2001, Weiner 2004, Manschadi et al. 2008, Henry et al. 2011, Palta et al. 2011). In nutrient-poor habitats or low-input agriculture, individual plant survival and growth depend directly on these morphological root traits that allow the plants to capture limited or ephemeral resources from the soil. The corresponding plant traits not only influence the productivity of natural and agricultural ecosystems (Hooper et al. 2005, Wolfe et al. 2008), they are also considered today as useful bases for advanced organic breeding programs and new sustainable crop practices in developing as well as in industrialized countries (Baenziger et al. 2011, Wasson et al. 2012).

The present study addresses the changes in plant morphology and growth patterns in response to natural or human selection, starting with the hypothesis that plants from nutrient-poor habitats or low-input agriculture have developed traits that enhance nutrient capture, particularly by the root system. To test for this, a comparative study was conducted to investigate the plant growth and rooting patterns during the vegetative growing phase of three Andean chenopod species, from wild and cultivated populations, in order to identify contrasting plant traits and their ecological significance.

## 2 MATERIAL AND METHODS

### 2.1 Studied species and experimental design

The study was conducted in a glasshouse at the Centre d'Ecologie Fonctionnelle et Evolutive (CEFE-CNRS, Montpellier, France, 43°38'19''N, 3°51'46''E), under uniform, non-limiting growth conditions. Considering a population as a group of interbreeding individuals from a same area, four *Chenopodium* populations, from contrasting habitats and with different domestication status were compared: domesticated *C. quinoa* from temperate, rainy lowlands of nutrient-rich soils in Chile (QC), domesticated *C. quinoa* from arid and cold highlands of nutrient-poor soils in southern Bolivia (QB), domesticated *C. pallidicaule* (PA) and wild *C. hircinum* (HI) both from semi-arid highlands of nutrient-poor soils in northern Bolivia (Table 1). In each population, seeds were selected for their size homogeneity, and sterilized with sodium hypochlorite (1%) for 10 min. Planning 9 harvests with 7 replicate plants per population, seeds were sown (7 seeds/pot) in 224 pots containing 7 L of a sandy substrate (extra silica: 99 %, Fe<sub>2</sub>O<sub>3</sub>: < 0.1 %, Al<sub>2</sub>O<sub>3</sub>: < 0.4 %, K<sub>2</sub>O: < 0.2 %, CaO: < 0.1 %, pH 7.5) watered with 1.2 L/pot of a nutritive solution (N: 16%, P: 10 %, K: 24 %, MgO: 3 %, micro-nutrients: 2.5 g/L). The substrate water content was maintained near 90 % of the field

capacity during the experiment. Over the course of the experiment, air temperature varied between 10 and 29 °C (mean = 25.1 °C ± 0.6) while soil temperature varied between 15 and 29 °C. Air relative humidity was maintained between 55 and 75 %. Light intensity at midday varied between 190 and 900 W m<sup>-2</sup> (mean = 633 W m<sup>-2</sup> ± 31). Seedling emergence was fairly uniform among the four populations since *C. quinoa* and *C. pallidicaule* are non-dormant species, and *C. hircinum*, having been harvested two years before, had lost its seed dormancy. After seedling emergence, pots were thinned to one plant per pot and placed in a randomized design with a density of 16 plants per square meter.

## 2.2 Plant sampling and measurements

In each population, seven plants were harvested weekly from 7 days after sowing (DAS) until 63 DAS. Each individual plant was divided into roots, main stem, secondary stems, leaves and, when present, floral buds. Leaves were scanned at 600 dpi (CanonScan LIDE 100) and the images were analyzed with the SigmaScan Pro 5 software (Systat Software Inc.) to determine the total leaf area per plant (LA, cm<sup>2</sup>).

Leaves, main stems, secondary stems and floral buds were then oven-dried at 70 °C for 48 h and their dry mass was weighted. Individual plant roots were gently washed from the sand and placed in plastic bags at -20 °C for later measurements. After defrosting, roots were stained with methylene blue (4 g L<sup>-1</sup>) for 5 min to increase contrast before scanning. In the three species, the herringbone root system comprises a single main root (tap root) and lateral branches (Fitter 1994). The entire root system of each sampled plant was digitalized up to the third harvest (28 DAS).

Table 1. Origin of the four studied *Chenopodium* populations

Species	Code	Provenance	Latitude	Longitude	Altitude (m)	Rainfall (mm/y)
<i>C. quinoa</i>	QB	Jirira, Bolivia	19°51'S	67°34'W	3.700	250
<i>C. quinoa</i>	QC	Cunco, Chile	38°56'S	72°03'W	200	1.200
<i>C. hircinum</i>	HI	Aranjuez, Bolivia	16°33'S	68°36'W	3.200	550
<i>C. pallidicaule</i>	PA	La Paz, Bolivia	17°30'S	68°36'W	3.600	550



From that harvest on, a representative root sub-sample was selected on a weight basis, composed of the entire main root and two complete lateral branches in each of three zones: the superficial zone (0-5 cm depth), the intermediate zone (15-25 cm depth) and the deep zone (> 40 cm). Roots were prepared according to Roumet *et al.* (2006) before scanning on a flatbed scanner at a resolution of 400 dpi (Acer Scan 300F, 6684 03A). The root system image was analyzed with the WinRhizo Pro2003a software (Regent Instruments Inc.) to measure main root length (MRL, m), lateral root length (LRL, m), total root length (TRL, m), average main root diameter (MRD, mm), average lateral root diameter (LRD, mm), and total root projected area (RA, cm<sup>2</sup>). Following these measurements, the entire root system was dried at 70 °C for 48 h and weighted.

### 2.3 Plant morphology and growth parameters

From the various mass and length measurements, the following values were calculated: mass fractions of the leaves, stems and roots relative to the whole plant dry mass (respectively: LMF, SMF, RMF, %), root to shoot mass ratio (R/S, g g<sup>-1</sup>), leaf area to whole plant mass ratio (LAR, m<sup>2</sup> kg<sup>-1</sup>), root area to leaf area ratio (RA/LA, cm<sup>2</sup> cm<sup>-2</sup>), specific leaf area (SLA, m<sup>2</sup> kg<sup>-1</sup>), and specific root length (SRL, m g<sup>-1</sup>). Following the recommendations of Hoffmann and Poorter (2002), the relative growth rates (RGR, mg g<sup>-1</sup> day<sup>-1</sup>) for the shoots (stems + leaves + floral buds) or the roots (main root + lateral roots) were calculated for successive harvests using natural log-transformed plant mass data with the equation:

$$\text{RGR} = (\ln(M_2) - \ln(M_1)) / (t_2 - t_1)$$

where  $\ln(M_x)$  stands for the average of the natural log of shoot mass or root mass at time  $x$ , and  $t$  stands for the time of harvest. The whole-plant net assimilation rate (NAR, g m<sup>-2</sup> day<sup>-1</sup>) was determined using the classical equation of Vernon and Allison (1963):

$$\text{NAR} = [(\ln(LA_2) - \ln(LA_1)) (M_2 - M_1)] / [(LA_2 - LA_1) (t_2 - t_1)]$$

where  $M_x$  and  $LA_x$  stand respectively for average total plant mass and average leaf area at time  $x$ .

### 2.4 Data analyses

Statistical procedures were applied using the Statistica version 7.1 software (StatSoft Inc.). Except for RGR and NAR (see below), differences in plant traits among populations were

tested using factorial analysis of variance (ANOVA) after log transformation of the data when necessary to improve normality and homoscedasticity. Tukey's HSD was used to compare population means. The means of shoot and root RGR and their standard errors were calculated using the method proposed by Causton (1991). As recommended by Coleman *et al.* (1994), plant growth data were plotted as a function of the natural logarithm of the whole plant biomass to avoid size effects when comparing the four populations. To account for these size effects, ANCOVA analyses were performed to compare plants growth traits among populations, with shoot RGR, root RGR and NAR as the dependent variables, plant biomass as a covariate, and testing for a possible interaction between the covariate and the factor "populations".

### 3 RESULTS

#### 3.1 Plant growth, biomass allocation and leaf traits

Average seed mass differed 8.1-fold among the four populations with  $4.89 \pm 0.10$  mg per seed for the quinoa of Bolivia,  $2.09 \pm 0.04$  mg for the quinoa of Chile,  $1.50 \pm 0.04$  mg for *C. hircinum* and  $0.60 \pm 0.01$  mg for *C. pallidicaule* (mean  $\pm$  S.E,  $n = 120$  in each population). At all harvests, *C. pallidicaule* had the smallest plants while the other three populations showed comparatively small differences among them (Fig. 1). By the end of the growing period, plants of *C. pallidicaule* were about a tenth the size of the other populations (LA =  $0.029$  m<sup>2</sup>/plant and TPM =  $1.6$  g/plant against averages of  $0.28$  m<sup>2</sup>/plant and  $22.2$  g/plant respectively in the other three populations).

The general patterns of leaf, root and whole plant growth were roughly similar in the four populations, with a slow growth until the fifth week (35 DAS), followed thereafter and nearly until the end of the experiment by an accelerated biomass production and leaf area expansion. Main root elongation differed from these growth responses, with the accelerated growth period limited to 42 DAS, followed by a stopping in the main root elongation in *C. pallidicaule* and a slow growth in the other three populations. In general, the quinoa from Bolivia showed a faster root growth than the other populations, reaching 1 m main root length between 42 and 49 DAS, one to two weeks earlier than in quinoa from Chile and *C. hircinum* (Fig. 1c). At each harvest, main root length in *C. pallidicaule* remained less than half that of the other three populations.

Initially, the biomass allocation to leaves, stems and roots was fairly similar in the four populations with a dominant part ( $\geq 50\%$ ) allocated to the leaves (Fig. 2). At 14 DAS, the leaf mass fraction was maximum in all populations, reaching values near 70%.

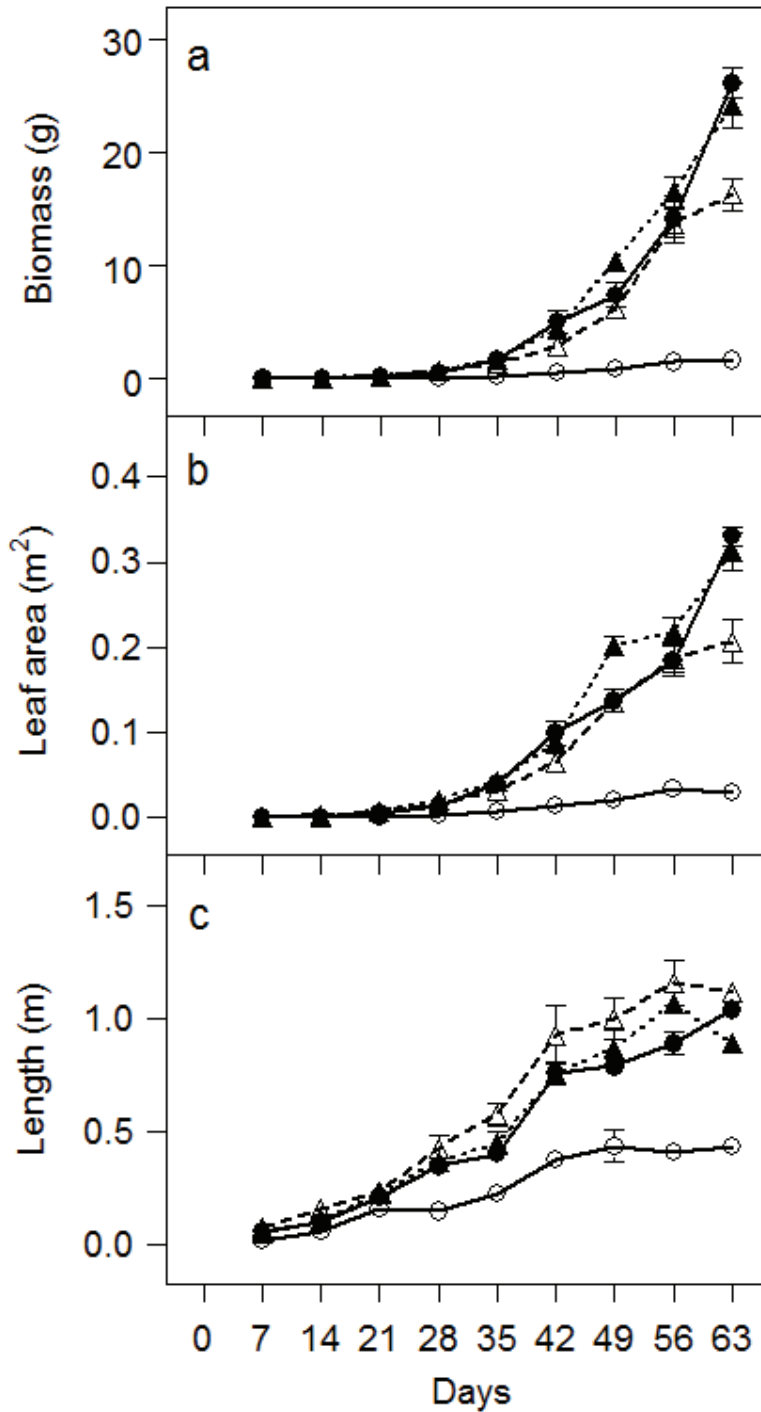


Fig. 1. Time variation in plant biomass (a), leaf area (b) and main root length (c) in *C. quinoa* from Bolivia ( $\Delta$ ), *C. quinoa* from Chile ( $\blacktriangle$ ), *C. hircinum* ( $\bullet$ ) and *C. pallidicaule* ( $\circ$ ) (mean  $\pm$  SE,  $n = 7$ , vertical bars show SE unless eclipsed by the symbol).

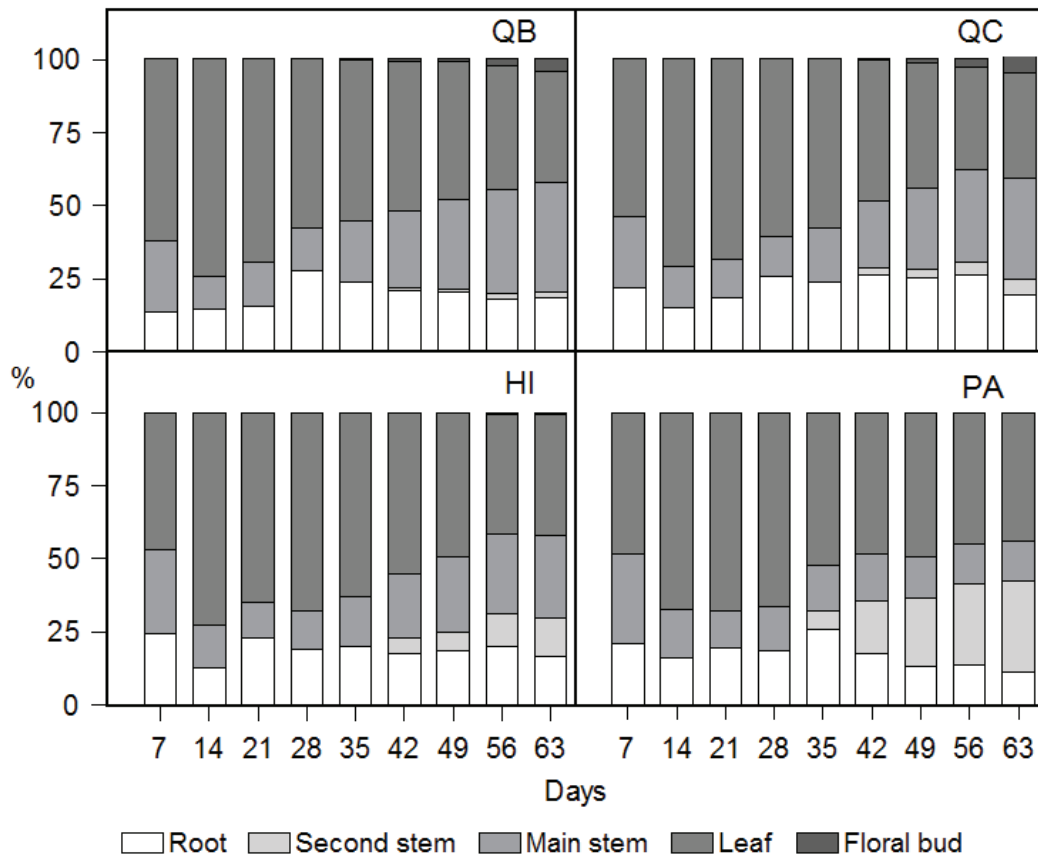


Fig 2. Time variation in plant biomass allocation in *C. quinoa* from Bolivia (QB), *C. quinoa* from Chile (QC), *C. hircinum* (HI) and *C. pallidicaule* (PA).

It decreased from then on, at the benefit of the roots for the next two or three weeks (28-35 DAS), and later at the benefit of the stems up to the eighth week (56 DAS). The stem mass fraction reached about 40 % by the end of the growing period, without noticeable difference among populations. However, in both cultivated quinoa populations the proportion of secondary stems remained negligible, while it counted for up to a third of the total stem biomass in *C. hircinum*, and two third of it in *C. pallidicaule*. Stem branching in *C. pallidicaule* after 35 DAS was at the cost of root allocation, as shown by a lower root mass fraction than in *C. hircinum* and the cultivated quinoas. Under non-limiting conditions, the root mass fraction never passed over 25 % of the total plant mass in the four populations. In this experiment centered on the vegetative growth phase, floral bud development remained negligible in the quinoa populations, and virtually absent in the other two populations.

The root to shoot ratio was highly variable in the first two weeks when plant size was very small and the ratio between minute biomass quantities resulted in erratic R/S values (Fig. 3a). R/S reached maximum values at 28 DAS (35 DAS in *C. pallidicaule*) and declined more

or less rapidly thereafter. The increase at 28 DAS was much more pronounced in the cultivated quinoas than in the other two populations ( $R/S \approx 0.25$  in average in these populations against 0.4 in the cultivated quinoas) and resulted in constantly higher  $R/S$  values in the quinoa populations until the end of the growing period. Root area was one to two times higher than leaf area in the first week (Fig. 3b). After 7 DAS, leaf area expanded rapidly and  $RA/LA$  declined to nearly 0.5, maintaining fairly constant values until 49 DAS without difference among populations. In the last two weeks of the experiment, the beginning of leaf senescence led to higher but also more variable  $RA/LA$  values.

The specific leaf area over the whole period ranged between  $30 \text{ m}^2 \text{ kg}^{-1}$  and  $65 \text{ m}^2 \text{ kg}^{-1}$  (Fig. 4a). Showing relatively high values in the smallest plants,  $SLA$  maintained values near  $45 \text{ m}^2 \text{ kg}^{-1}$  at medium plant size (ln TPM between 5 and 9) without statistical differences among the four populations, before dropping again in the biggest plants. Regarding the leaf area ratio (Fig. 4b), after an initial increase over the first two harvests, it declined continuously as plant size increased, without noticeable differences among the four populations. Considered over the four populations and the entire vegetative growth period,  $SLA$  and  $LAR$  showed a high positive correlation ( $r = 0.72$ ,  $n = 36$ ,  $P < 0.001$ ).

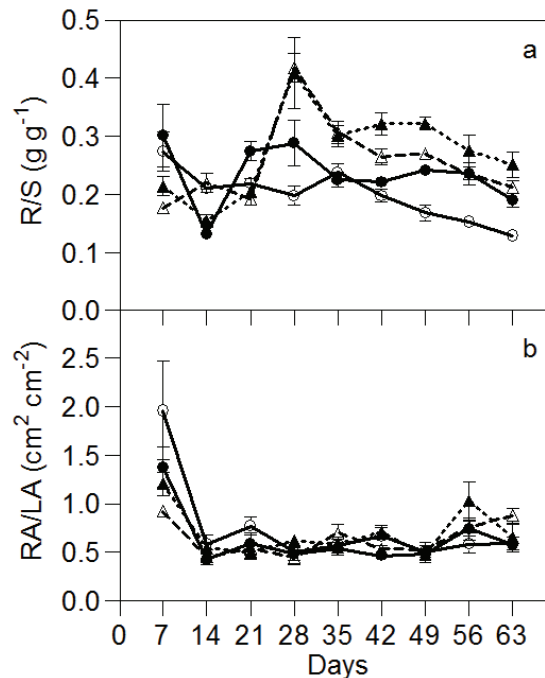


Fig. 3. Time variation in root to shoot biomass ratio ( $R/S$ ) and root to leaf area ratio ( $RA/LA$ ) in *C. quinoa* from Bolivia ( $\triangle$ ), *C. quinoa* from Chile ( $\blacktriangle$ ), *C. hircinum* ( $\bullet$ ) and *C. pallidicaule* ( $\circ$ ) (mean  $\pm$  SE,  $n = 7$ , vertical bars show SE unless eclipsed by the symbol).

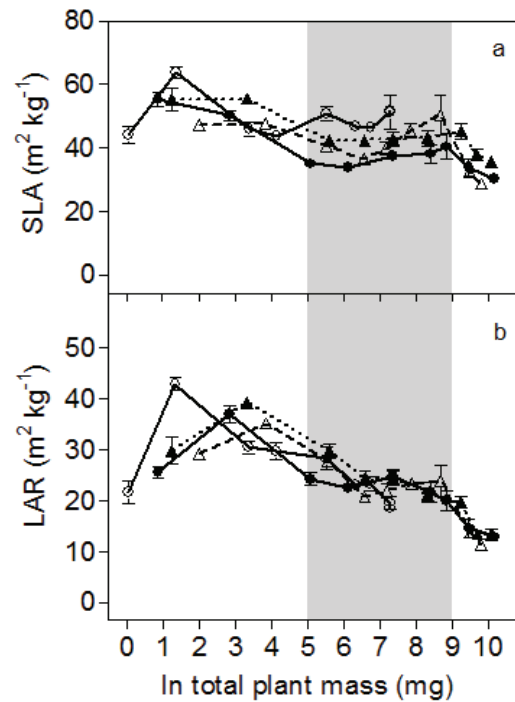


Fig. 4. Variation of specific leaf area (SLA) and leaf area ratio (LAR) in relation to total plant mass in *C. quinoa* from Bolivia ( $\Delta$ ), *C. quinoa* from Chile ( $\blacktriangle$ ), *C. hircinum* ( $\bullet$ ) and *C. pallidicaule* ( $\circ$ ) (mean  $\pm$  SE,  $n = 7$ , vertical bars show SE unless eclipsed by the symbol).

### 3.2 Relative growth rates and net assimilation rate

In the four populations, relative growth rates showed similar patterns of variation in relation to the total plant biomass (Fig. 5), with a highly significant correlation between root and shoot RGR ( $r = 0.89$ ,  $n = 32$ ,  $P < 0.001$ , for all populations). In all cases, shoot and root RGR had similar high values around  $300 \text{ mg g}^{-1} \text{ d}^{-1}$  at small plant size, and stabilized near  $150 \text{ mg g}^{-1} \text{ d}^{-1}$  at medium plant size (dashed area in Fig. 5), before decreasing again by the end of the growing period. The gradual decline in both shoot and root RGR was associated with decreases in SLA and LAR at similar plant sizes (Fig. 4).

Interestingly, in the two cultivated quinoas, the decline in shoot did not parallel that of root RGR since shoot RGR dropped at  $\ln \text{TPM} > 5$  ( $\text{TPM} > 0.15 \text{ g}$ ) while root RGR remained at or near their high initial values up to  $\ln \text{TPM} = 6$  ( $\text{TPM} = 0.40 \text{ g}$ ). The transition from high to intermediate shoot RGR at  $\ln \text{TPM} \approx 5$  was similar for *C. hircinum* and the two quinoa populations, while in *C. pallidicaule* shoot (and root) RGR dropped for  $\ln \text{TPM}$  as low as 3 ( $\text{TPM} = 0.02 \text{ g}$ ).

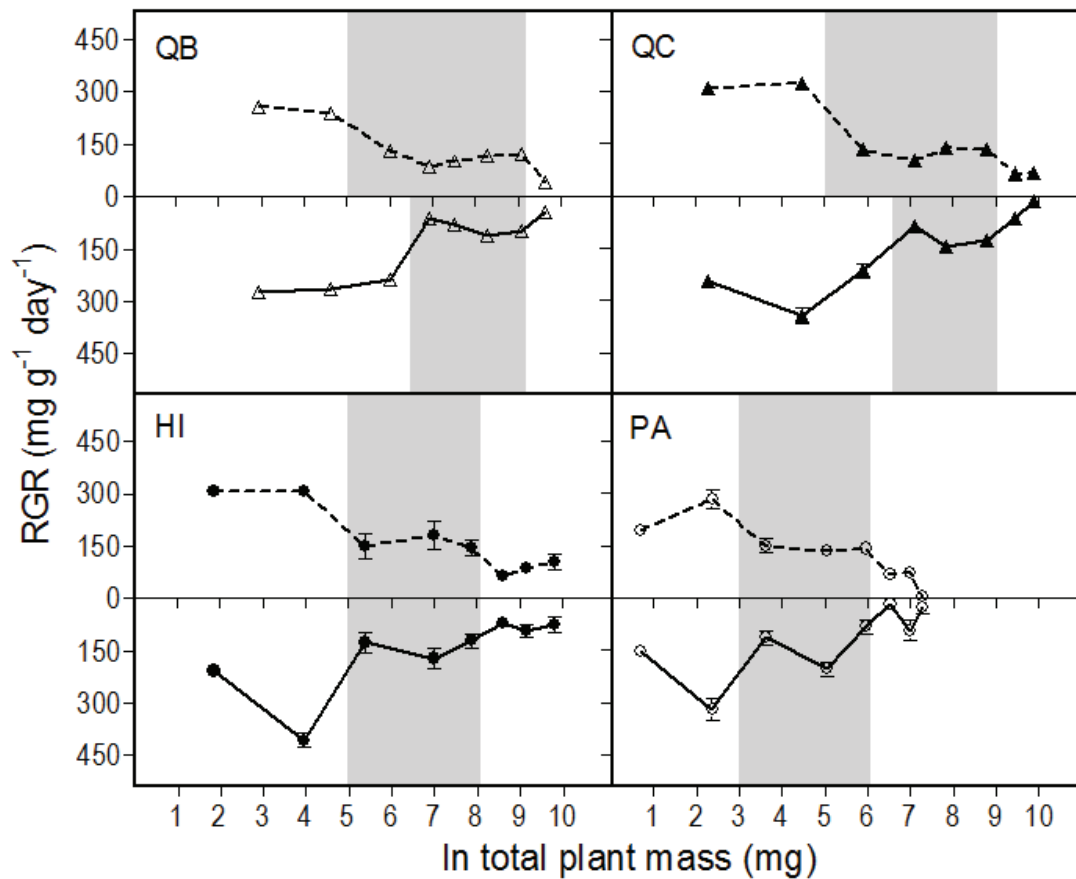


Fig. 5. Relationship of shoot RGR (dashed lines) and root RGR (solid lines) to the total plant mass in *C. quinoa* from Bolivia (QB), *C. quinoa* from Chile (QC), *C. hircinum* (HI) and *C. pallidicaule* (PA) (mean  $\pm$  SE,  $n = 7$ , vertical bars show SE unless eclipsed by the symbol). Data points in the shaded area are statistically different from those in the neighboring areas.

Thus, the studied populations differed less in their absolute values of shoot or root RGR than in the dynamics of RGR in relation to plant size, with *C. pallidicaule* showing RGR reductions at smaller plant size than the other three populations, and cultivated quinoas maintaining high root RGR in plants of medium size when this trait was already reduced by half in *C. hircinum* and *C. pallidicaule*.

The net assimilation rate (NAR) varied between 1.7 and 12  $\text{g m}^{-2} \text{d}^{-1}$  for all populations, with declining values as plant biomass increased (Fig. 6). At any given value of plant biomass, *C. pallidicaule* showed significantly lower NAR ( $P < 0.001$ ) than the other three populations, these latter showing no differences among them.

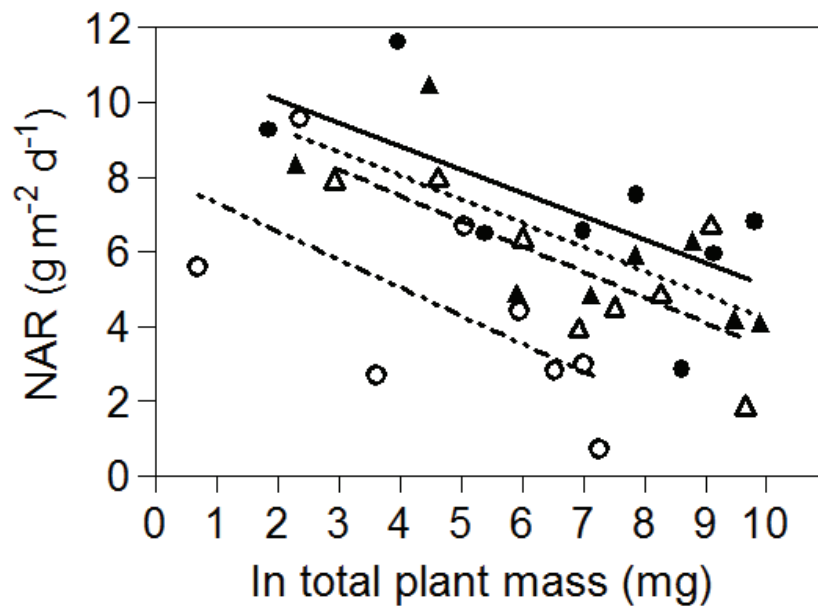


Fig. 6. Relationship between NAR to the total plant mass in *C. quinoa* from Bolivia ( $\Delta$  ---  $y = -0.68x + 10.25$ ,  $r^2 = 0.54^{**}$ ), *C. quinoa* from Chile ( $\blacktriangle$  ....  $y = -0.68x + 10.58$ ,  $r^2 = 0.56^{**}$ ), *C. hircinum* ( $\bullet$ —  $Y = -0.63x + 11.35$ ,  $r^2 = 0.46^*$ ) and *C. pallidicaule* ( $\circ$  ·····  $y = -0.75x + 8.05$ ,  $r^2 = 0.54^{**}$ )  
\*, \*\* significant at  $P < 0.10$  or  $P < 0.05$  respectively,  $n = 8$ ; each point is the mean of 7 samples.

### 3.3 Root growth and distribution

Reflecting the overall difference in plant size, *C. pallidicaule* had a much smaller root system than the other populations throughout the experiment (Fig. 7a). By the end of the growing period, the total root length of *C. pallidicaule* reached about 60 m/plant against an average of 620 m/plant in the other three populations, these latter showing little differences among them (Fig. 7a). In all populations, the accelerated growth period for the whole root system was between 35 and 56 DAS. This corresponded to a slowing of the main root elongation from 42 DAS on (Fig. 1c) compensated by the fast development of the lateral roots which represented up to 98 % of the total root length and total root biomass from 14 DAS on.

The specific root length (SRL) varied among populations and over time (Fig. 7b). From 21 DAS on, SRL in *C. pallidicaule* was significantly higher than the mean of the other three populations ( $290.7 \pm 10.6 \text{ m g}^{-1}$  vs  $174.0 \pm 12.6 \text{ m g}^{-1}$ ,  $P < 0.001$ ).

Over the growing period, the average main root diameter remained limited to 0.5 mm in *C. pallidicaule*, which was nearly twice lower than in the other three populations (Fig. 7c). In these populations, the main root went through two phases of root thickening: first in the young plants (until 21 DAS) and then in plants older than 42 days.



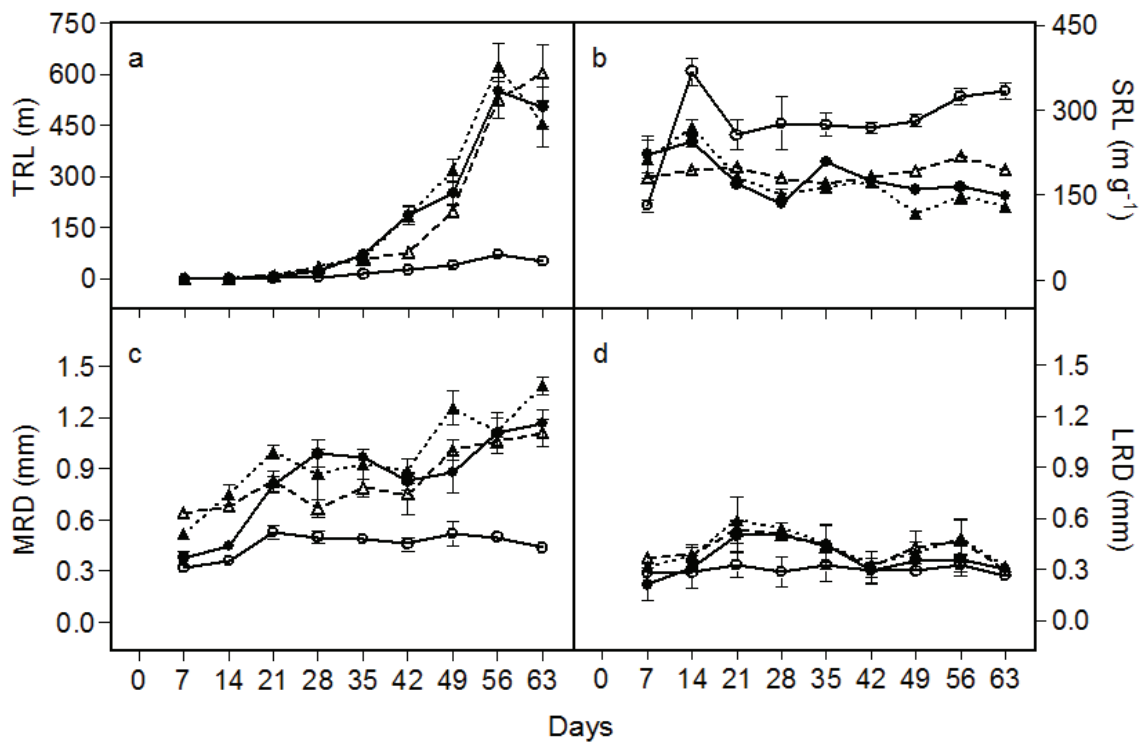


Fig. 7. Total root length (a), specific root length (b), tap root diameter (c) lateral root diameter (mm) (d) in *C. quinoa* from Bolivia (△), *C. quinoa* from Chile (▲), *C. hircinum* (●) and *C. pallidicaule* (○) (mean  $\pm$  SE,  $n = 7$ , vertical bars show SE unless eclipsed by the symbol).

The second phase of main root thickening coincided with the period of slower main root elongation (Fig. 1c) and faster lateral root elongation (Fig. 7a). Compared to the main root, lateral roots had a lower average diameter (*ca.* 0.3 mm) without much variation either among populations or over time (Fig. 7d).

In all populations, most of the roots were initially finer than 0.3 mm, representing up to 75 % of the total root length in *C. hircinum* and *C. pallidicaule* at 7 DAS (Fig. 8). Then the fraction of thick roots (diameter > 0.3 mm) increased rapidly up to 50 % until 21 DAS, except in *C. pallidicaule* where it remained nearly constant and limited at 25 % over the growing period.

This increase of the thickest root fraction corresponded to the elongation and first thickening phase of the main root (Figs. 1c and 4c) and was one week faster in the two quinoa populations compared to *C. hircinum*. The finest root fraction (diameter < 0.2 mm) increased continuously, reflecting the progressive branching of the root system in all populations (Fig. 8).

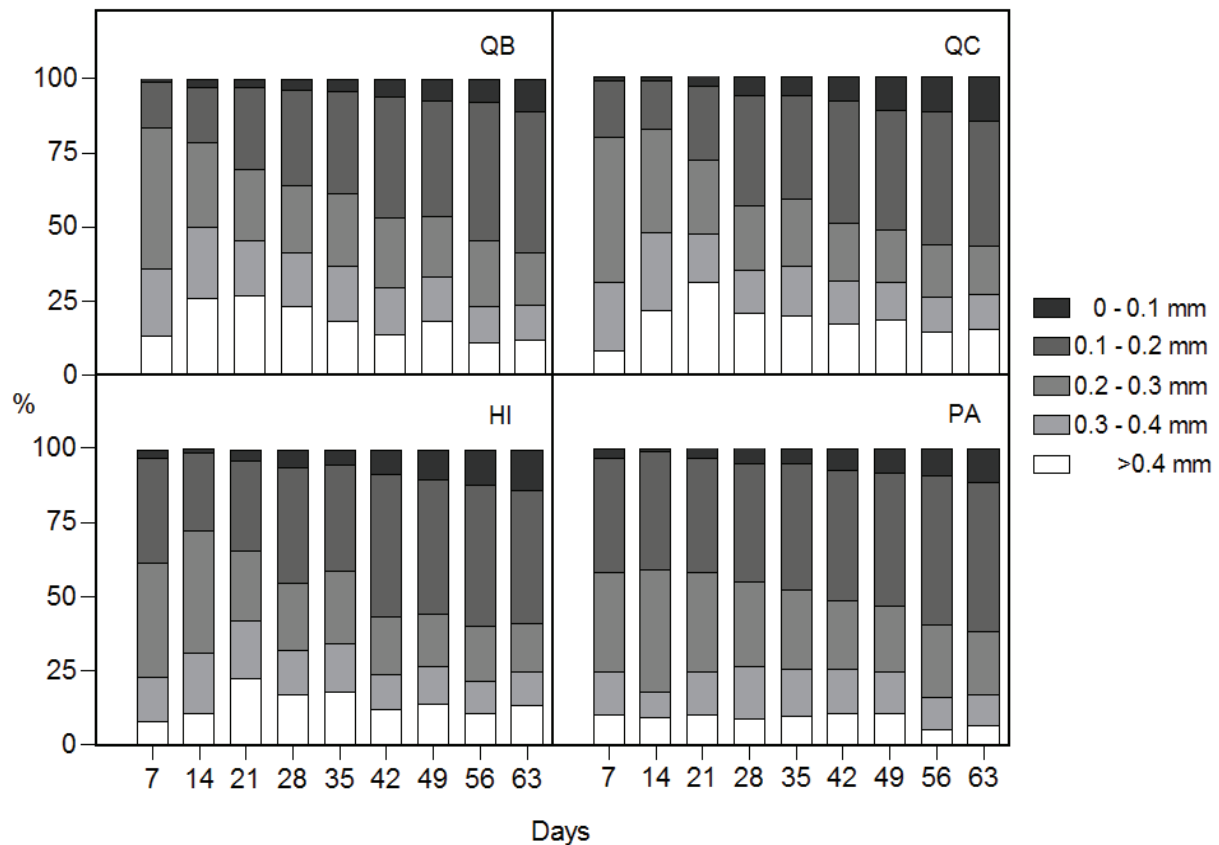


Fig. 8. Time variation in the total root length distribution among five classes of root diameter in *C. quinoa* from Bolivia (QB), *C. quinoa* from Chile (QC), *C. hircinum* (HI) and *C. pallidicaule* (PA).

## 4 DISCUSSION

This experiment comparing four *Chenopodium* populations postulates that some populations show consistent phenotypic trait associations related to cultivated or to low-resource environments or to both. The specific hypothesis is that, to allow for survival in resource-poor habitats or improved productivity in crop fields, changes in plant morphology and growth should have occurred that enhance soil resource capture, particularly by the root system. This comparative study is thus framed by two contrasts: one between wild and cultivated species, the other one between low-resource and high-resource habitats.

### 4.1 Wild versus cultivated species

In the present study, seed mass, plant morphology and seedling growth clearly differentiated the four populations, with the two quinoa populations showing the largest seeds, the least branching stems, and the most vigorous seedlings as regards biomass accumulation (Fig. 1, 2). Considering the morphological and growth traits, the cultivated quinoas appeared closer to

their wild progenitor, *C. hircinum*, than to their cultivated relative, *C. pallidicaule* (Figs. 1-4) as already found by Bruno (2003), Bruno and Whitehead (2006), and Mujica and Jacobsen (2006). Interestingly, the stem branching characteristic of the wild *C. hircinum* developed within the same "pool" of total stem biomass as for the quinoa populations (*ca.* 40 % of the total plant biomass at 63 DAS in the two species, Fig. 2). It thus appears that domestication of quinoa did not change the overall proportions of leaf, stem and root mass already present in the wild progenitor but it changed significantly the length and number of stems (data not show).

As regards *C. pallidicaule*, the total stem mass fraction was similar to that in the other two species but stem branching remained largely dominant despite domestication. This branched growth habit in *C. pallidicaule* is still present, although marginally so, in the quinoa populations, and should give these cultivated species a higher potential of adaptability against microsite, weather, and biotic variations (Janssens *et al.* 1990). As shown by Nishimura *et al.* (2010) in *Chenopodium album*, stem branching is also reduced by plant competition in dense stands. Considering that plant competition is random in natural habitats while it is the rule in crop fields, the branching habits of the three studied species are consistent with the stand densities commonly observed in their originating environments, where quinoa fields show higher plant densities than *C. pallidicaule* stands, themselves generally denser than wild populations of *C. hircinum* (authors' personal observation).

The fact that domestication in the studied species did not change the overall biomass allocation among the main plant organs in the vegetative phase supports the assessment of Janssens *et al.* (1990) that cultivated crops in low-input agriculture retain a high proportion of ancestral characteristics. Indeed, the four studied populations shared a large number of morphology and growth traits, starting with the trajectory of biomass allocation during the vegetative phase. From seed germination to floral bud bursting, all populations showed the same sequence of rapidly substituting the seed reserves by an autonomous carbon fixation through the expansion of cotyledons and leaves in the first 14 DAS, followed by the fast exploration of deep soil layers by the main root until 28 to 35 DAS, this latter opening the way to the subsequent proliferation of lateral roots which sustains the accelerated shoot growth by the end of the vegetative period (Fig. 2). This temporal pattern of biomass allocation observed under non-limiting conditions in wild as well as cultivated populations conforms the general ontogenetic trend for vegetative eudicots described by Poorter *et al.* (2011). It also corresponds to the fast early growth of quinoa seedlings observed by Rosa

(2009) and Ruffino *et al.* (2009). It should be noted that the initial priority to leaf growth in terms of biomass did not imply a reduced role of the root system since high RA/LA values at the earliest growth stage show that, in emerging seedlings, the exchange area was equal or even larger in roots than in leaves, suggesting a balance between soil foraging and light capture in the early plant life of the four studied populations (Fig. 3b).

Several other growth parameters such as SLA, RA/LA, LAR, and the maximum values of root or shoot RGR were similar in the four populations (Fig. 5, Fig. 3b, 4ab). The decrease in SLA over the period of vegetative growth agrees with the declining trend reported by Jensen *et al.* (2000) for field grown quinoa although the values observed in the present study were nearly twice as high. Lower light irradiance and higher air temperature in glasshouse compared to field experiments could be the cause of this difference in SLA (Garnier and Freijssen 1994, Poorter *et al.* 2009). The high correlation between LAR and SLA over a range of plant size suggests that the influence of SLA on variations in LAR observed across species (Poorter *et al.* 2011) also holds when considering the ontogenetic variations in LAR associated to changes in plant size and leaf morphology during the vegetative growth phase.

In view of the many similarities in the morphology and growth patterns in the studied species, the major contrast between them appeared associated to their ploidy level, the tetraploid *C. hircinum* and *C. quinoa* being much more productive than the diploid *C. pallidicaule* in terms of total plant biomass, total root length and NAR (Figs. 1, 3, 4a). As regards root morphology, both tetraploid species showed root axes significantly thicker than *C. pallidicaule*, which results in lower SRL values (Fig. 7bcd). These traits contribute to the penetration capacity of the roots and, thus, to the competitive ability of the plants (Materechera *et al.* 1992, Drenovsky *et al.* 2008). Plants with roots of low SRL tend to have lower root respiration and lower root turnover rates than those with roots of high SRL (Roumet *et al.* 2006). The two tetraploid species showed generally the highest values of root to shoot ratio (Fig 2, 3a). This higher investment in the root system, together with a lower cost of root system maintenance, might explain the higher overall productivity and slower RGR decrease in *C. hircinum* and *C. quinoa* compared to *C. pallidicaule*.

Seed mass and RGR can be positively correlated as is the case of *Medicago sativa*, *Hordeum muranum* and *Polygonum convolvulus* (Grime and Hunt 1975, Bell 2005), or weakly and negatively correlated (Shipley and Peters 1990), or no correlated at all (Villar *et*

al. 1998). In the present study, the small-seeded *C. pallidicaule* and the medium-seeded *C. hircinum* showed maximum values of root and shoot RGR similar to those in the large-seeded quinoa populations, thus without correlation with seed mass. Other cases of small seed mass associated to high RGR have been described in *Urtica dioica*, *Stellaria media*, or comparing wild and cultivated *Hordeum* (Grime and Hunt 1975, Chapin et al. 1989). It is a general observation that crop domestication did not result in any consistent difference in RGR among wild and cultivated species, with the consequence that relative differences in seedling vigour associated with initial differences in seed mass may persist for several weeks (Evans 1993). The present study showed that, despite similar maximum values of root or shoot RGR, the four populations differed in the changes of these growth parameters in relation to plant size (Fig. 5). As regards *C. pallidicaule*, the lower threshold values for the progressive decrease in root or shoot RGR were associated with the lower early vigour and general lower productivity of this species, themselves associated to its lower seed mass. Ploidy level could play a role in the association between seed mass and vigour in early plant growth, although a direct relationship remains difficult to establish (Evans 1993, Meerts and Garnier 1996). Among the studied species of larger seed mass, prolonged high values of root RGR at medium plant size were characteristic of the cultivated quinoa populations and were associated to a vigorous root growth for a longer time period than the wild *C. hircinum*. This could be related to the higher main root diameter and higher proportion of coarse root (> 0.3 mm) in the cultivated quinoas until 21 DAS (Figs. 4c, 5), both traits indicating a root system with stronger morphology in the young quinoa plants compared to their wild ancestor.

The present work showing interspecific differences in root growth rate and root morphology under a common environment suggests that these traits are genetically controlled in the studied species and that root system in quinoa may have been positively influenced by human selection under low-input agriculture and/or low-resource environments. Similar conclusions have been drawn by Siddique *et al.* (1990) and Palta *et al.* (2011) comparing old and modern wheat cultivars grown under drought-prone environments. These studies on wheat, as the present experiment on Andean chenopods, found that human selection and improvement of seed crops has indirectly favoured the development of a strong root system in the early stages of the vegetative period, notably through the rapid growth of coarse main root and secondary root branches. Such root morphology at early growth stage is an advantage for the rapid colonization of deeper soil layers, enhancing the acquisition of soil nutrients and water (Wasson *et al.* 2012), and thus contributing to better seedling competitiveness and early

vigour in domesticated plants (Janssens et al. 1990, Wolfe et al. 2008).

#### 4.2 *Low-resource versus high-resource habitats*

In the present study, the contrast in plant traits between populations from low- and high-resource habitats is mainly illustrated comparing quinoa from the Bolivian highlands with quinoa from the Chilean lowlands, while some traits characteristic of *C. pallidicaule* bring insights on traits characteristic of extremely high and cold habitats. This comparison assumes that: i) differences in environmental conditions have been consistent over time and plant dispersal was limited enough to allow genetic differentiation among quinoa populations, ii) genetic differentiation is expressed even under uniform and non-limiting growth conditions. While the first assumption seems reasonably satisfied when comparing the arid and cold Bolivian highlands to the rainy and temperate Chilean lowlands *ca.* 2100 km apart, the fulfilment of the second assumption remains more uncertain.

In fact, under non-limiting conditions, the two quinoa populations showed only marginal differences in their morphological and growth patterns despite the contrast in their originating environments. The most notable difference between them lies in the seed mass, significantly higher in the quinoa from low-resource habitat compared to that from high-resource habitat. Baker (1972) states that large-seeded species tend to be found in environments where seedlings are exposed to drought, although a lack of correlation between seed mass and annual rainfall has also been reported (Villar et al. 1998). In quinoa, phenotypic variation in seed mass has been documented at the continental scale by Bhargava *et al.* (2007), but the lack of precise location of the origin of the 27 studied lines precludes inferring any biological adaptation. Within the Bolivian germplasm, Rojas (2003) finds larger seeds in quinoa from the southern altiplano compared to quinoa from less cold and dry regions. Since seed mass is in direct relation with energy availability in the endosperm and early exposition of the cotyledons after emergence (Moles and Leishman 2008), larger seeds should confer better plant vigour and competitive ability in young seedlings of quinoa from southern Bolivia. However, under non-limiting controlled conditions, this population displayed superiority only in its constantly higher main root length. This trait, jointly with the high fraction of coarse roots, might reflect a priority to continued colonization of the soil at depth, a rooting pattern adaptive to soil water capture in arid habitats. Similar differences in root depth and distribution have been observed in legume species from climatically contrasted locations compared under uniform conditions (Bell 2005).

In an ecological perspective, the low productivity displayed by *C. pallidicaule*, together with its branched growth habit, low NAR and high SRL, could be part of a syndrome of plant growth adapted to cold, high altitude habitats (Poorter et al. 2011) as, in fact, Simmonds (1965) and Gade (1970) have postulated for *C. pallidicaule*. In particular, small, branched plants are less exposed to the night radiative frosts frequent in the Andean highlands (Winkel et al. 2009, Pouteau et al. 2011). As for the high value of SRL, indicative of a strategy of rapid resource acquisition (Picon-Cochard et al. 2012), it could partly compensate for the limited soil foraging capacity in this species.

### 5 CONCLUSION

This study shows that wild and cultivated populations of *Chenopodium* from contrasted ecological origins display both differences and similarities in plant morphology and growth patterns. Interspecific variation under common non-limiting conditions suggests that these morphological differences are genetically controlled. Seed mass was the primary factor controlling early growth and allocation patterns among the studied *Chenopodium* species. Biomass production in the seedlings of cultivated quinoa was not higher than that of their wild relative, but shoot branching architecture was modified. The morphology and growth dynamics of the root system was also changed in the cultivated populations, improving their capacity of exploration of deep soil layers in the early stages of growth. While the present experimentation in a common environment under non-limiting conditions allowed to infer a genetic basis for the phenotypic variations observed among species, the adaptive value of these variations should still be assessed along agroenvironmental gradients.

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## CHAPITRE 2

# ONTOGENETIC CHANGES IN ROOT GROWTH AND SOIL FORAGING IN THREE ANDEAN *CHENOPODIUM* SPECIES

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## ABSTRACT

*Background and aims.* Phenotypic variation in root foraging ontogeny should hold ecological and evolutionary significance since early plant growth and survival depend on the acquisition of essential soil water and nutrient resources by the root system. Natural and anthropic selection could have influenced these root foraging patterns, particularly in low-resource habitats or low-input agriculture where fast root growth at depth would be beneficial for seedling establishment. To test for this, temporal root growth was compared in two cultivated ecotypes of *Chenopodium quinoa*, one cultivated population of *C. pallidicaule*, and one population of their wild relative *C. hircinum*, all originating from contrasted habitats in the Andes.

*Methods.* Plants were grown in rhizotrons under non limiting soil water and nutrient conditions over a 42-day period of vegetative growth. Entire root systems were scanned weekly, allowing to calculate topological indices, and to characterize temporal changes in the vertical distribution of the root system. Above and below-ground biomass, root:shoot ratio, and specific root length were determined at the end of the experiment.

*Results.* Despite large differences in plant biomass production and morphology of the aerial plant part, all studied populations showed a similar herringbone root system topology. Compared to *C. hircinum* and *C. pallidicaule*, *C. quinoa* showed faster taproot elongation, thicker roots and deeper lateral root proliferation, a set of root traits resulting in a significantly higher total root length and an improved soil foraging capacity in that species. Among the two *C. quinoa* ecotypes, the one originating from the driest habitat showed the highest ranking in all the root system traits.

*Conclusion.* These results show that, while the root system topology was independent of the ecological background in the studied *Chenopodium* populations, their root foraging capacity appeared as an intrinsic attribute which was significantly improved in the dry-habitat ecotype of the cultivated *C. quinoa*.

**Keywords:** *Chenopodium quinoa*, *Chenopodium hircinum*, *Chenopodium pallidicaule*, ontogeny, rhizotron, root elongation, root system architecture, root system topology, specific root length.

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## 1 INTRODUCTION

Root system architecture has profound implications for plant growth and evolution, enabling individual plants to cope with changing environmental conditions and plant species to thrive in different ecological niches (Nibau et al. 2008). Variations in the size and shape of plant root systems are brought about largely by variations in root branching which, in turn, are controlled by a suite of genetic factors, and hormonal and environmental signals (De Dorlodot et al. 2007, Osmont et al. 2007, Nibau et al. 2008). In the case of crop species, it is most likely that plant domestication has indirectly selected for root traits adaptive to different agricultural systems and environments (Wasson et al. 2012). Exploring the phenotypic variation in root branching, and particularly the contrast between wild and cultivated species, would help defining root system ideotypes that optimize water and nutrient uptake and, thus, contribute to increased crop productivity in a context of global climate change and scarcity of water and nutrients (De Dorlodot et al. 2007).

Examining the various patterns of root system branching or topology, theoretical and experimental works suggest that a root system consisting of only a main axis and primary laterals (herringbone topology) would favour nutrient acquisition in low-resource environments because it minimizes intraplant root competition (Fitter et al. 1991, Fitter & Stickland 1991, Taub & Goldberg 1996). In some species, plasticity in root architecture enhances the capacity of roots to acquire limited nutrient resources by developing either greater branching complexity or longer main root segments, depending on the mobility of the considered nutrient (Arredondo & Johnson 1999).

Among the root system traits enabling the plants to access deep water and nutrients, the early elongation rate and the total root length at seedling stage show extensive genetic variation which might be related, in some species, to seed mass and early plant vigor (Richards et al. 2007). In several crop species, genotypic differences in rooting density, rooting depth, or root branching are associated with differences in drought resistance of cultivars from contrasting environments (Henry et al. 2011, Kashiwagi et al. 2006, Liu et al. 2011, Manshadi et al. 2008). In wild species also, morphological and functional root traits reflect major ecological groupings according to soil resource acquisition, not only between contrasting functional types (Paula & Pausas 2011, Roumet et al. 2006, Taub & Goldberg 1996), but also among species of the same growth form (Leva et al. 2009). Several theoretical and experimental studies show that under rich soil conditions, these morphological and

functional root traits include shorter root links (the segments of root between two branches, or between a node and a root tip), greater link number, and more dichotomous root branching (Arredondo & Johnson 1999, Arredondo & Johnson 2011, Berntson 1994, Fitter et al. 1991, Taub & Goldberg 1996). On the opposite, species from low-resource environments are expected to show a more herringbone architecture (*ibid.*), although this trend may be significant only when the plants are effectively grown under low-resource conditions (Taub & Goldberg 1996).

A previous study assessed the aerial and root growth in wild and cultivated Andean chenopods under non-limiting conditions (Alvarez-Flores et al. 2012). It found genotypic and temporal variations in several root morphological traits such as total root length, specific root length, and root diameter. It also concluded that changes in both the shoot and root morphology between wild and cultivated chenopods should have enhanced the capacity of cultivated species seedlings to explore the soil, particularly in the ecotypes from low-resource habitats. However, the destructive sampling of plants grown in containers did not allow for a detailed study of temporal changes in root topology, morphology and branching patterns.

By means of a rhizotron experiment, the present study addresses the ontogenetic changes in root system architecture in three Andean species of *Chenopodium* conforming four populations of different agroecological backgrounds. The starting hypothesis is that *Chenopodium* populations from low-resource natural environments or low-input agriculture have developed root traits that enhance root foraging in deep soil layers at early growth stage.

## 2 MATERIALS AND METHODS

### 2.1 Study site and plant populations

The study was conducted outdoor at the CEFÉ-CNRS experimental field (Montpellier, France, 43°38'19''N, 3°51'46''E), from the 18<sup>th</sup> of March to the 20<sup>th</sup> of April 2011. Four *Chenopodium* populations (wild and cultivated) from contrasting habitats were compared: cultivated *C. quinoa* from temperate and rainy lowlands with nutrient-rich soils in Chile, cultivated *C. quinoa* from the arid and cold highlands with nutrient-poor soils in southern Bolivia, cultivated *C. pallidicaule* and wild *C. hircinum* both from the semi-arid highlands with nutrient-poor soils in northern Bolivia (Table 1). The cultivated *C. quinoa* populations were local landraces without pedigree. In each population, seeds collected directly at the specified sites were selected for their size homogeneity, and sterilized with sodium

hypochlorite (1%) for 10 mn before sowing in rhizotrons at 3 cm depth.

## 2.2 Rhizotrons

Rhizotrons measuring 85 x 34 x 2.3 cm were made of white PVC plates with a pane of glass on one side. The windowpane was protected from light incidence with a mobile polystyrene plate 1.5 cm thick. Each rhizotron was filled with 7 L of a soil substrate humidified at field capacity (see below), sown with two seeds of the same population and then disposed outdoor under a transparent roof for rain protection. The rhizotrons were disposed with a 15° inclination in relation to the vertical to favor the root growth on the windowpane.

## 2.3 Soil substrate and growth conditions

The soil substrate was made of 84.5 % of sand, 6.3 % of clay, 4.5 % of silt, 3.05 % of organic matter, with pH 8.08, CEC Metson 9.02 cmol+/kg, and an 11.2 C/N. It was dried and sterilized to 120 °C for 48 hours. The volumetric water content of the substrate was calculated from its granulometric composition using the equations of Saxton and Rawls (2006). Water content was estimated to be 0.12 cm<sup>3</sup> water/cm<sup>3</sup> soil at field capacity and 0.05 cm<sup>3</sup> water/cm<sup>3</sup> soil at wilting point. The substrate was humidified just before filling the rhizotrons, using a nutritive solution (N: 16%, P: 10 %, K: 24 %, MgO: 3 %, micro-elements: 2.5 g/L).

The process of substrate humidification and rhizotron filling took place at night within a closed room to avoid water loss due to evaporation in the day. The experiment started with the rhizotrons watered at field capacity, and continued without re-watering until the end of the study.

Species	Code	Provenance	Latitude	Longitude	Altitude (m)	Rainfall (mm/y)
<i>C. quinoa</i>	QB	Jirira, Bolivia	19°51'S	67°34'W	3700	250
<i>C. quinoa</i>	QC	Cunco, Chile	38°56'S	72°03'W	200	1200
<i>C. hircinum</i>	HI	Aranjuez, Bolivia	16°33'S	68°36'W	3200	550
<i>C. pallidicaule</i>	PA	La Paz, Bolivia	17°30'S	68°36'W	3600	550

Table 1. Origin of the four studied *Chenopodium* populations.



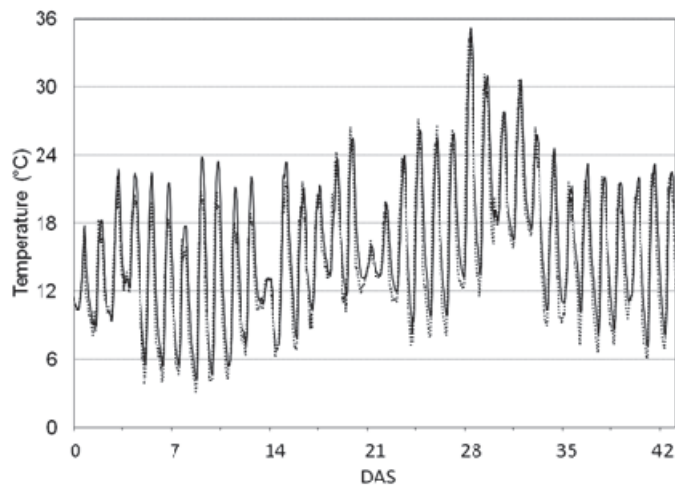


Figure 1. Air temperature at 150 cm height (solid line) and soil temperature at 25 cm depth (dotted line) during the course of the experiment.

Thermic probes measured the temperature of the substrate at a 25 cm depth in two rhizotrons, while air temperature and humidity were measured at a 150 cm height near the center of the experimental plot. These data were stored in a Campbell CR-21 data logger with a frequency of 30 minutes. Over the course of the experiment, air temperature varied between 3.0 and 35.2 °C (mean = 15.5 °C ± 0.1) while soil temperature varied between 4.8 and 35.2 °C (mean = 14.9 °C ± 0.05) (Fig. 1). Air relative humidity varied between 30 and 89 % (mean = 61 % ± 0.5).

#### 2.4 Plant sampling and measurements

Three days after emergence, young plants were thinned to one seedling per rhizotron, and five replicate plants per population were followed weekly from 7 to 42 days after sowing (DAS) for non-destructive growth measurements. Aerial growth was recorded measuring the plant height (pH, cm) as the length of the main stem from the root collar to the shoot apex. Root growth of each replicate plant was recorded by tracing on a transparent acetate sheet the root axes visible through the windowpane of the rhizotron. Weekly records were made using 0.5 mm sharp point permanent markers of different colors. By the end of the experiment, the acetate sheets with the root system image were scanned at a resolution of 400 dpi (Acer Scan 300F, 6684 03A) (Fig. 2).

The root system images were then analyzed with the morphological and architectural procedures of the WinRHIZO Pro2009 software (Regent Instruments Inc., Quebec, Canada), calculating the following root traits: total tip number (TTN), total link number (TLN), total root length (TRL, m), total root length per link type.

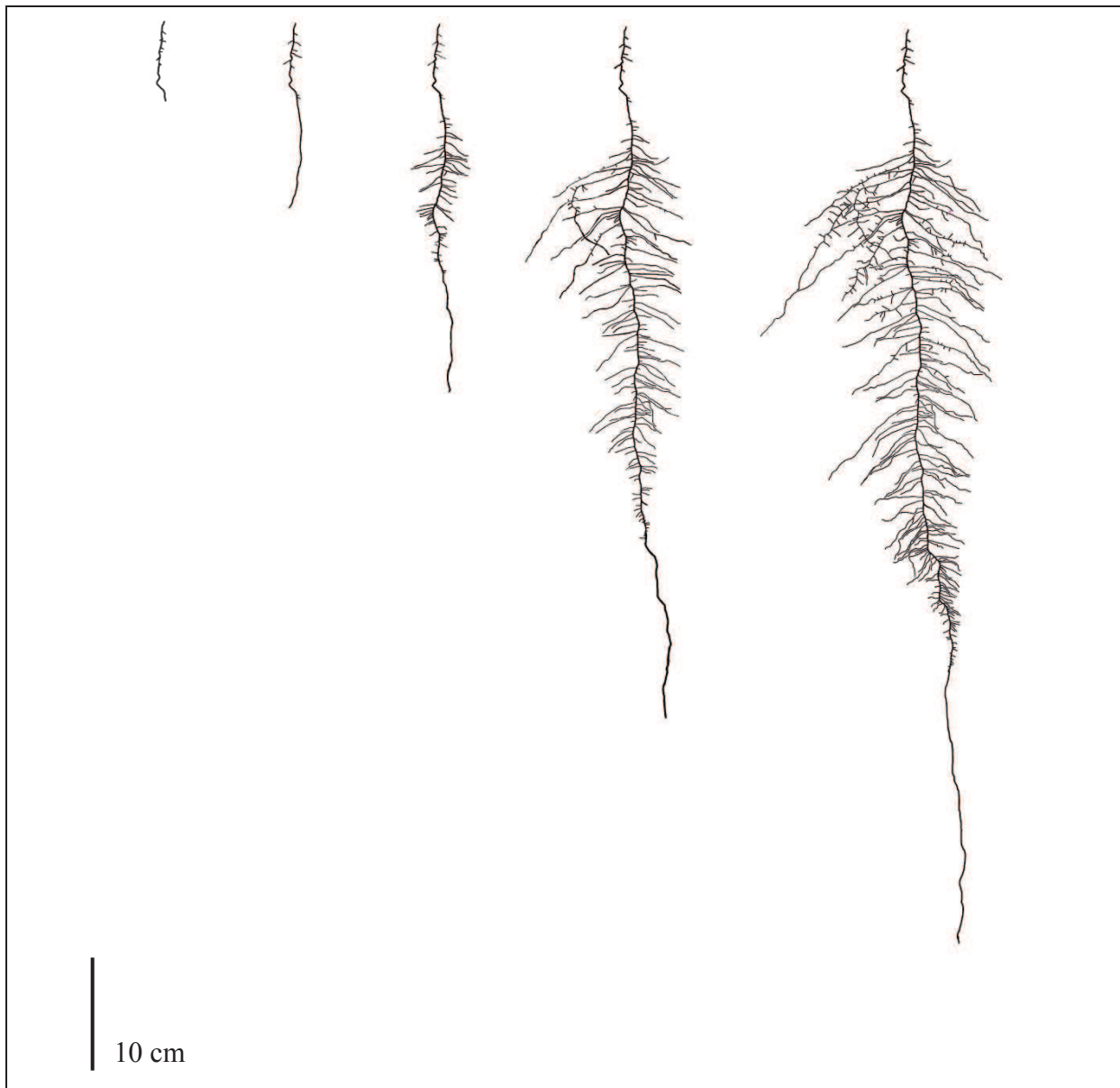


Figure 2 Time course of *Chenopodium hircinum* root system from week 2 to week 6

Links are defined as root segments between two branch junctions or a branch junction and a meristem. After Fitter (1987), link types are classified as external-external (EE) when external links join other external links, external-internal (EI) when external links join internal links, and internal-internal (II) when links do not terminate in a meristem (Fig. 3).

Following Glimskär (2000), two root topological indices were calculated. The quotient index  $\log(\alpha)/\log(\mu)$  was calculated for individual plants at the end of the experiment, with  $\alpha$  representing the altitude of the root system (i.e. the number of links of the longest path from the root collar to an external tip) and  $\mu$  its magnitude (i.e. the total number of tips in the root

system). The slope index (TI) was also determined as described by Fitter and Stickland (1991), calculating the slope of the regression of  $\log(\alpha)$  on  $\log(\mu)$  for the set of altitude and magnitude values observed in each studied population during the time of the experiment. Large values of both topological indices show a more "herringbone" structure of the root system, with branching mostly confined to the main axis.

By the end of the experiment (42 DAS), after completing the non-destructive aerial and root growth measurements, root collar diameter was measured with an electronic caliper (Mitutoyo, Andover, UK) and each individual plant was cut at the level of the root collar. Leaves were separated from the stems, scanned at 600 dpi (CanonScan LIDE 100) and the images were analyzed with the SigmaScan Pro 5 software (Systat Software Inc.) to determine the total leaf area per plant (LA, cm<sup>2</sup>). Leaves and stems were then oven-dried at 65 °C for 48 h and their dry mass was weighted. The substrate containing the root system was divided into six depth levels (0-5 cm, 5-20 cm, 20-35 cm, 35-50 cm, 50-65 cm, 65-80 cm) and roots within each layer were washed, separating the roots growing onto the windowpane from those growing inside the substrate. After drying at 65 °C for 48 hours, root dry mass was weighted allowing to calculate: i) the specific root length (SRL, m/g) from the roots growing onto the windowpane, and ii) the root/shoot ratio (R/S, g/g) from the bulk of the root system biomass.

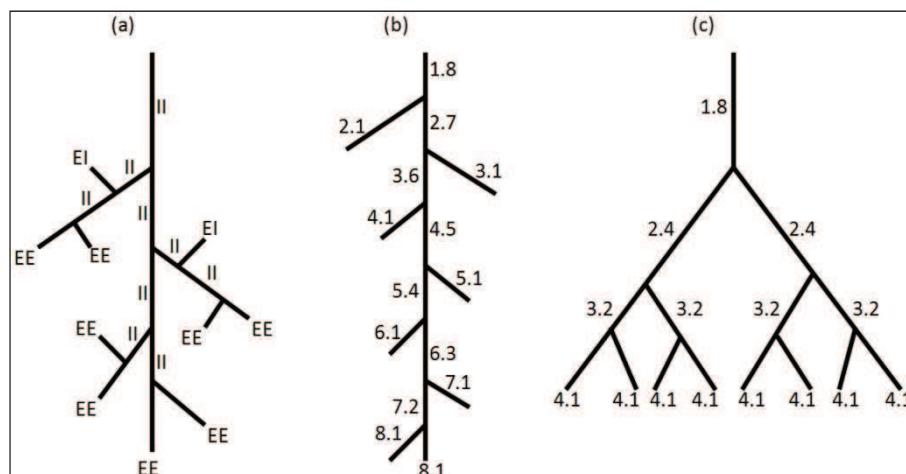


Figure 3. Root system topology: (a) Link types: EE (External-External), EI (External-Internal), II (Internal-Internal). (b) Maximally herringbone topology: magnitude = altitude = 8. (c) Maximally dichotomic topology: magnitude = 8, altitude = 4. In (b) and (c), the first number in each pair is the path length to the root collar and the second the link magnitude (modified from Arrendondo & Johnson 1999).

## 2.5 Data analyses

Statistical procedures were applied using STATISTICA version 7.1 (Statsoft Inc., Tulsa, USA). Differences in plant traits among populations were tested using factorial analysis of variance (ANOVA) after log transformation of the data when necessary to improve normality and homoscedasticity. Tukey's HSD was used to compare population means at  $P < 0.05$ .

## 3 RESULTS

### 3.1 Plant size, biomass production and root/shoot ratio

Comparing the four populations, seedlings of *C. quinoa* from Bolivia showed the most vigorous growth, resulting in higher plant height and deeper root system during the course of the experiment, and statistically higher leaf area, root collar diameter and biomass production at 42 DAS (Table 2).

At that time, *C. pallidicaule*, the least vigorous of the studied populations, was only 6.5% the size of *C. quinoa* from Bolivia in terms of total plant mass (1.9 g smaller), 10% its size in terms of leaf area (88 cm<sup>2</sup> smaller), and 20% its size in terms of total root length (19.5 m shorter). *C. quinoa* from Chile and *C. hircinum* showed intermediate values, with the wild *C. hircinum* always less productive than the cultivated *C. quinoa* from Chile although not statistically different in plant height and total root length. At 42 DAS, *C. hircinum* was 46% the size of *C. quinoa* from Chile in terms of total plant mass, 40% its size in terms of leaf area (32 cm<sup>2</sup> smaller), and 77% in terms of total root length (3.4 m shorter).

Despite contrasted growth capacities, *C. quinoa* from Bolivia and *C. pallidicaule* showed similar biomass allocation patterns with R/S ratio of 28% and 31% respectively, significantly lower than those observed in *C. quinoa* from Chile (45%) and *C. hircinum* (39%).

### 3.2 Root segment and root system morphology

The morphology of individual root segments varied greatly among the studied populations, with *C. pallidicaule* showing roots three-fold finer on average than those of the Bolivian population of *C. quinoa*, the other two populations having intermediate values. As regards the shape of the root system, the topological slope index TI showed statistical differences only among *C. quinoa* from Bolivia (0.72) and *C. hircinum* (0.87), while the topological quotient index did not showed any difference among the four studied populations (Table 2).

Table 2. Mean values of plant and root system traits of four *Chenopodium* populations at 42 DAS. TPM: total plant mass (g/plant), LM: leaf mass (g/plant), LA: leaf area (cm<sup>2</sup>/plant), PH: plant height (cm), R/S: root shoot ratio (g/g), RCD: root collar diameter (mm), SRL: specific root length (m/g), TRL: total root length (m/plant), TTN: total tip number, TLN: total links number,  $\log(\alpha)/\log(\mu)$ : topological quotient index, TI: topological slope index. (S.E. = standard error, n = 5, values followed by the same letter within rows are not statistically different at P = 0.05).

Traits	<i>C. quinoa</i> (Bolivia)		<i>C. quinoa</i> (Chile)		<i>C. hircinum</i>		<i>C. pallidicaule</i>	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
TPM	2.00	a 0.37	0.87	b 0.10	0.40	c 0.08	0.13	d 0.03
LM	1.38	a 0.28	0.55	b 0.03	0.24	c 0.03	0.09	d 0.02
LA	98.0	a 3.7	54.0	b 1.3	21.7	c 4.8	10.0	d 2.3
PH	8.4	a 0.5	5.9	b 0.5	4.5	bc 0.4	3.5	c 0.4
R/S	0.28	a 0.01	0.39	b 0.02	0.45	b 0.01	0.31	a 0.03
RCD	4.90	a 0.49	3.47	a 0.49	2.31	b 0.28	1.34	c 0.27
SRL	57.9	a 3.82	75.1	ab 12.2	106.5	b 10.0	179.7	c 35.5
TRL	24.1	a 3.4	16.4	ab 2.6	12.7	b 2.0	4.8	c 0.4
TTN	525	a 14.3	663	a 12.4	449	b 11.2	140	c 8.16
TLN	1122	a 64.6	1392	a 115	816	b 61.2	283	c 46.1
$\log(\alpha)/\log(\mu)$	0.77	a 0.01	0.77	a 0.01	0.77	a 0.01	0.77	a 0.01
TI	0.72	a 0.04	0.80	ab 0.04	0.87	b 0.03	0.85	ab 0.04

The regression of  $\log(\alpha)$  on  $\log(\mu)$  showed that the strong linear relationship over the range of observed plant size hides a tendency for the slope of the regression to decrease sharply at high magnitude values (namely,  $\log(\mu)$  close to 3) (Fig. 4).

The topological quotient index was negatively related to the root dry weight at 42 DAS, with statistically significant correlations for *C. hircinum* and *C. pallidicaule* (Fig. 5). Compared to the other three populations, the relationship observed in *C. pallidicaule* was shifted towards lower values, but the slope of its regression was not statistically different from that of the other populations (P = 0.74).

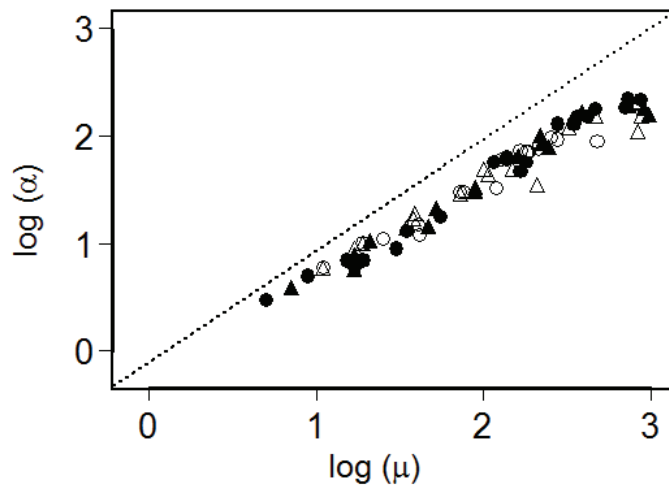


Figure 4. Regression of  $\log(\alpha)$  on  $\log(\mu)$  observed during the experiment in *C. quinoa* from Bolivia ( $\Delta$ ), *C. quinoa* from Chile ( $\blacktriangle$ ), *C. hircinum* ( $\bullet$ ) and *C. pallidicaule* ( $\circ$ ) ( $y = 0.81x + 0.08$ ,  $r^2 = 0.96$ ,  $n = 80$ ; the dotted line corresponds to a maximally herringbone topology).

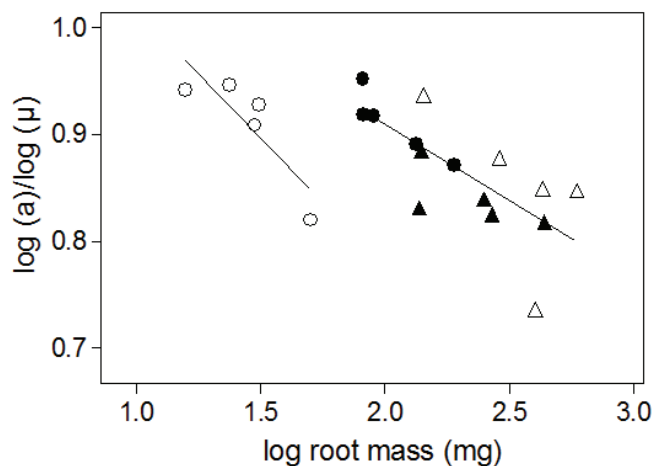


Figure 5. Relationship between  $\log(\alpha)/\log(\mu)$  and  $\log$  root mass at 42 DAS in individual plants of *C. quinoa* from Bolivia ( $\Delta$ ), *C. quinoa* from Chile ( $\blacktriangle$ ), *C. hircinum* ( $\bullet$ ) and *C. pallidicaule* ( $\circ$ ). Regression for *C. pallidicaule* is:  $y = -0.24x + 1.26$ ,  $r^2 = 0.74$ ;  $n = 5$ . Regression for the other three populations is:  $y = -0.14x + 1.19$ ,  $r^2 = 0.53$ ,  $n = 15$ .

### 3.3 Root system architecture

Despite a similar herringbone morphology, the studied populations differed greatly in the details of their root system architecture: *C. quinoa* from Bolivia and Chile produced higher total root length and had significantly greater numbers of root links and root tips than *C. hircinum* which, in turn, showed nearly three times more root branches and root tips than *C. pallidicaule* (Table 2).

A thorough analysis of the different kinds of root links (II, EI, EE, see Fig. 3a) reveals contrasted dynamics of root branching among the studied populations. Considering the cumulated root length of the three types of links (Fig. 6), the highest increase was observed between 28 and 35 DAS in *C. quinoa* from Bolivia, particularly in EI links which, by the end of the experiment, totalized 8 m per plant on average (53% of the total root length).

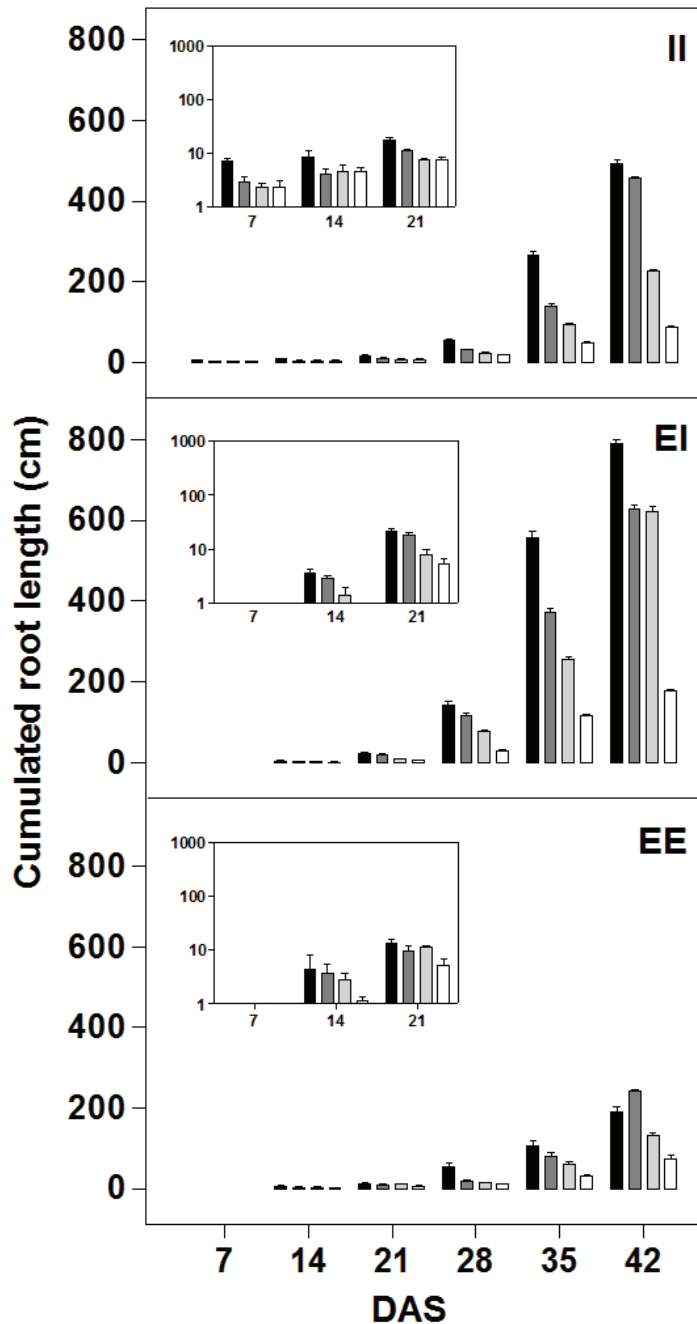


Figure 6. Mean cumulative length of the internal-internal (II), external-internal (EI) and external-external (EE) root links in the four studied *Chenopodium* populations (*C. quinoa* from Bolivia: black, *C. quinoa* from Chile: dark grey, *C. hircinum*: light grey, *C. pallidicaule*: white) (n = 5, vertical bars show standard errors, insets show root length on a log scale for a better appreciation of changes at early stages).

In comparison, EI links in *C. pallidicaule* at the same stage totalized only 2 m per plant on average, though they amounted to a similar proportion of the total root length. Except in the first two weeks when the taproot made for the major part of the root system, EI links (corresponding to secondary and higher orders branches) concentrated most of the total root length in the studied species.

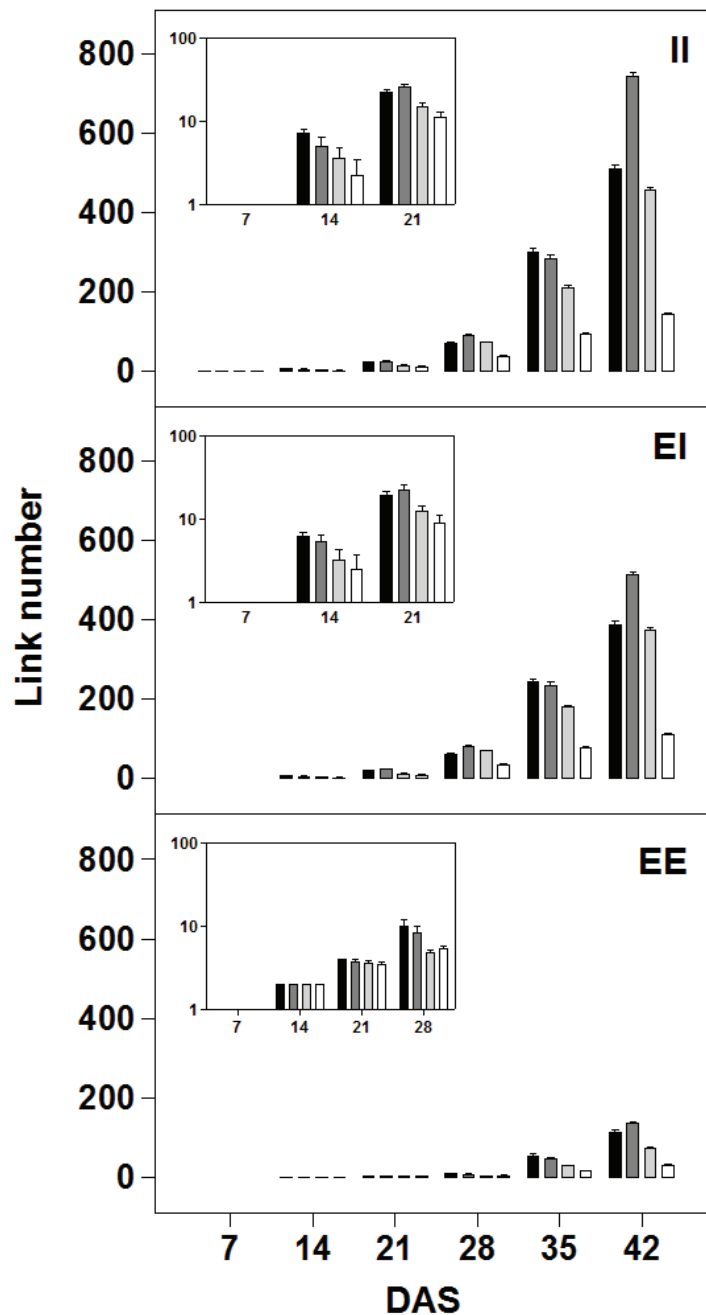


Figure 7. Mean number of internal-internal (II), external-internal (EI) and external-external (EE) root links in four *Chenopodium* populations (same color chart as in Fig. 5) ( $n = 5$ , vertical bars show standard errors, insets show link number on a log scale for a better appreciation of changes at early stages).

Decomposing the total root length in its two components (namely: the mean number and the mean length of the different links), it appeared that temporal changes in total root length reflected mostly the changes in link number ( $r = 0.84$ ,  $n = 65$ ,  $P < 0.001$ ) and not those in mean link length ( $r = 0.02$ ,  $n = 65$ ).



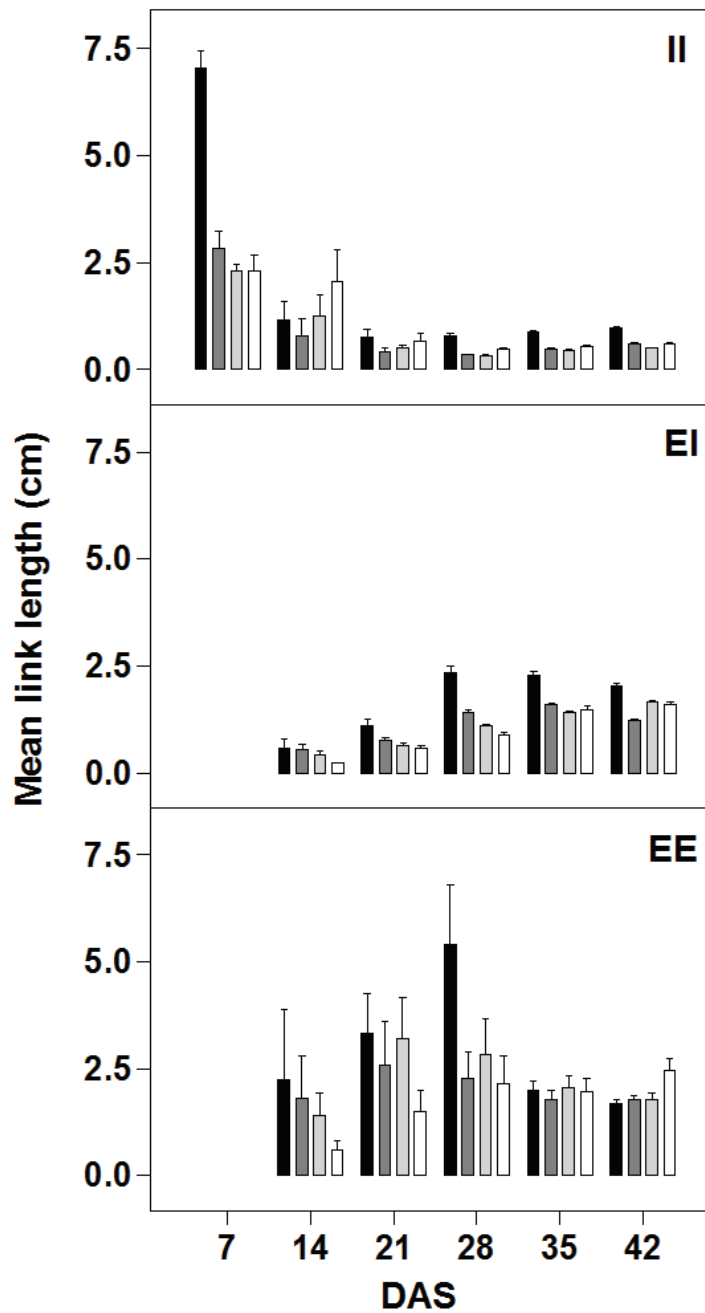


Figure 8. Mean length of the internal-internal (II), external-internal (EI) and external-external (EE) root links in the four studied *Chenopodium* populations (same color chart as in Fig. 6) (n = 5, vertical bars show standard errors).

The number of EI and EE links increased progressively from 14 DAS on, with EI links always in a higher proportion than EE links in all populations (Fig. 7). The appearance of new links reached its highest rate by the end of the experiment and, apparently, could have been lasting for a still longer time period (see Alvarez-Flores et al. submitted). At that time, *C. quinoa* from Chile showed the highest rate of root branching.

Regarding the mean length of the different links, considerable variation was shown with maximal values of nearly 7 cm observed in the young taproot of the Bolivian population of *C. quinoa*, while the taproot of the other populations was limited to about 2 cm at that early developmental stage (Fig. 8). From 14 DAS on, the mean length of II links decreased rapidly as a consequence of the emergence of new lateral roots. While mean link length did not showed much variations in time and among populations from 28 DAS on, it should be noted that *C. quinoa* from Bolivia maintained slightly but constantly higher link lengths than the other three populations.

### 3.4 Root system profile and branching dynamics

After six weeks of plant growth (42 DAS), the vertical distribution of the root system showed significant differences among populations and among soil layers (Fig. 9). In each soil layer, the number of root tips was highly correlated to the root length ( $r = 0.97$ ,  $P < 0.001$ ,  $n = 24$ ) and both traits showed similar vertical distributions, with noticeable amounts of roots only beyond 5 cm depth. *C. quinoa* from Bolivia and Chile had similar root profiles down to 65 cm depth, with maximum root development in the 20-35 cm soil layer (ca. 5 m root length in that layer, equivalent to a root length density ca. 0.35 cm root/cm<sup>3</sup> soil). *C. quinoa* from Bolivia differed noticeably from the Chilean population beyond a 65 cm depth, showing high root colonization at depth (ca. 4 m of root against 0.5 m respectively). *C. hircinum* and *C. pallidicaule* showed significantly less root development, although the former still colonized deep layers while the latter was virtually limited to the 35-50 cm layer (Fig. 9).

These patterns of vertical root distribution at 42 DAS resulted from the progressive proliferation of the root system through the soil profile. The sequential analysis of the number of new lateral branches appearing on the main root during the experiment revealed contrasted dynamics among the studied populations (Fig. 10). In *C. quinoa* from Chile and *C. hircinum*, root branching proceeded in a similar way, with new lateral roots successively appearing in ever deeper soil layers down to -75 cm at 42 DAS. The main difference between these two populations was in a more extended period of root proliferation in *C. quinoa* from Chile, starting one week earlier than in *C. hircinum*. By contrast, in *C. quinoa* from Bolivia, root branching was maximum at -75 cm as soon as 35 DAS, when none of the other populations add reached that depth yet. In *C. pallidicaule*, root branching occurred over the same lapse of time than in the other populations but at a much lower rate, and it hardly passed the -30 cm soil depth.

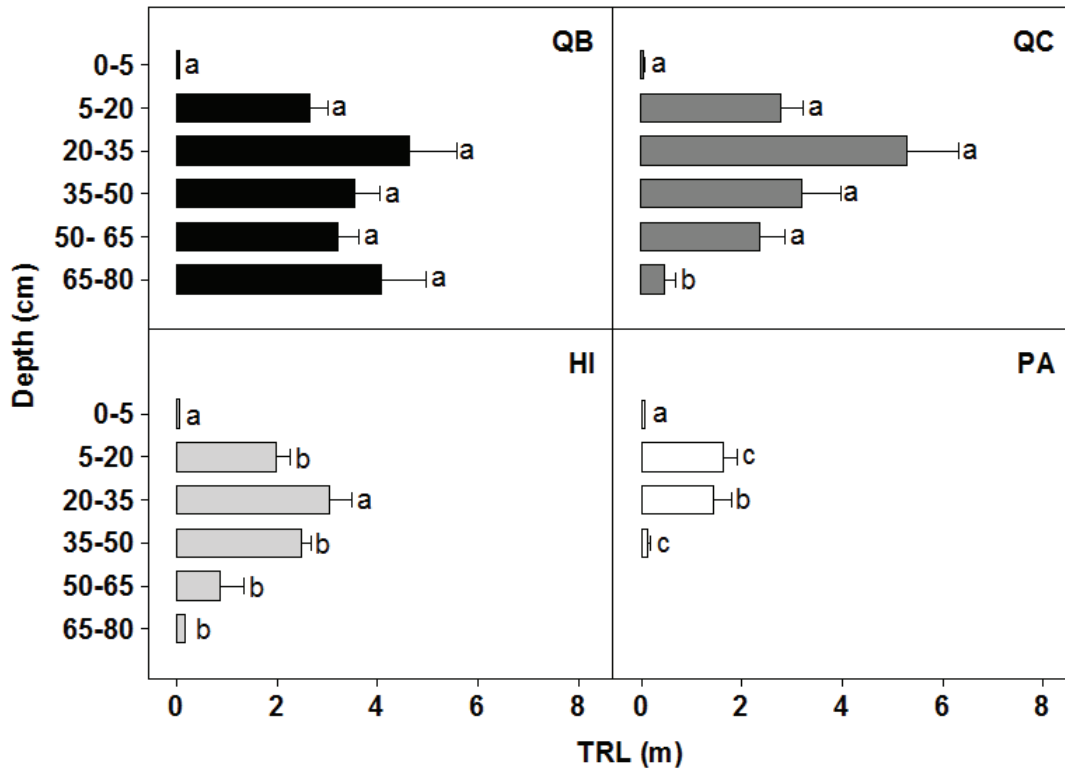


Figure 9. Total root length distribution in the soil profile at 42 DAS in *C. quinoa* from Bolivia (QB), *C. quinoa* from Chile (QC), *C. hircinum* (HI) and *C. pallidicaule* (PA) (mean  $\pm$  S.E, n = 5, letters show statistical differences between populations at a given depth, P < 0.05).

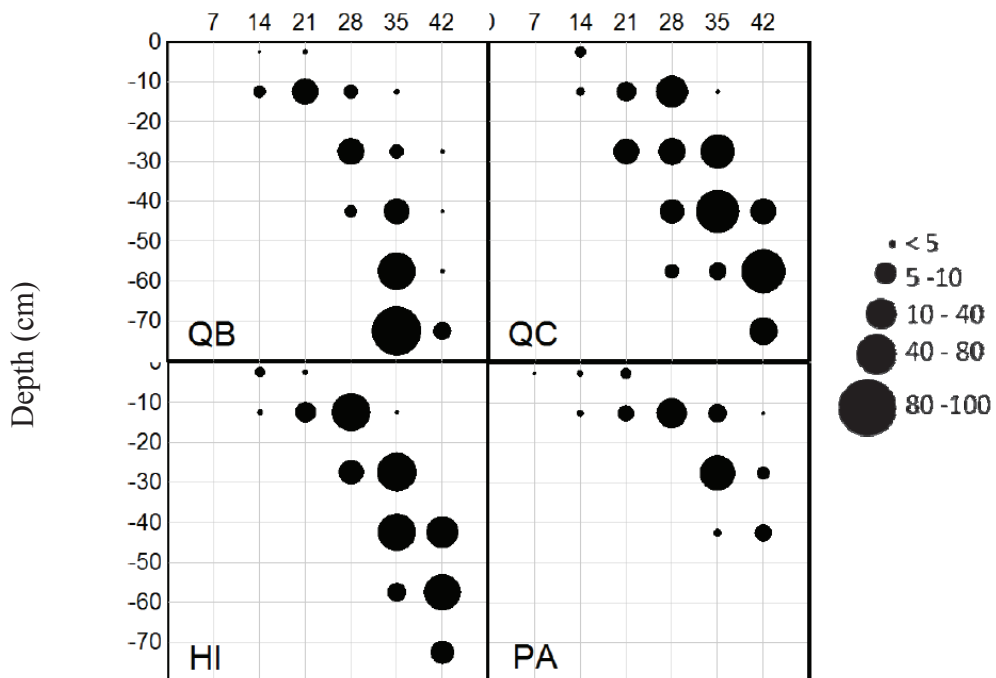


Figure 10. Vertical distribution of the mean number of new lateral roots appearing during the experiment in *C. quinoa* from Bolivia (QB), *C. quinoa* from Chile (QC), *C. hircinum* (HI) and *C. pallidicaule* (PA) (each point is the mean of n = 5 plants).

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## 4 DISCUSSION

### *4.1 Root topology in different chenopod species: plant size and ontogenetic effects dominate ecological background*

This study found significant variation in total biomass production among three Andean *Chenopodium* species, *C. quinoa* being two to five-fold more productive than *C. hircinum* and up to fifteen-fold more productive than *C. pallidicaule* (Table 2). As already mentioned by Alvarez-Flores et al. (submitted.), this difference in plant vigor seems related to the interaction between the ploidy level of the species and their wild vs cultivated status, the cultivated and diploid *C. pallidicaule* being noticeably less vigorous than the wild tetraploid *C. hircinum*, this one being less productive than the cultivated tetraploid *C. quinoa*.

This contrast between species did not affect the overall shape of the root system: the three studied species showed quite similar root topological indices (Table 2) with values characteristic of herringbone root systems, a feature commonly observed in chenopod species (Fitter 1987). The similarity in the topological indices of the studied species could reflect their close phylogenetic relationship. In fact, *C. quinoa* was probably domesticated from *C. hircinum*, this latter being also a wild relative of *C. pallidicaule* (Mujica & Jacobsen 2006). An alternative explanation arises when comparing our results to those of Glimskär (2000). In his study on five forbs and grass species grown at two different nitrogen levels, a quite general relationship emerges between  $\log(\alpha)$  and  $\log(\mu)$ , with a curvature in the altitude / magnitude relation suggesting a general size effect. Interestingly, the curvature in Glimskär's data appears at  $\log(\mu)$  near 3, with  $\log(\alpha)$  leveling near 2.4, both values which are quite close to our own data on three chenopod species. While the ecological significance of this size effect remains hypothetical (Glimskär 2000), an ontogenetic explanation of the leveling in the altitude / magnitude relation could be found in the continued branching of a not strictly herringbone root system: after an initial period of seedling growth dominated by the elongation and branching of the main root, lateral roots emerge directly from internal links, thus forming new external links (EI) which increase the root system magnitude but without further increase of its altitude. In our study, such new lateral roots of second and higher orders emerge in great number from 28 DAS on (Figs. 6 and 9). The generality of the altitude / magnitude relation observed in forbs and grasses under different growth conditions (Arredondo & Jonhson 1991, Fitter 1991, Glimskär 2000, Paula et al. 2011, Roumet et al 2006), suggests a general ontogenetic shift in the root topology of annual plant species, with

lateral roots emerging in ever greater numbers (increasing magnitude) as soon as  $\log(\alpha)$  exceeds a value of 2 approximately.

Due to this ontogenetic effect, the curvature in the altitude / magnitude relation points to a limitation of the topological slope index (TI) as proposed by Fitter & Stickland (1991). Assuming a linear relation over a large size range, TI overemphasizes small deviations from linearity (Glimskär 2000), which makes it a poorly suited index to discriminate root branching patterns in growing seedlings (Paula & Pausas 2011). As an alternative index, Glimskär (2000) proposed the  $\log(\alpha)/\log(\mu)$  quotient calculated on individual plants, with a range of values from 0.69 to 0.79 for five different species, embracing our estimate of 0.77 found in three Andean chenopods (Table 2). This author advocates that the graph of the quotient index *versus* root biomass (Fig. 5) is the safest and most sensitive method to evaluate species differences. In our case, Figure 4 shows that the topological quotient index decreases as plant root mass increases, in the same proportion for the three studied species. Glimskär (2000) also observes a decrease in the topological quotient index with plant size and, yet, he regards this index as relatively size-independent in comparison to TI. Considering our results together with those of Glimskär, we suggest a general ontogenetic shift as a possible component of this size effect. Therefore, when evaluating species differences in root topology, it could be recommended to compare not only plants belonging to a narrow size interval (Glimskär 2000), but also plants of similar developmental stages. As for the ecological interpretation of this ontogenetic-size effect on root topology, Glimskär (2000) as well as Paula and Pausas (2011) put forward that the more herringbone topology of young plants could be related to their need to quickly reach deep soil layers, in a context of competition with other plants. This assumption similarly applies to the growth of seedling plants in low-resource environments where fast root growth at an early developmental stage would secure nutrient capture by young plants (León et al. 2011, Palta et al 2011, Richards et al. 2007). The following discussion on the root foraging capacity observed in the studied species should bring some insights into this assumption.

#### *4.2 Root foraging: a suite of traits significantly improved in the cultivated dryland ecotype*

While the herringbone topology remained virtually unchanged among the three Andean chenopods of the present study, their root systems were quite different as regards the branching architecture and the progressive proliferation within the soil. *C. quinoa* produced

significantly higher total root length than *C. hircinum* and *C. pallidicaule* (Table 2). The detailed analysis of root links revealed that external links (EI and EE) were not only in higher proportion than internal links, which is characteristic of herringbone topology (Fitter 1987): they were also more abundant and longer in *C. quinoa* than in the other two species (Figs. 6 and 7). As these external links are the most absorptive root region (Paula & Pausas 2011), their abundance should give the quinoa populations a physiological advantage for nutrient uptake that their wild progenitor, *C. hircinum*, or their relative, *C. pallidicaule*, do not display.

Among the *C. quinoa* populations, the one from the arid altiplano of Bolivia had slightly fewer root tips (therefore fewer root branches) but consistently greater mean link length than the population from humid regions of Chile, which resulted in more total root length in the Bolivian population. Such compensation between link number and link length bears a general ecological significance as shown by Nicotra et al. (2002) in a comparison of 11 phylogenetically independent contrasts, namely: species originating from dry environments usually have fewer root links but with greater link length than species from high rainfall environments. Differences in root architecture among populations from arid and humid habitats suggest an adaptive response of the plants for soil water capture by means of an improved foraging capacity of the root system in the populations from dry environments (Reynolds et al. 2004). In fact, the greater root link length in *C. quinoa* from Bolivia, particularly at the very beginning of the root system growth, was associated with a fast and vigorous growth of the taproot in the early days after seed germination. The quinoa population from the arid area thus rapidly developed a higher soil foraging capacity than the population from the more humid area (Fig. 10).

Taking into account the water distribution in the soil profile and the scarcity of rainfall in such arid areas, a fast growth of the root system at depth seems more adapted to drought than multiplying root branches in the upper soil layers (Reader et al 1993; Padilla & Pugnaire 2007). Indeed, the vertical root elongation allows the plants to access water resources at depth, while dense root colonization in the upper soil layers, in the absence of new rainfall events, would rapidly led to root competition and complete water depletion. For the plant mineral nutrition, however, a dense root system in the superficial layers, where soil nutrients generally accumulate, seems more favorable. In fact, most part of the root system in the three studied species appeared concentrated between 5 and 50 cm depth, with a maximum in the 20-35 cm soil layer. But in the filtrating sandy soils typical of the southern altiplano of Bolivia, such a mobile element as nitrate moves easily at depth (Austin et al. 2004). In that

case, a dense and fast root growth beyond 50 cm depth would be useful to better capture water as well as nitrogen before they move beyond the root zone (Richards et al. 2007). This feature was indeed observed in the Bolivian population of *C. quinoa* which produced abundant lateral roots at -75 cm depth as early as the fifth week of growth, when none of the other populations had reached that depth (Fig. 10). These observations substantiate those made in an independent experiment previously conducted on the same species, where *C. quinoa* from Bolivia reached 1 m taproot length between 42 and 49 DAS, one to two weeks earlier than *C. quinoa* from Chile and *C. hircinum*, while *C. pallidicaule* remained limited to a 40 cm soil depth (Alvarez-Flores et al. submitted).

Specific root length (SRL) is another root trait of importance for root foraging, as it influences the capacity of the root system to proliferate through the soil, with thick roots penetrating more easily than fine ones into compacted or dry soil layers (Paula & Pausas 2011). As already found in our previous experiment (Fig. 5b in Alvarez-Flores et al. submitted), *C. pallidicaule* had significantly higher SRL values than the other two species. These two experiments gave similar species ranking, but with species mean values for SRL at 42 DAS about two-fold lower in the present study. This suggests a higher proportion of coarse roots in the root system of plants grown in rhizotrons placed outdoors compared to the previous experiment with plants in containers within a glasshouse. This difference could be due to the dryer and cooler soil conditions in the experiment with rhizotrons (mean air temperature =  $15,5 \pm 0,1^{\circ}\text{C}$  against  $25,1 \pm 0,6^{\circ}\text{C}$  in the glasshouse experiment). In an ecological perspective, while high SRL in *C. pallidicaule* could compensate for its low foraging capacity by a higher nutrient transport efficiency (Paula & Pausas 2011, Picon-Cochard et al. 2012), the low SRL of *C. quinoa* should enhance its ability to penetrate deeply tough soil layers, and at the same time improves the anchoring of plants significantly taller than those of *C. hircinum* and *C. pallidicaule* (see plant height and collar root diameter values in Table 2).

As a whole, the very fast root elongation, with thicker roots allowing an efficient deep soil penetration, and abundant and long external links of high absorptive capacity, make a suite of architectural root traits that should contribute significantly to the improved soil foraging of *C. quinoa* compared to *C. hircinum* and *C. pallidicaule*. This should be particularly true for the *C. quinoa* population from the southern altiplano of Bolivia, which showed the highest ranking in these traits of root foraging, and which actually thrives in a region of very poor soil and dry climate where initial seedling growth and establishment are

crucial.

## 5 CONCLUSION AND PERSPECTIVES

The present study brings the first detailed report of root system architecture and growth pattern in three Andean species of *Chenopodium*, including two cultivated ecotypes of *C. quinoa*. It makes up for a lack of information particularly in the root system of *C. quinoa* which up to now remains roughly parameterized in the few crop models developed for this species (see: AquaCrop by Geerts et al. 2007, and STICS by Lebonvallet 2008). Despite different growth conditions, our experiments in pots and rhizotrons found similar temporal patterns of root growth with the same ranking among the studied populations, which suggest that these patterns of the root system architecture are intrinsic to the studied *Chenopodium* populations (Ingram & Malamy 2010). When the present results obtained in controlled conditions will have been validated under real field conditions, the integration of these root elongation and branching parameters into plant growth models could allow for a more accurate description and understanding of the different capacities of root foraging and nutrient uptake in related species or ecotypes (Pagès 2011).

From an evolutionary perspective, one should bear in mind that natural selection as well as crop domestication relies on phenotypic selection (Lynch & Brown 2012). Considering that the higher root branching and foraging capacity of *C. quinoa* is associated to a higher biomass production than in its wild progenitor, *C. hircinum*, or its diploid relative, *C. pallidicaule*, it could be assumed that these root system traits have been indirectly selected during the polyploidization and domestication processes. Recent works on improved root systems in water-limited crops suggest that such indirect impacts on root branching and foraging might indeed have occurred in several crop species (Hammer et al. 2009, Palta et al. 2011, Wasson et al. 2012).

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## CHAPITRE 3

### ROOT ARCHITECTURE IN THREE ANDEAN *CHENOPODIUM* SPECIES: RESPONSES TO SOIL WATER DEFICIT

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## 1 INTRODUCTION

Soil water availability is a critical environmental cue for plants growing in arid regions or exposed to intermittent drought in more humid areas. The process of germination once engaged, seedling survival and early growth is directly dependent on the water supply in the shallow soil layers (e.g. León et al. 2011). Thereafter, the maintenance and growth of the young plants will rely on their capacity to acquire water and nutrient resources from a soil volume extended enough to meet their needs. Here, the architecture and the dynamics of the root system are of paramount importance for the exploration and exploitation of the soil by the growing plants, in particular in arid or semiarid environments where soil resources are limited or ephemeral (Janssens et al. 1990). In these water pulse-driven and often patchy environments, water and nutrient acquisition by plants becomes both spatially and temporally aleatory as it depends on complex interactions between the soil and the climate (the rainfall, principally) (Schwinning and Sala 2004). Under such unpredictable conditions, plant growth and survival requires not only adequate root system morphology, but also some degree of functional plasticity to modulate the root system architecture in response to changing conditions. Hodge (2004), Osmont et al. (2007), Hodge (2009), and Ingram and Malamy (2010) give comprehensive reviews of the functional role of root plasticity which they regard as a major mechanism for plant survival and species adaptation to environmental heterogeneity.

Defining a root system ideotype that optimize water and nutrient uptake is a difficult task, due to the multiple interactions among root traits, and between them and the environment (De Dorlodot et al. 2007, Osmont et al. 2007, Ingram and Malamy 2010). For example, while it is generally considered that root elongation is restricted under dry conditions (e.g. King and Bush 1985, Ogawa et al. 2005), there is plenty of evidence that root length can be increased under such conditions (e.g. Raja and Bishnoi 1990, Schmidhalter et al. 1998). The discrepancy in these results might arise because of interactions with the plant developmental stage or the degree of soil drying (Ito et al. 2006). Yet, some evidences have emerged regarding advantageous traits of root system architecture in low-resources environments. A herringbone architecture, for example, is recognized as more beneficial in these habitats as it reduces the intraplant root competition when nutrient resources are limited (Fitter et al. 1991, Fitter and Stickland 1991, Taub and Goldberg 1996). Similarly, fast root growth at early developmental stage, or long individual root segments are valuable traits that enhance the capacity of the root system to forage water and nutrients at depth (León et al.

2011, Palta et al 2011, Richards et al. 2007). Genotypic variation has been reported for many of these root traits such as root angle in sorghum lines (Singh et al 2011, 2012) or root length at seedling stage in different temperate cereals (Richards et al. 2007). This genotypic variation in root traits is now included in crop breeding programs (Lynch and Brown 2012), and also serves to classify functional plant types in natural ecosystems (Leva et al. 2009).

In an unpredictable environment, however, a rigid architectural design would make the root system poorly reactive, whereas developmental plasticity of the root system seems desirable to adaptatively alter the root system architecture shaped by the plant genome. The modular structure and relatively simple anatomy of the roots are key features that allow for highly adaptive responses of the root system to changing conditions, mostly through mere modifications in the root branching (Nibau et al. 2008, Ingram and Malamy 2010). These modifications are mediated by hormonal signals and, ultimately, by genetic factors (Osmont et al. 2007, Ingram and Malamy 2010, De Smet et al. 2012). As such, developmental plasticity should be a character like others attributes, subject to natural and anthropic selection to fit species to the particular conditions of different environments (Bradshaw 2006). Therefore, comparative studies of the variation in root branching across phenotypes from different ecological backgrounds would contribute to untie the 'rules of response' developed by plant species to cope with environmental variability (Hodge 2009 PCE Root decisions).

An interesting model for exploring such questions is provided by the vegetation of the Andes where the highly variable environment of tropical mountains extending over considerable ranges of latitude and altitude has generated an exceptional biodiversity (Troll 1968, Murra 1992a, b). Rising from the ocean level to more than 6000 m height, exposed to the changing influences of the ENSO on the western coast, the Amazonian basin on the east, and the polar air circulation from the south, the Andes present environmental gradients that range from rainy temperate lowlands of nutrient-rich soils on the Pacific coast and the Amazonian piedmont, to extremely arid and cold highlands of poor soils in the Cordillera. This variety of habitats, and the ancient civilizations that thrive there for millennia, have produced hotspots of biodiversity both in native and in crop species (National Research Council 1989, Zimmerer 1998, Tapia 2000). Among others, several *Chenopodium* species, wild or cultivated, are distributed from the Pacific coast to the Andean highlands where they face different environmental conditions, notably varying soil water resources. Comparing two ecotypes of *C. quinoa* and two populations of *C. hircinum* and *C. pallidicaule* from different ecological and agricultural backgrounds, previous studies have found great variation at the

population level in several traits controlling the root system architecture of the studied species and populations. It was concluded assuming that several of these traits might have enhanced the root foraging capacity of the plants at early growth stage, particularly in the cultivated ecotype from the low-resources habitat (Alvarez-Flores et al. 2012a, b). This feature would be of critical value for plants exposed to water deficit, especially in their early seedling developmental phase when plantlets are vulnerable to the drought spells typical of the erratic onset of the rainy season in the arid areas of the Andes.

To test for this, a comparative study was conducted to investigate the root system architecture and the rooting dynamics during the early growing phase of four Andean chenopods, from wild and cultivated populations, in order to identify contrasting architectural patterns in response to water deficit.

## 2 MATERIALS AND METHODS

The study site, plant populations, and other details of the experimental design are described in a previous paper (Alvarez-Flores et al. 2012b). It is just recalled here that a rhizotron experiment was conducted outdoors from the 18<sup>th</sup> of March to the 20<sup>th</sup> of April 2011 at the CEFÉ-CNRS experimental field (Montpellier, France, 43°38'19''N, 3°51'46''E), comparing four Andean *Chenopodium* populations: cultivated *C. quinoa* from the temperate and rainy lowlands with nutrient-rich soils in Chile, cultivated *C. quinoa* from the arid and cold highlands with nutrient-poor soils in Bolivia, cultivated *C. pallidicaule* and wild *C. hircinum* both from the semi-arid highlands with nutrient-poor soils in Bolivia (see Table 1 in Alvarez-Flores et al. 2012b).

### 2.1 Soil substrate and water deficit treatments

Rhizotrons were filled with a sandy substrate of a composition comparable to that of the soils commonly found in the arid areas of the Bolivian altiplano (Table 1). Following the equations of Saxton and Rawls (2006), the granulometric composition of the substrate was used to estimate its water content at field capacity and at the wilting point, showing values of 0.12 cm<sup>3</sup> water/cm<sup>3</sup> soil and 0.05 cm<sup>3</sup> water/cm<sup>3</sup> soil respectively. The substrate was humidified just before filling the rhizotrons, using a nutritive solution (N: 16%, P: 10 %, K: 24 %, MgO: 3 %, micro-elements: 2.5 g/L). The process of substrate humidification and rhizotron filling took place at night within a closed room to avoid water loss due to evaporation in the day.

Table 1. Physical properties of the substrate used in the present experiment compared to mean values observed in soils from the arid altiplano of Bolivia (n=123) and the rainy littoral of Chile (n=9).

Soil composition	This experiment	Bolivia		Chile	
		Mean	Range	Mean	Range
Clay (%)	6.3	4,9	1.4 to 12	25	23 to 68
Limon (%)	4.5	11,5	11 to 46	30	20 to 48
Sand (%)	84.8	83,7	28 to 96	29	15 to 34
Organic matter (%)	3.0	2,6	1 to 7	15.9	21 to 13
Total nitrogen (%)	0.1	0,07	0.02 to 0.2	n.a.	n.a
Organic Carbon (%)	1.1	1,5	0.7 to 5.0	n.a.	n.a.
C/N	11.2	29,6	4 to 100.35	n.a.	n.a.
pH	8.1	7,4	6 to 8	5.1	4 to 5
CEC	9.0	17,9	3 to 91	7.0	3 to 11

Sources of the field soil data: Cardenas and Choque (2008) for Bolivia, personal data for Chile.

All rhizotrons were watered at field capacity (water content = 12% vol.) in the 5-cm deep seeding bed (Fig. 1). Beneath the seedbed, the substrate was humidified at field capacity over the entire rhizotron depth in the control treatment (T0), while in the water deficit treatments, a layer of 15 cm (T1), 30 cm (T2), or 77 cm (T3), was filled with a substrate near the wilting point (water content = 7% vol.). Dry seeds were sown directly in the moist seedbed, with a 2 cm cap of dry sand at the top of the rhizotron to limit evaporation. Plants were grown from the water stored in the rhizotrons, without additional irrigation in the course of the 6-week experiment. The substrate water content was controlled two times and in two depths during the experiment using the classical gravimetric method (Table A-2.).

Thermic probes measured the temperature of the substrate at a 25 cm depth in two rhizotrons, one in the control and one in the T3 treatment. These temperature data were stored in a Campbell CR-21 data logger with a frequency of 30 minutes. Over the course of the experiment, soil temperature varied between 4.2 and 35.3 °C in T0 (mean = 15.3 °C ± 0.1) and between 3.8 and 35.8 °C in T3 (mean = 15.1 °C ± 0.1), without significant difference between the control and the dry treatment (mean difference = 0.2°C, P = 0.12).



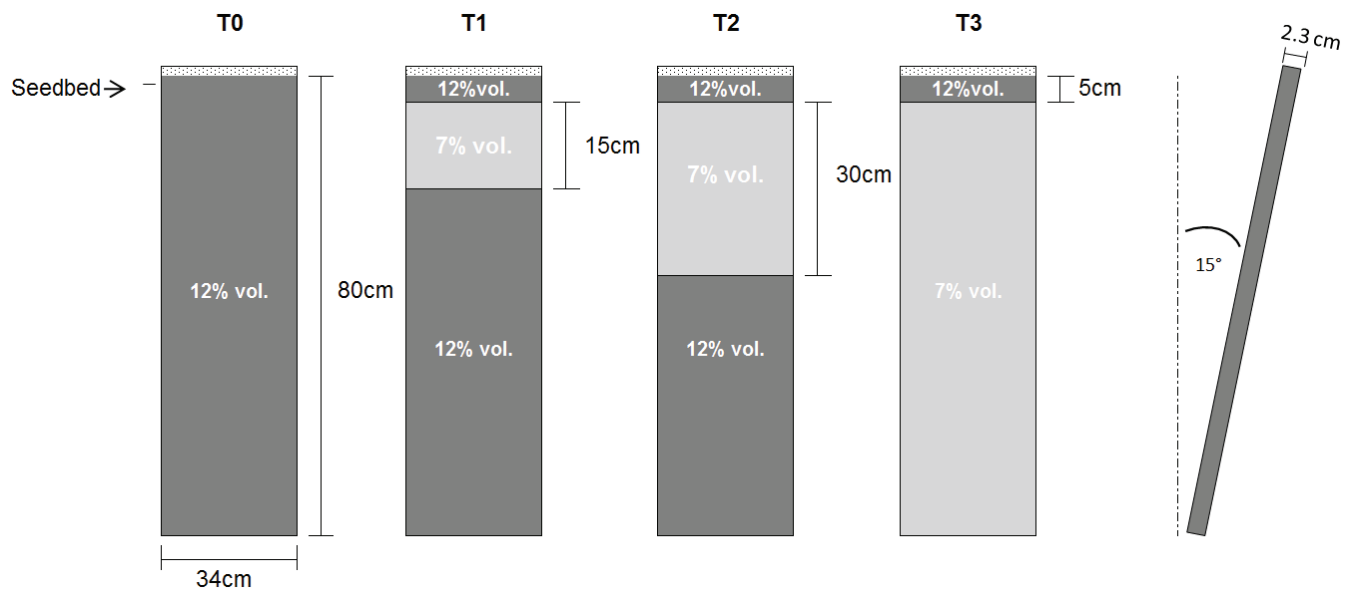


Figure 1. Diagram of the control (T0) and water deficit treatments (T1 to T3) applied in the rhizotron experiment.

## 2.2 Plant sampling and measurements

With reference to Alvarez-Flores et al. (2012b) for more experimental details, it is recalled that five replicate plants per population were followed weekly from 7 to 42 days after sowing (DAS) for non-destructive growth measurements. Regarding the root system growth and development, after scanning the image of the root system visible through the rhizotron, the morphological and architectural procedures of the WinRHIZO Pro2009 software (Regent Instruments Inc., Quebec, Canada) allowed to calculate weekly values of the following root system traits: total tip number (TTN), total link number (TLN), total root length (TRL, m), total root length per link type, maximum rooting depth (MRD, cm). Links are defined as root segments between two branch junctions or a branch junction and a meristem, and are classified as external-external (EE) when external links join other external links, external-internal (EI) when external links join internal links, and internal-internal (II) when links do not terminate in a meristem (Fitter 1987). The sum of the numbers of EI and EE links is equivalent to the total tip number (or magnitude) of the root system.

Two root system topological indices were calculated. After Glimskär (2000), the topological quotient index (TQI) was calculated from the altitude  $\alpha$  of the root system (i.e. the number of links of the longest path from the root collar to an external tip) and its magnitude  $\mu$  (i.e. the total number of tips in the root system), calculating the average of:

$$\text{TQI} = \log(\alpha)/\log(\mu) \quad (\text{Eq. 1})$$

for the sets of altitude and magnitude values observed over the course of the experiment in each population x treatment combination. The same set of data allowed calculating the topological slope index (TI) as the slope of the regression of  $\log(\alpha)$  on  $\log(\mu)$  (Fitter and Stickland 1991). For both topological indices, values close to 1 indicate a more "herringbone" structure of the root system, with branching mostly confined to the main axis.

The mean inter-root distance (cm) was calculated with the following equation (Tennant 1975) where L is root density (cm root cm<sup>-3</sup> soil) in the considered soil volume:

$$C = 2 (\pi L)^{-1/2} \quad (\text{Eq. 2})$$

By the end of the experiment (42 DAS), destructive aerial and root growth measurements included leaf, stem and root dry masses, total leaf area per plant, specific leaf area, root collar diameter, specific root length from the roots growing onto the windowpane, and root/shoot ratio from the bulk of the root system biomass.

### 2.3 Data analyses

Statistical procedures were applied using STATISTICA version 7.1 (Statsoft Inc., Tulsa, USA). Differences in plant traits among populations and treatments were tested using factorial analysis of variance (ANOVA) after log transformation of the data when necessary to improve normality and homoscedasticity. Tukey's HSD was used to compare population and treatment means at  $P < 0.05$ .

## 3 RESULTS

### 3.1 Aerial growth and bulk root parameters

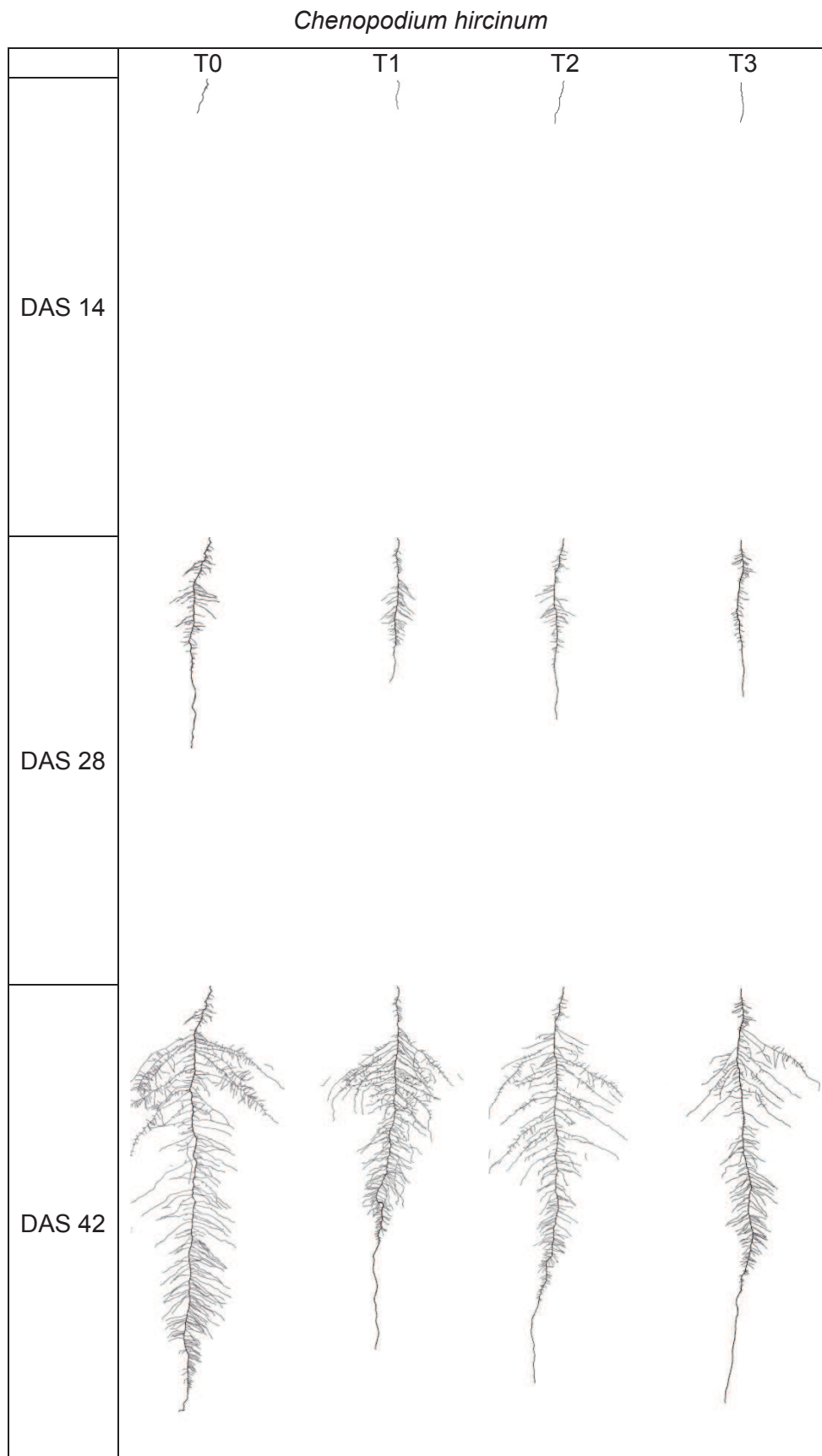
Under well-watered conditions, great differences in total biomass production at 42 DAS were observed among the studied populations, with a decreasing ranking as follows: *C. quinoa* from dry habitat, *C. quinoa* from rainy habitat, *C. hircinum*, and *C. pallidicaule* (see a detailed analysis of the T0 control treatment in Alvarez-Flores et al. 2012b). The studied populations appeared fairly insensitive to the lowest level of water deficit corresponding to T1 since plants in T1 did not show any significant reduction in biomass production compared to the control (Table 2). On the contrary, all populations showed significantly reduced plant biomass production in response to lower water availability in treatments T2 and T3.

In comparison to well-watered plants, droughted plants in T3 showed lower values of leaf area ratio in *C. quinoa* from Chili and *C. pallidicaule*, while no difference was observed for *C. quinoa* from dry habitat or *C. hircinum* (Table 2). Whatever the population, treatments T1 and T2 did not show any effect on LAR. Similarly, specific leaf area of droughted plants in T3 was lower (*ca.* 25% less) than in well-watered plants for *C. quinoa* from Chili and *C. pallidicaule*, while no difference was observed in the other two populations. Whatever the population, the two intermediate stress treatments (T1, T2) had no effect on SLA (Table 2).

The total root length in plants of treatment T1 remained similar to the control, while it was significantly decreased in the droughted plants of T2 and T3 (Table 3, Fig. 2, 3). The reduction in TRL in the different populations followed their ranking of total biomass production, with the lowest reduction in *C. quinoa* from dry habitat (-39% and -38% in T2 and T3 respectively), and the highest in *C. pallidicaule* (-48% and -85% in T2 and T3 respectively). As a consequence, the root:shoot ratio increased significantly in T3 for all populations except HI where it decreased (Table 3). The effect of treatments T1 and T2 was indefinite and generally non significant.

Noticeably, the mean specific root length was not changed by the water deficit in any of the studied populations (Table 3). The ranking observed in well-watered plants was thus maintained under water deficit, with *C. quinoa* from dry habitat having the coarsest roots and *C. pallidicaule* the finest ones.

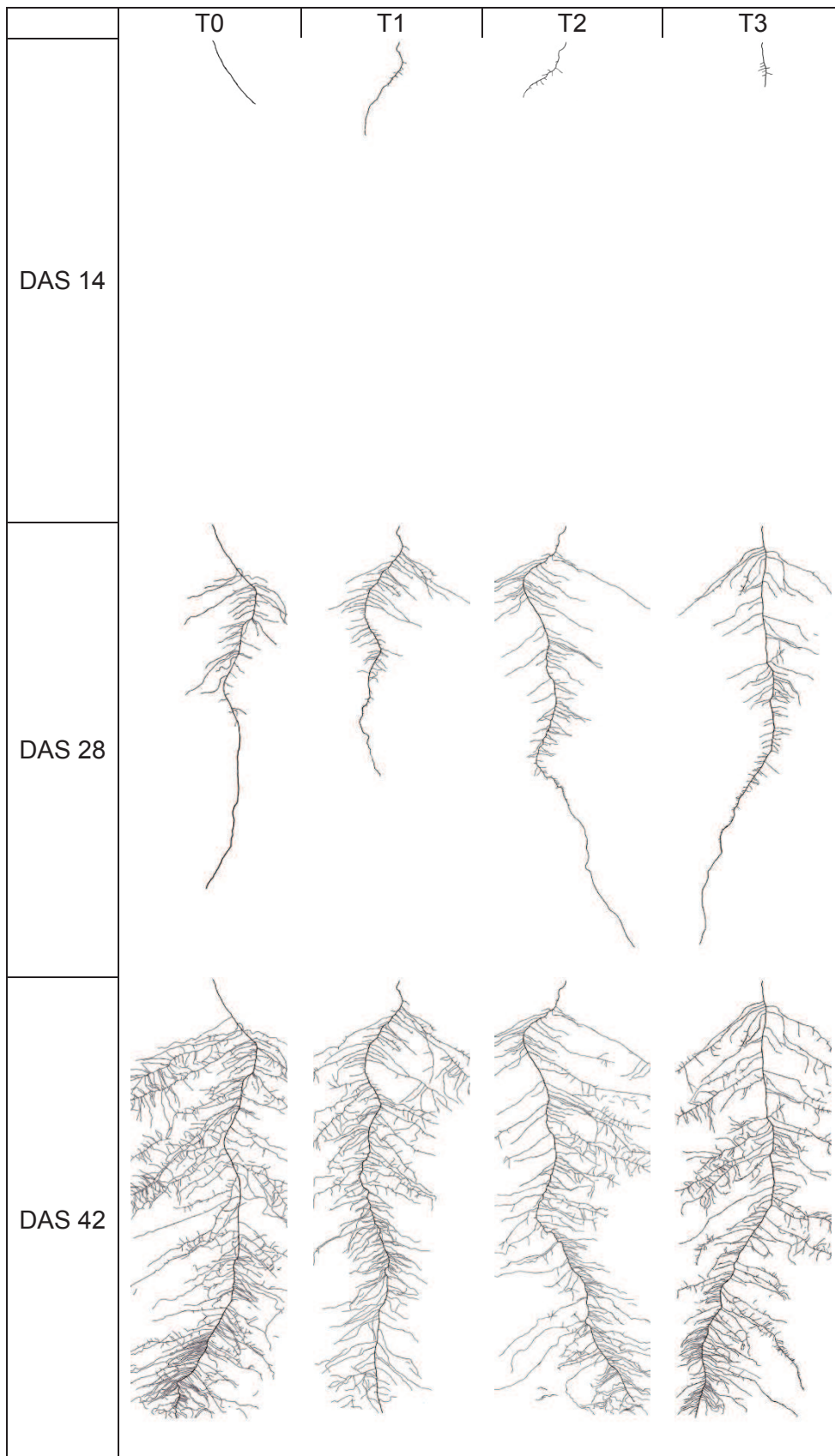
Figure 2. Time-course of the root system at DAS 14, 28 and 42 for the 4 treatments (see text for details).



*Chenopodium pallidicaule*

	T0	T1	T2	T3
DAS 14				
DAS 28				
DAS 42				

*Chenopodium quinoa* Bolivie



*Chenopodium quinoa* Chili

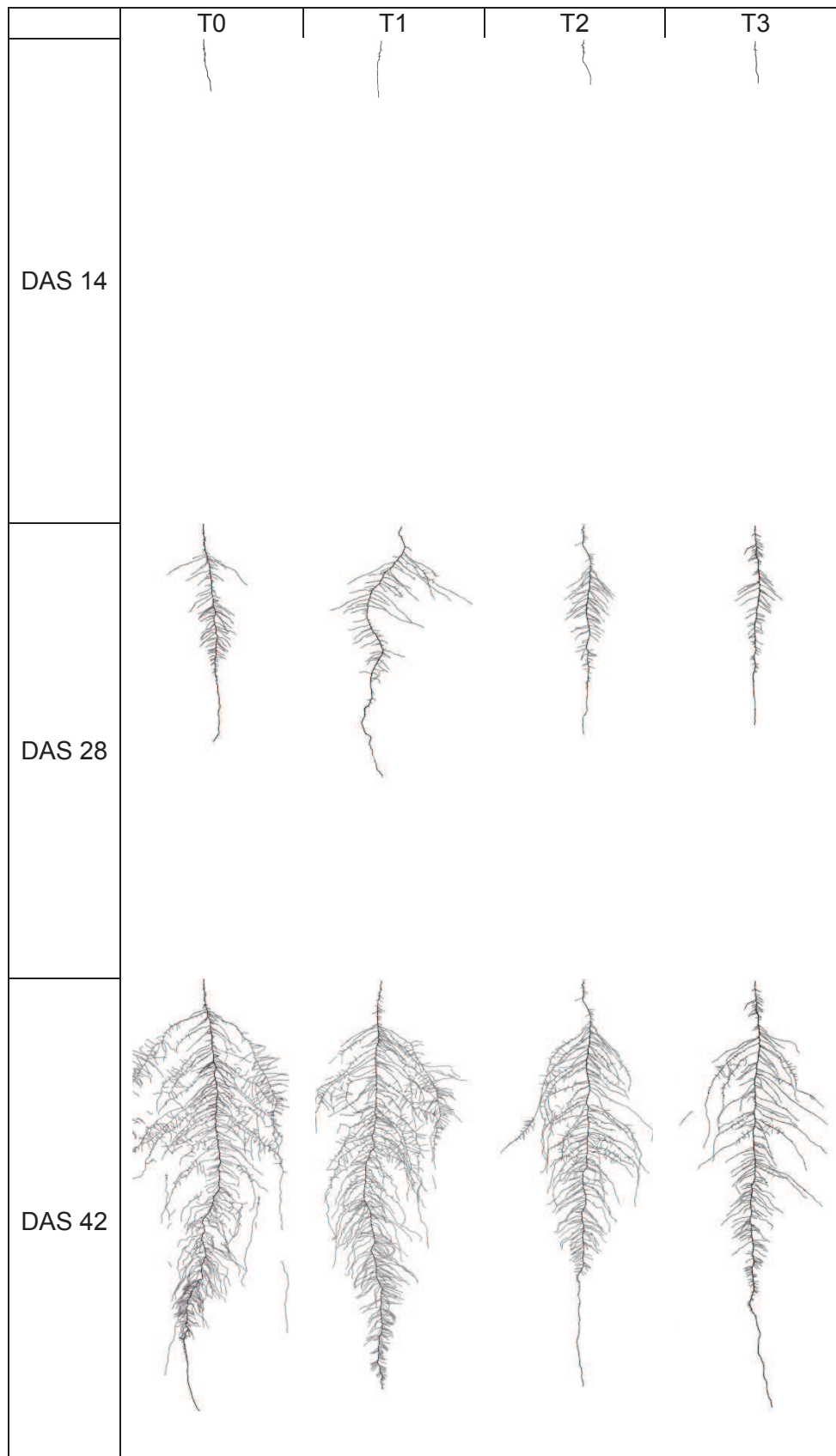


Table 2. Mean values of plant and root system traits of four *Chenopodium* populations at 42 DAS in the four treatments (see Material & Methods for a description of the treatments). LA: leaf area (cm<sup>2</sup>/plant), SLA: specific leaf area (m<sup>2</sup>/g), LAR: leaf area ratio (m<sup>2</sup>/g total plant mass), TM: total plant mass (g/plant), R/S: root shoot ratio (g/g) (S.E. = standard error, n = 5; within columns, values followed by the same letter across treatments are not statistically different at P = 0.05).

Treatment	Trait	Unit	<i>C. quinoa</i> dry habitat		<i>C. quinoa</i> rainy habitat		<i>C. hircinum</i>		<i>C. pallidicaule</i>					
			Mean	SE	Mean	SE	Mean	SE	Mean	SE				
Control	LA	cm <sup>2</sup>	97,99	3,74	a	53,98	1,28	a	21,66	4,79	a	9,98	2,26	a
	SLA	cm <sup>2</sup> g <sup>-1</sup>	76,21	13,46	a	99,11	3,00	a	89,00	3,21	a	113,09	3,85	a
	LAR	cm <sup>2</sup> g <sup>-1</sup>	52,12	8,49	a	63,62	5,32	a	52,77	4,94	a	76,71	4,62	a
	TM	g	2,00	0,37	a	0,87	0,10	a	0,40	0,08	a	0,13	0,03	a
	R/S	g g <sup>-1</sup>	0,28	0,01	a	0,39	0,02	a	0,45	0,01	a	0,31	0,03	a
T1	LA	cm <sup>2</sup>	72,88	8,45	ab	45,61	8,63	a	18,83	1,71	a	7,51	1,63	a
	SLA	cm <sup>2</sup> g <sup>-1</sup>	77,46	7,85	a	104,17	4,19	a	80,01	4,88	a	116,92	3,24	a
	LAR	cm <sup>2</sup> g <sup>-1</sup>	53,40	5,24	a	64,40	2,59	a	48,96	1,91	a	69,94	3,67	ab
	TM	g	1,37	0,10	ab	0,71	0,14	ab	0,39	0,04	ab	0,11	0,02	ab
	R/S	g g <sup>-1</sup>	0,25	0,03	a	0,37	0,03	a	0,39	0,02	a	0,36	0,04	a
T2	LA	cm <sup>2</sup>	57,77	7,07	bc	25,85	1,84	b	16,34	0,89	b	3,38	0,33	b
	SLA	cm <sup>2</sup> g <sup>-1</sup>	88,13	2,87	a	105,94	3,26	b	96,91	16,86	a	86,54	12,15	b
	LAR	cm <sup>2</sup> g <sup>-1</sup>	55,34	4,46	a	62,32	4,41	a	62,00	9,49	a	52,86	7,27	b
	TM	g	1,07	0,16	b	0,52	0,10	b	0,28	0,04	b	0,07	0,01	b
	R/S	g g <sup>-1</sup>	0,34	0,06	a	0,48	0,05	ab	0,37	0,05	b	0,42	0,10	b
T3	LA	cm <sup>2</sup>	41,72	2,93	c	16,70	1,44	c	9,45	0,41	c	0,70	0,11	c
	SLA	cm <sup>2</sup> g <sup>-1</sup>	91,94	6,83	a	75,04	2,09	c	90,48	5,54	a	82,12	19,38	b
	LAR	cm <sup>2</sup> g <sup>-1</sup>	56,39	2,63	a	44,21	1,24	b	62,41	3,01	a	35,47	7,94	c
	TM	g	0,74	0,05	c	0,38	0,03	c	0,15	0,01	c	0,03	0,01	c
	R/S	g g <sup>-1</sup>	0,43	0,04	b	0,49	0,04	b	0,33	0,03	c	0,71	0,14	c

### 3.2 Effect of the soil water deficit on temporal changes in rooting depth

Facing a dry soil layer more or less thick, the studied populations exhibited different responses in the progress of the taproot at depth (Fig. 2). Maximum rooting depth was most limited in *C. pallidicaule*, reaching 50 cm depth as a maximum when the other three populations all reached 80 cm under favorable conditions.



Table 3. Mean values of plant and root system traits of four *Chenopodium* populations at 42 DAS in the four treatments (see Material & Methods for a description of the treatments). TRL: total root length (m/plant), SRL: specific root length (m/g), TQI: topological quotient index. (S.E. = standard error, n = 5; within columns, values followed by the same letter across treatments are not statistically different at P = 0.05).

Treatment	Trait	Unit	<i>C. quinoa</i> dry habitat			<i>C. quinoa</i> rainy habitat			<i>C. hircinum</i>		<i>C. pallidicaule</i>			
			Mean	SE		Mean	SE		Mean	SE	Mean	SE		
Control	TRL	m	24,15	3,39	a	16,41	2,68	a	12,65	2,00	a	4,77	0,41	a
	SRL	m g <sup>-1</sup>	57,98	3,82	a	75,10	12,23	a	106,47	10,00	a	179,70	35,52	a
	TQI		0,77	0,01	a	0,77	0,01	a	0,77	0,02	a	0,77	0,01	a
T1	TRL	m	18,50	1,20	ab	17,74	2,91	ab	8,85	0,36	ab	3,67	0,22	a
	SRL	m g <sup>-1</sup>	69,30	7,27	a	98,34	12,08	a	84,44	6,79	a	109,44	14,74	a
	TQI		0,88	0,02	b	0,90	0,01	b	0,88	0,01	b	0,89	0,01	b
T2	TRL	m	14,78	0,50	b	12,88	0,66	b	6,93	0,27	bc	2,49	0,11	b
	SRL	m g <sup>-1</sup>	62,22	12,78	a	116,07	24,82	a	97,02	4,13	a	149,21	47,07	a
	TQI		0,87	0,02	b	0,89		b	0,88	0,13	b	0,89	0,01	b
T3	TRL	m	14,96	0,48	b	7,11	1,00	c	4,65	0,20	c	0,72	0,09	c
	SRL	m g <sup>-1</sup>	73,37	5,51	a	69,90	1,87	a	125,18	7,45	a	142,12	18,57	b
	TQI		0,90	0,02	b	0,90	0,02	b	0,93	0,01	b	0,87	0,02	b

### 3.2 Effect of the soil water deficit on temporal changes in rooting depth

Facing a dry soil layer more or less thick, the studied populations exhibited different responses in the progress of the taproot at depth (Fig. 2). Maximum rooting depth was most limited in *C. pallidicaule*, reaching 50 cm depth as a maximum when the other three populations all reached 80 cm under favorable conditions.

Under well-watered conditions, the four populations displayed a similar rooting pattern with a first 3-week period of moderate taproot elongation at an average vertical growth rate of *ca.* 1 cm depth day<sup>-1</sup> (0.5 cm day<sup>-1</sup> in *C. pallidicaule*), followed by another 3-week period of fast vertical growth at nearly 3 cm depth day<sup>-1</sup> down to 75-80 cm (respectively 2 cm depth day<sup>-1</sup> and 50 cm in *C. pallidicaule*). Vertical taproot growth in that second period was still faster in *C. quinoa* from dry habitat (*ca.* 4 cm day<sup>-1</sup>), allowing the plants to reach 80 cm depth as early as the fifth week of growth.

The temporal rooting pattern observed in well-watered plants was little affected by the intermediate stress treatments T1 and T2, except in *C. quinoa* from dry habitat. In that population, T1 slowed the taproot progress in the fourth and fifth weeks to a rate similar to that shown in *C. quinoa* from Chili or *C. hircinum*. In T2, on the contrary, the plants of *C. quinoa* from dry habitat responded to the lower water availability by an accelerated taproot growth as early as the third and fourth weeks (with mean vertical rates of 2.6 and 4.3 cm day<sup>-1</sup> respectively). This accelerated growth of the taproot allowed the Bolivian *C. quinoa* in T2 to escape the dry soil layer from 21 DAS on and to reach 60 cm depth at 28 DAS.

Treatment T3 exhibited the most contrasting responses among populations as regards plant rooting depth in dry soil. In *C. pallidicaule* the vertical growth of the taproot was virtually stopped from 28 DAS on, the rooting depth hardly reaching half the value of control plants. In *C. hircinum*, the soil water deficit did not change the temporal pattern of maximum rooting depth observed in well-watered plants.

On the contrary, both populations of *C. quinoa* showed an accelerated taproot growth at low water availability, a process observed not before the fifth week in the Chilean ecotype but occurring as early as the third week in the Bolivian one.

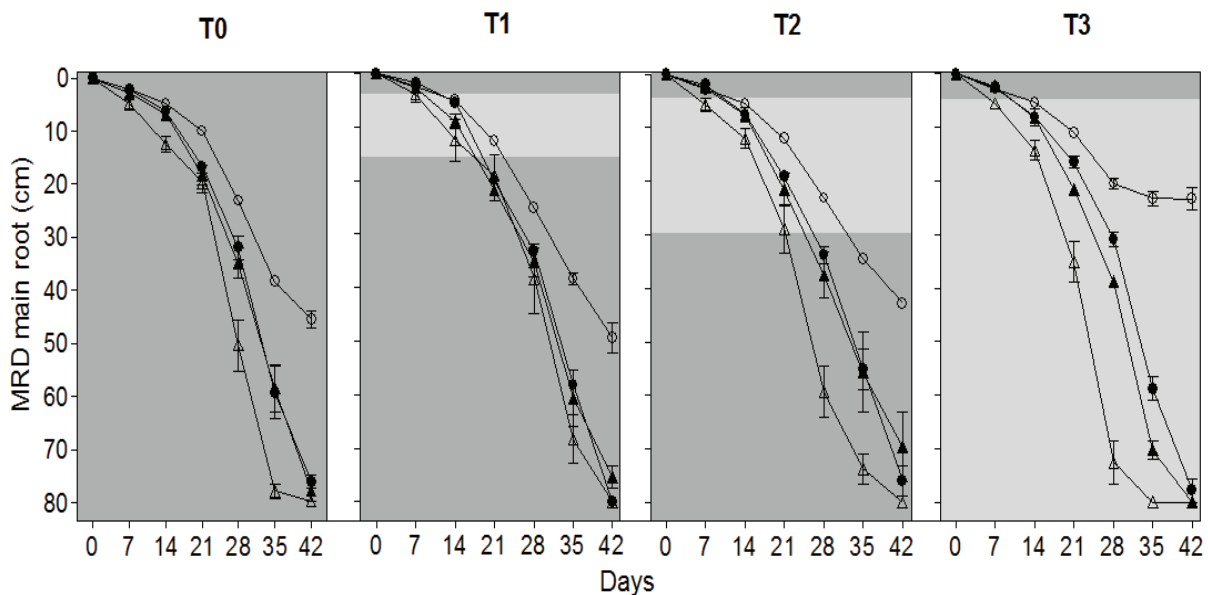


Figure 3. Dynamic of maximal root depth (MRD) in the four studied populations of *Chenopodium quinoa* from dry habitat ( $\Delta$ ), *C. quinoa* from rainy habitat ( $\blacktriangle$ ), *C. hircinum* ( $\bullet$ ) and *C. pallidicaule* ( $\circ$ ) (n = 5, vertical bars show standard errors, light grey areas show the substrate layers with low water content).

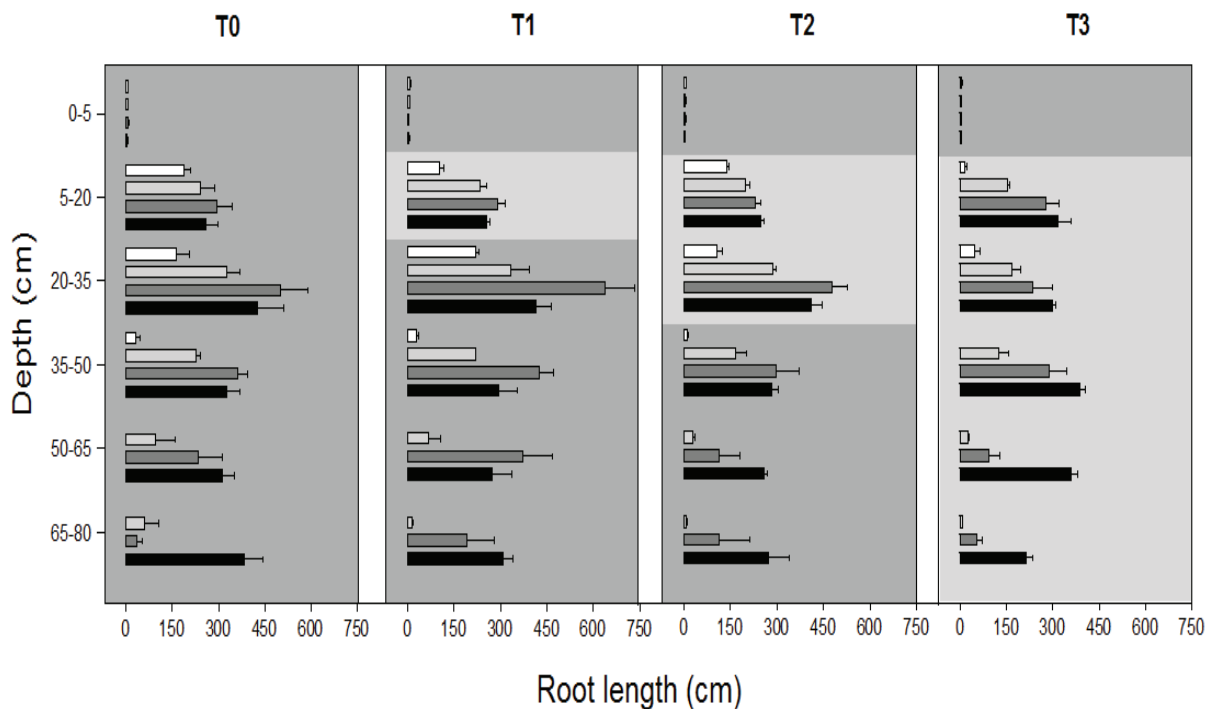


Figure 4. Vertical distribution of root length at 42 DAS in the four studied *Chenopodium* populations and the four treatments (*C. quinoa* from dry habitat: black, *C. quinoa* from rainy habitat: dark grey, *C. hircinum*: light grey, *C. pallidicaule*: white) (n = 5, horizontal bars show standard errors, light grey areas show substrate layers with low water content).

Compared to the control treatment, the accelerated taproot growth of *C. quinoa* from dry habitat in T3 allowed the plants to explore 15 cm more of the soil profile at 21 DAS, and 20 cm more at 28 DAS.

### 3.3 Effect of the soil water deficit on the vertical root distribution

Under well-watered conditions, the 20-35 cm layer concentrated the greatest root length in all the studied populations, but these showed differences in the distribution of their root system at depth: while *C. pallidicaule* was virtually absent beneath 35 cm depth, *C. quinoa* from rainy habitat and *C. hircinum* decreased progressively down to 80 cm, and *C. quinoa* from dry habitat densely occupied the substrate at depth with a root length still amounting 350 cm in the deepest layer (Fig. 2, 4, Table A-1).

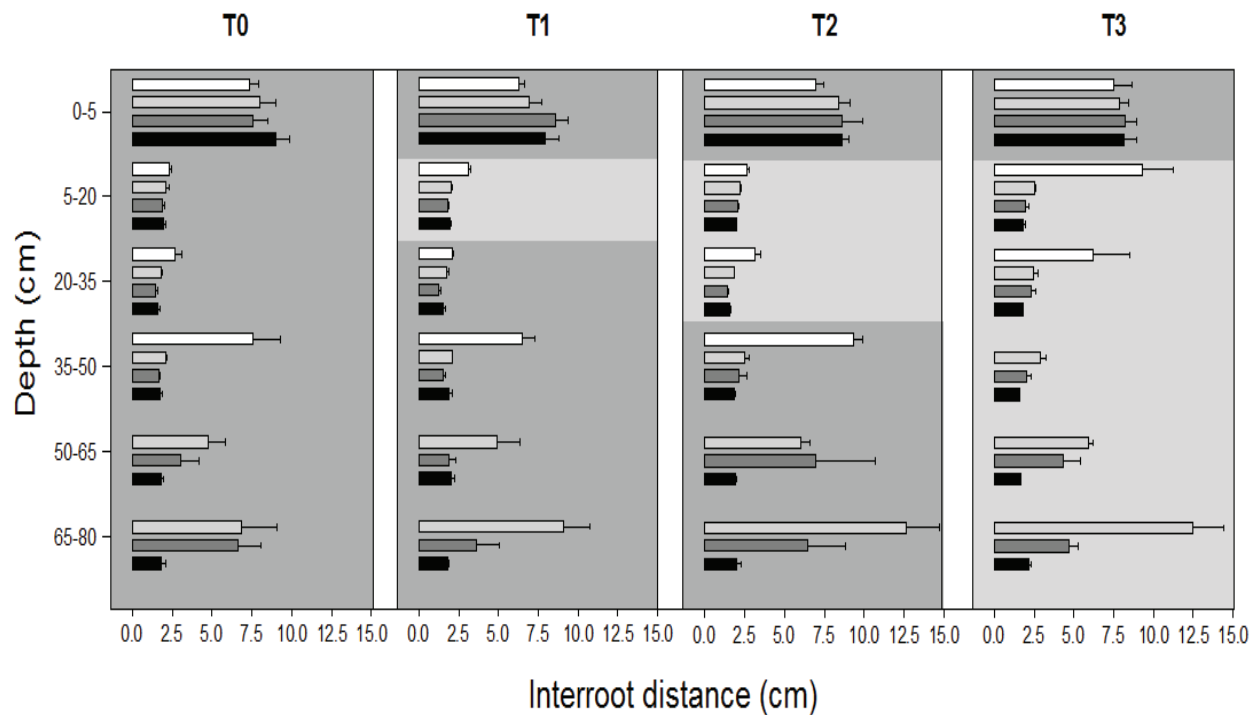


Figure 5. Vertical distribution of inter-root distance at 42 DAS in the four studied *Chenopodium* populations and the four treatments (*C. quinoa* from dry habitat: black, *C. quinoa* from rainy habitat: dark grey, *C. hircinum*: light grey, *C. pallidicaule*: white) (n = 5, bars show standard errors).

The more uniform rooting pattern in *C. quinoa* from dry habitat resulted in values of inter-root distance ranging narrowly between 1.6 and 2 cm among the different substrate layers (except the 0-5 cm layer), while this trait varied by a factor of at least three in the other populations (Fig. 5).

This vertical rooting pattern was little affected by the intermediate water deficit treatments, and the 20-35 cm layer still concentrated the largest amount of root length even when water availability in that layer was reduced by treatment T2. Root length in the dry substrate layers of T1 and T2 was not reduced in comparison to the control in any of the studied populations and, as a result, inter-root distance did not change in these dry layers (Fig. 5). Root proliferation beneath these dry layers was unchanged and even stimulated in the case of *C. quinoa* from rainy habitat in T1.

Root length was reduced in T3 in almost all substrate layers and all populations. The reduction was the highest in the 20-35 cm layer, resulting in a more uniform root length distribution down to 50 cm depth (except in *C. pallidicaule* which stopped growing beneath 35 cm depth). The two ecotypes of *C. quinoa* maintained high root length in the 35-50 cm

layer. *C. quinoa* from dry habitat in particular showed dense soil colonization at depth despite the low water availability. As a consequence, *C. quinoa* from dry habitat maintained inter-root distances near those in the control treatment, whereas the other three populations showed markedly less root density under dry conditions (Fig. 5).

### 3.4 Root system architecture

Under well-watered conditions, all the studied *Chenopodium* populations showed a herringbone topology (see Alvarez-Flores et al. 2012b for a detailed analysis). This root topology was reinforced in case of water deficit, all populations showing significantly higher values of the topological quotient index (Table 2) and of the slope of the regression of  $\log(\alpha)$  on  $\log(\mu)$  in treatment T3 (Fig. 6).

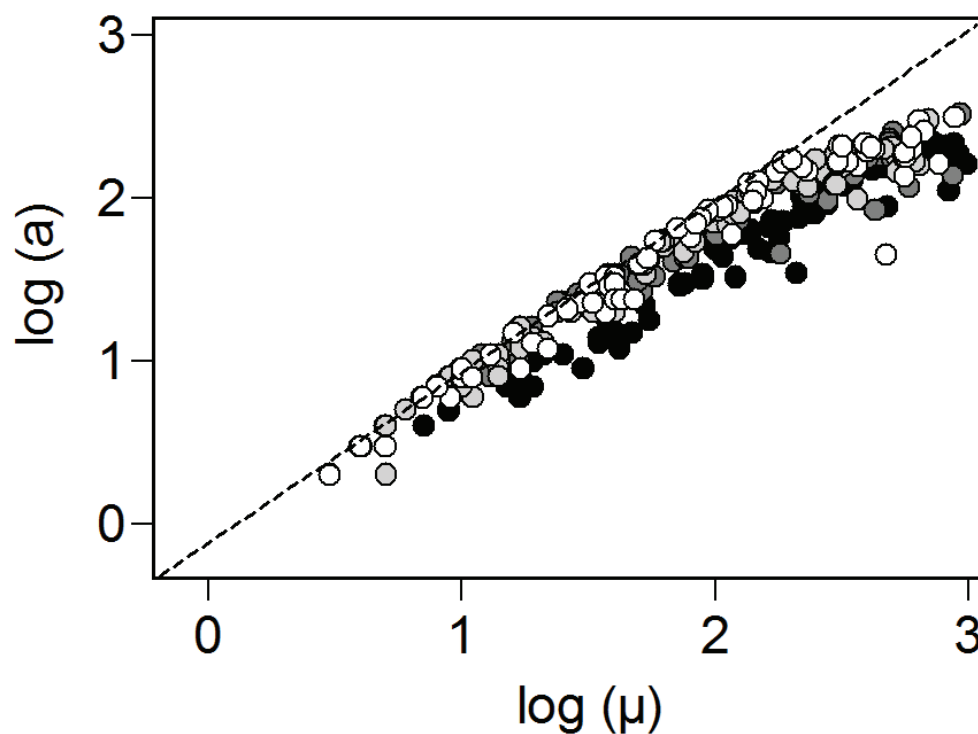


Figure 6. Regression of  $\log(\alpha)$  on  $\log(\mu)$  observed during the experiment in four *Chenopodium* populations (*C. quinoa* from dry habitat, *C. quinoa* from rainy habitat, *C. hircinum* and *C. pallidicaule*) in four water treatments (black circles: control; dark grey circles: T1, light grey circles: T2, white circles: T3; populations were gathered by treatment) (regressions in the control ( $y = 0.81x - 0.07$ ,  $r^2 = 0.96$ ,  $n = 81$ ) and the T3 treatments ( $y = 0.86x + 0.07$ ,  $r^2 = 0.94$ ,  $n = 81$ ) were not statistically different  $F = 4.87$ ,  $n = 162$ ,  $dl = 1$ ,  $P = 0.03$ ).

In general, the mean link length was not affected by water deficit and, when present, the effects were contradictory: in *C. quinoa* from rainy habitat, II links in T1 and EI links in T2 and T3 were significantly longer than in the control; whereas in *C. hircinum*, EI links in T1 were significantly shorter than in the control (Table 4). Whatever the water treatment, *C. quinoa* from dry habitat showed generally higher mean length of II and EI links than the other populations.

Mean link number was reduced by water deficit, marginally so in T1 but more importantly in T2 and T3 where all populations were affected (although to a lesser extent in *C. quinoa* from dry habitat) (Table 5). The reductions in link numbers concerned EI and EE links equally. They resulted in decreased total tip numbers (or root system magnitude) in T3 compared to the control, with values lower by 14% and 21% in *C. quinoa* from dry and from rainy habitat respectively, and by 42% and 61% in *C. hircinum* and *C. pallidicaule*.

Table 4. Mean link length (cm) by link type at 42 DAS in four *Chenopodium* populations. (II: Internal – Internal links, EI: External – Internal links, EE: External – External links) (S.E. = standard error, n = 5; within columns, similar letters show similar values across treatments at P = 0.05).

Treatment	Link type	<i>C. quinoa</i> (dry)		<i>C. quinoa</i> (rainy)		<i>C. hircinum</i>		<i>C. pallidicaule</i>	
		Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
T0	II	1,0a	0,03	0,6a	0,02	0,5a	0,01	0,6a	0,03
	EI	2,0a	0,05	1,2a	0,03	1,7a	0,04	1,6a	0,08
	EE	1,7a	0,09	1,8a	0,11	1,8a	0,15	2,4a	0,28
T1	II	1,0a	0,03	0,7b	0,02	0,5a	0,02	0,6a	0,03
	EI	2,2a	0,06	1,3ab	0,03	1,2b	0,04	1,5a	0,07
	EE	1,8a	0,10	1,6a	0,10	1,9a	0,22	2,1a	0,28
T2	II	1,1a	0,04	0,7ab	0,02	0,5a	0,02	0,6a	0,03
	EI	2,3a	0,06	1,5bc	0,05	1,6ab	0,05	1,8a	0,08
	EE	1,7a	0,11	2,1a	0,16	2,3a	0,22	2,3a	0,31
T3	II	1,1a	0,03	0,6a	0,02	0,5a	0,02	0,7a	0,05
	EI	2,2a	0,05	1,6c	0,04	1,6a	0,06	1,5a	0,12
	EE	1,5a	0,07	2,0a	0,19	1,2a	0,20	1,8a	0,42

Table 5. Mean link number by link type at 42 DAS in four *Chenopodium* populations. (II: Internal – Internal links, EI: External – Internal links, EE: External – External links) (S.E. = standard error, n = 5; within columns, similar letters show similar values across treatments at P = 0.05).

Treatment	Link type	<i>C. quinoa</i> (dry)		<i>C. quinoa</i> (rainy)		<i>C. hircinum</i>		<i>C. pallidicaule</i>	
		Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
T0	II	622,2a	10,2	743,5a	7,9	456,8a	7,9	142,3a	5,5
	EI	473,4a	8,0	511,8a	7,9	374,2a	7,0	110,5a	4,7
	EE	167,5a	6,4	152,0a	4,2	75,0a	5,0	29,87a	3,4
T1	II	534,3b	24,6	827,5a	13,7	458,0a	28,8	145,8a	16,9
	EI	444,3a	19,8	621,5a	20,3	355,0a	19,0	120,5a	6,2
	EE	140,3ab	17,3	143,5ab	14,2	64,7ab	15,5	22,8a	4,0
T2	II	536,0b	16,5	501,3b	27,1	410,0a	17,6	118,0a	17,8
	EI	422,5a	26,8	402,0b	26,3	358,8a	17,9	98,3a	2,5
	EE	111,8b	15,6	92,3b	18,6	50,8b	6,3b	18,3ab	4,5
T3	II	608,0ab	28,2	549,5b	35,2	285,3b	17,6	67,3b	15,8
	EI	459,5a	19,0	460,5b	34,1	229,0b	29,7	55,7b	6,8
	EE	93,8b	15,2	68,3b	11,2	31,3b	6,8	12,7b	4,1

### 3 DISCUSSION

This study is the first detailed report of root system responses to increasing water deficit among *C. pallidicaule*, *C. hircinum* and two ecotypes of *C. quinoa*. In any of these four populations, a 15-cm thick layer of dry substrate did not reduce the production of biomass in 6-week old seedlings. Instead, in a substrate at wilting point over a 30-cm thick layer or down to 80 cm depth, significant biomass losses were observed, but with different intensities depending on the population.

The tolerance of the plants to the soil water deficit in treatment T1 could be related the root growth and proliferation remaining unaffected both within and beneath the dry substrate layer, which resulted in virtually unchanged values of total root length, inter-root distances and total tip numbers in T1 compared to the control (Fig. 2, 4, 5, Tables 3 and 5). On a methodological point of view, it could be objected that a water content of 7% in a sandy substrate might still be enough to provide the plants with adequate water availability. Besides, high capillarity in the sandy substrate could have allowed the dry layers to become humidified from the moist substrate beneath them. However, checking the dry substrate layers for their water content during the course of the experiment suggests that capillary rise did not occur in the present study. Besides, the significantly reduced plant biomass in treatments T2 and T3

clearly proves the negative effect of a substrate water content as low as 7% for the plant growth and biomass production in the studied species. Hence, it is considered that the present results indeed illustrate the capacity of young chenopod seedlings to tolerate a limited water availability in the soil layer just beneath the seedbed. Such a situation is frequent in arid areas or in drought years when some isolated rainfall events may be sufficient to promote seed germination but not enough to connect the superficial soil layer where seedling root growth begins to the deeper soil layers where water reserve may have accumulated in the previous rainfall season (Schwinning and Sala 2004). In a study on shrub seedling establishment in the Atacama Desert (Chile), León et al. (2011) showed that in arid environments driven by water pulses, seedling survival is strongly dependent on the plant capacity to quickly reach moist soil layers at depth. The present study demonstrates the high rooting capacity of *C. quinoa* seedlings in their very early developmental stages, confirming the high drought tolerance of young *C. quinoa* plants commonly observed in the fields of the arid Andean altiplano (Geerts et al. 2012). This capacity to develop roots through a dry soil layer up to 15 cm thick was equally displayed by the young plants of *C. hircinum* and *C. pallidicaule*.

A limit to the tolerance of the plants to water deficit was reached in the drier T2 and T3 treatments showing significant reductions in total biomass production in all the studied populations. Total root length was also significantly reduced in these two treatments although to a lesser extent than aerial biomass. This resulted in increased values of the root:shoot ratio (except in *C. hircinum*), a response to water deficit also observed in *C. quinoa* by Bosque et al. (2003). Root elongation was less affected by water deficit in *C. quinoa* than in the other two species, with the dry-habitat ecotype of *C. quinoa* maintaining the highest root length (Table 2, Fig. 2, 3). Under dry conditions, the relative loss in root elongation among the studied populations thus reflected their ranking in biomass production, showing the importance of root growth for maintaining plant productivity under soil water deficit. In particular, preserving a high rooting capacity allowed the *C. quinoa* from dry habitat to keep relatively low values of inter-root distance compared to the other three populations (Fig. 5). In association to constantly longer II and EI links (Table 4), this root foraging trait should benefit the *C. quinoa* from dry habitat with an comparatively more efficient exploitation of the soil resources at depth (Nicotra et al. 2002, Paula and Pausas 2011).

The number of root tips is another important determinant of the plant's ability to absorb water and nutrients from the soil (Liu 2011, FCR). In the three studied species, this trait was fairly unaffected in the moderate water deficit (T1), while in treatment T2 *C. quinoa*



of both ecotypes showed reduced number of root tips (Table 5). In face of very low water availability in the root zone (T3), the number of root tips was reduced in all populations. This reduction in the external branching of the root system corresponded to a lower root system magnitude, which should affect the root system topology. In fact, the topological indices in the dry treatments were significantly higher than in the control (Table 3), which implies that the root system altitude (i.e. the longest root system path length) was proportionately less reduced by the water deficit than magnitude. Similar observations were made by Fitter et al. (1991) and Taub and Goldberg (1996). This resulted in a more herringbone topology of the root system under dry conditions in all the studied species. It thus appears that, under severe soil water deficit, lateral root branching was more restricted than main root axis growth. For plants facing water deficit at an early developmental stage, vertical elongation of the taproot is indeed considered as more suitable to improve the acquisition of scarce soil resources at depth than a densely branched root system, restricted to shallow soil layers and rapidly exhausting the resources available in a limited soil volume (Reader et al 1993; Padilla and Pugnaire 2007). Our results show that the three studied species were capable of altering their root system topology in response to low water availability in such an adaptive way. Yet, under very dry soil conditions, the studied species showed distinct responses, with *C. hircinum* and *C. pallidicaule* reducing the emission of root tips much more than *C. quinoa*. Among the two *C. quinoa* ecotypes, the one from dry habitat appeared less affected than the one from rainy habitat. This trait, associated to lower inter-root distances, suggest an adaptive foraging pattern particularly improved in the *C. quinoa* originating from the driest habitat.

Specific root length may also contribute to the higher capacity of the dry habitat ecotype of *C. quinoa* to cope with soil water deficit by means of an improved root foraging of the soil. The ability to penetrate soil is significantly influenced by SRL, and water extraction at depth has been associated with root thickness (Price et al. 2002). In this regards, SRL differences among the studied populations show an advantage for *C. quinoa* from dry habitat which had the coarsest roots, while *C. pallidicaule* had the finest ones (Table 2). Similar differences in SRL were observed in an independent experiment with well-watered plants of the same chenopod populations (Alvarez-Flores et al. 2012a) and, since SRL showed little changes under dry conditions, it is suggested that this root morphological trait is intrinsic to each population or species.

Differential root penetration capacity among populations should partly explain the temporal patterns of maximal rooting depth observed during the experiment, with *C.*

*pallidicaule* limited to 50 cm depth in well-watered conditions (and barely more than 25 cm in the driest treatment), while the two other species reached 80 cm depth whatever the water regime (Fig. 2). Both the specific root length and the maximum rooting depth showed differences among species which could be associated to differences in the overall plant vigour and, finally, to the seed mass characteristic of each studied species (Alvarez-Flores et al. 2011a). According to Richards et al. (2007), the size of the embryo is likely to be the most important trait for root vigour. Contrary to the specific root length, maximal rooting depth also showed plasticity under dry conditions, *C. quinoa* from dry habitat having significantly accelerated taproot growth compared to well-watered plants of the same ecotype or plants of the other populations. Ingram and Malamy (2011) state that taproot growth, together with lateral root branching, is directly affected by the soil water availability, and has direct impact on the reproductive success of the plants. The plasticity in taproot growth allowed the plants of the dry-habitat ecotype of *C. quinoa*, when exposed to low soil water availability, to potentially explore quickly a much greater soil volume than under well-watered conditions. The observations made on the vertical distribution of the roots and their inter-root distance at depth showed that this was indeed the case (Fig. 4, 5), which should confer this dry-habitat ecotype a marked advantage over the plant populations or species deprived of such fast and deep root foraging capacity.

In this study, it was also observed that specific leaf area of water-stressed plants of *C. quinoa* from rainy habitat and *C. pallidicaule* was significantly lower than in well-watered plants (Table 2). This apparent plasticity in SLA may be associated with adjustments in leaf morphology as stress increases, as assumed by Bell et al. (2007) in a comparative study on three leguminous species. However, in the present study, this effect was observed in the driest treatment only and for only two of the four studied populations. These contrasted responses to water deficit could result from interfering ontogenetic changes in SLA in young developing plants. Jensen et al. (2000) reported lower SLA due to water stress in young *C. quinoa* plants, but this also coincided with an abrupt reduction in SLA in well-watered plants at the transition between branching and flowering stages (35 to 45 DAS). As similar ambiguous effects of water deficit were observed on the leaf area ratio, it is inferred that leaf morphology both at the leaf and the whole-plant level was relatively insensitive to water deficit in seedling plants of the studied species. At this early developmental stage, the plant response to water deficit appeared more related to a decrease in total leaf area proportionate to the whole plant biomass reduction (Table 2). This reduced leaf area could have an impact on root growth

since it has been demonstrated in *Arabidopsis* plants that small delays in leaf development can cause major differences in lateral roots density, the leaves being the source of auxin to promote root growth (De Smet et al. 2012).

#### 4 CONCLUSION

Distinct root system responses to water-limiting conditions were observed among two *C. quinoa* ecotypes from dry vs. rainy habitats, and two of their Andean relatives: *C. pallidicaule* and *C. hircinum*. These responses encompassed a set of morphological root traits, some of them, like SRL or mean link length, appearing as intrinsic to the plant populations, while others traits, like lateral root branching or taproot length, showed plastic variations depending on the intensity of the soil water deficit. The integration of these intrinsic traits and plastic processes controls the root architecture and the root foraging dynamics and, thus, directly influences the functionality of the whole root system (Liu et al. 2011, Ingram and Mamaly 2010). The present study, focused on the responses to water deficit in the crucial phase of seedling establishment, found that these root traits may contribute significantly to the greater drought tolerance in the *C. quinoa* ecotype from the dry habitat.

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## SUPPLEMENTARY MATERIAL

Table A-1. Vertical distribution of root length at 42 DAS in the four studied *Chenopodium* populations and the four treatments (cm)

		QB	QB	QC	QC	HI	HI	PA	PA
	Depth	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T0	0-5	4,4	1,1	6,6	1,9	5,6	1,1	6,3	0,9
	5--20	259,9	36,6	293,8	49,0	241,2	46,4	187,7	22,1
	20-35	425,9	84,3	502,3	88,4	326,1	44,3	164,3	42,5
	35-50	325,5	44,9	360,3	33,7	226,1	16,5	30,2	16,2
	50-65	310,6	39,0	231,9	80,33	95,4	61,9	1,6	0
	65-80	383,5	60,3	34,91	16,2	59,6	47,8	0	0
T1	0-5	5,6	1,1	4,7	0,9	7,2	1,7	8,5	1
	5-20	253,7	13,6	291,0	25,8	235,4	20,0	104,6	12,4
	20-35	414,9	489,0	637,9	93,1	332,0	62,3	221,3	9,3
	35-50	292,9	61,1	424,1	46,2	218,7	2,3	27,6	9,4
	50-65	272,5	65,7	371,4	98,0	68,4	38,6	10,5	0
	65-80	308,2	32,2	190,1	89,8	13,9	4,0	0	0
T2	0-5	4,4	0,4	5,0	1,1	4,9	0,7	7,1	1,0
	5-20	247,0	10,5	231,8	15,4	197,5	15,1	137,2	9,4
	20-35	411,4	32,9	476,9	49,9	286,2	11,6	104,1	19,0
	35-50	284,2	20,2	297,5	74,9	168,0	32,2	11,6	1,6
	50-65	257,1	10,8	112,5	68,9	28,6	5,1	2,4	0
	65-80	273,5	64,4	112,2	99,7	8,3	3,5	0	0
T3	0-5	5,5	1,0	5,2	0,9	5,3	0,8	6,7	1,3
	5-20	317,9	41,5	276,3	43,2	154,6	7,7	16,9	7,5
	20-35	297,8	10,4	236,8	62,6	166,3	28,2	49,6	16,2
	35-50	388,3	17,8	287,3	57,2	125,4	31,5	0	0
	50-65	358,1	21,9	93,8	36,8	28,3	2,5	0	0
	65-80	212,5	23,4	55,2	16,2	7,1	2,1	0	0

Table A-2. Soil water content at two dates and to soil depths. Pop. = populations; QB = *C. quinoa* (Bolivia); QC = *C. quinoa* (Chile); HI = *C. hircinum*; PA = *C. pallidicaule*. SWC = Water content (3%). DAS = Days After Sowing.

		DAS 1		DAS 28	
Treatments		Pop.	SWC (%)	SWC (%)	
Depth (15 cm)	T0	QB	12,0	7,8	
		QC	11,5	7,6	
		HI	11,5	8,2	
		PA	11,5	9,3	
	T1	QB	6,6	5,4	
		QC	7,8	5,5	
		HI	6,7	5,6	
		PA	6,9	6,0	
	T2	QB	7,2	5,5	
		QC	7,5	5,6	
		HI	7,4	5,5	
		PA	6,9	5,6	
T3	QB	6,7	4,8		
	QC	7,5	4,9		
	HI	7,0	5,1		
	PA	7,2	5,3		
Depth (45 cm)	T0	QB	12,3	9,2	
		QC	12,4	10,0	
		HI	11,8	10,0	
		PA	12,3	11,4	
	T1	QB	12,5	9,3	
		QC	12,4	9,6	
		HI	12,1	9,7	
		PA	11,7	11,5	
	T2	QB	12,4	8,7	
		QC	11,7	9,2	
		HI	12,4	9,5	
		PA	12,3	11,1	
T3	QB	7,4	5,2		
	QC	6,7	5,2		
	HI	7,0	5,1		
	PA	7,3	5,4		





## DISCUSSION GÉNÉRALE

Ce travail a eu pour objectif d'explorer la plasticité phénotypique du système racinaire de quatre populations de *Chenopodium* des Andes sous l'hypothèse que les plantes provenant d'un habitat à faible disponibilité de ressources dans le sol ou d'un système de culture à faible usage d'intrants ont développé des traits racinaires qui leurs permettent d'augmenter l'acquisition de ressources du sol. Des expériences en conditions contrôlées nous ont permis d'examiner la morphologie et le développement du système racinaire pendant la phase cruciale d'installation des plantes, période pendant laquelle les jeunes plantules sont exposées, selon leur milieu d'origine, à des risques climatiques plus ou moins sévères.

### INTERPRÉTATION ÉCOLOGIQUE DES VARIATIONS DE TYPOLOGIE RACINAIRE

Dans des sols aux ressources en eau et en nutriments limitées, les plantes ayant une typologie racinaire *herringbone* sont réputées bénéficier d'un potentiel compétitif plus élevé que celles ayant un système racinaire dichotomique (Taub and Goldberg 1996), la présence d'un axe racinaire fort leur permettant d'explorer plus efficacement les horizons profonds du sol. Selon Glimskär (2000) et Paula et Pausas (2011), cette capacité d'approfondissement serait notamment décisive dans les stades initiaux du développement des plantes.

Parmi les quatre populations étudiées, les variations d'architecture racinaire observées en conditions non limitantes suggèrent une différenciation de nature génotypique, liée à la sélection dans des milieux aux niveaux de ressources contrastés combinée, dans le cas des écotypes cultivés, à la sélection récurrente par les agriculteurs. Les deux écotypes de *C. quinoa* développent ainsi des longueurs totales de racines généralement supérieures à celle de leur ancêtre sauvage *C. hircinum* et, entre ces deux écotypes, celui provenant du milieu le plus aride présente une vitesse d'élongation de la racine principale et une densité de colonisation racinaire en profondeur nettement plus élevée. Le fait que ces traits s'expriment même en absence de déficit hydrique suggère qu'ils sont intrinsèques à l'écotype de milieu aride et ont été sélectionnés par le jeu des contraintes du milieu et de la domestication agricole. Des valeurs élevées de vitesse d'élongation initiale et de densité racinaire en profondeur sont en effet des avantages décisifs dans les conditions de culture de l'altiplano aride. Dans ces régions, la pluviométrie annuelle comprise entre 150 et 300 mm selon les secteurs, ne peut satisfaire les besoins d'un cycle entier de culture de quinoa (Geerts et al.

2012). Les agriculteurs contournent cette contrainte en ne cultivant les parcelles de quinoa qu'une année sur deux, les précipitations reçues pendant l'année sans culture s'accumulent dans le sol pour augmenter d'autant le stock disponible pour la culture l'année suivante. Les sols sableux caractéristiques de cette région étant très filtrants, cette stratégie de culture suppose que les plantes accèdent rapidement aux horizons profonds du sol même si, comme c'est fréquent dans ces climats arides, les pluies du début de la saison de culture sont erratiques et insuffisantes pour garantir à elles seules une bonne installation de la culture. Plusieurs auteurs parmi lesquels Austin et al. (2004), Schwinning et Sala (2004), ou León et al. (2011), ont examiné en détail le fonctionnement hydrodynamique des sols et celui de la végétation dans ces milieux dépendants des *pulses* de précipitations. Tous soulignent l'importance cruciale pour la survie des plantules d'un accès rapide des racines aux couches profondes du sol, suivi d'une colonisation la plus dense possible de ces horizons où s'accumulent l'eau mais aussi les nutriments mobiles comme l'azote. Un système racinaire de type *herringbone* sera alors optimal puisqu'il combine une croissance rapide de l'axe principal et une compétition minimale entre racines d'un même individu. Ces critères adaptatifs sont effectivement présents chez l'écotype de *C. quinoa* cultivé dans l'altiplano aride et lui permettent, grâce à l'élongation rapide de sa racine principale et la prolifération de racines secondaires jusqu'à près d'un mètre de profondeur, de supporter des épisodes de sécheresse de plus de deux mois en début de cycle quand la partie aérienne des plantules ne dépasse pas 3 à 5 cm de hauteur (Joffre, Winkel, comm. pers.). Signalons qu'avec une profondeur de 60 cm atteinte en 60 jours, les paramètres empiriques de croissance racinaire utilisés par Geerts et al. (2007) dans leur modèle de simulation de culture de la quinoa sont proches des valeurs observées dans notre essais en rhizotrons. Ces traits de croissance racinaire observés chez l'écotype de *C. quinoa* de région aride ne sont pas présents chez l'écotype des régions humides, pas plus que chez les populations testées de *C. hircinum* et *C. pallidicaule* qui, en cas de déficit hydrique important, présentent toujours une productivité inférieure.

### SYNDROMES DE TRAITS ARCHITECTURAUX DES PLANTES

L'interprétation écologique des traits de morphologie et de croissance déployés par les plantes conduit à rechercher les combinaisons de traits, ou syndromes, rencontrées plus fréquemment dans certains milieux et donc susceptibles de présenter une valeur adaptative par rapport aux spécificités de ces milieux (Wright and Westoby 1999). De tels syndromes ont été identifiés à partir de traits racinaires, avec une valeur adaptative pour l'acquisition rapide ou la

conservation des ressources limitantes du milieu (Picon-Cochard et al. 2012). À partir de nos résultats sur la morphologie des systèmes racinaires de divers *Chenopodium*, deux syndromes particuliers émergent : l'un caractérisant les plantes de régions arides aux sols profonds, l'autre plus spécifique des plantes de régions de très haute altitude, froides et aux sols superficiels (Alvarez-Flores et al. 2012a).

Dans la première situation, une croissance aérienne rapide assure une capture et un stockage important des ressources du milieu au détriment des espèces compétitrices. Dans la partie souterraine, une élongation très rapide de l'axe racinaire principal, avec des racines de SRL élevé facilitant la pénétration du sol en profondeur, et des segments racinaires de longueur moyenne élevée, composent une suite de traits qui augmentent nettement la capacité d'enracinement en profondeur et donnent un avantage sélectif dans les milieux de climat sec et au sol pauvre, où la croissance initiale et l'établissement des plantules sont vitaux pour l'accomplissement du cycle de vie des plantes. L'écotype de *C. quinoa* de région aride montre ce syndrome qui lui confère une supériorité décisive par rapport à d'autres écotypes cultivés, ou d'autres espèces compétitrices.

Dans les milieux d'altitude, froids et aux sols peu développés, des plantes de faible hauteur, ramifiées, échappent plus facilement aux effets du vent et du gel. Sur ces sols superficiels, des racines peu profondes mais avec un SRL élevé permettent une acquisition rapide des ressources du sol. Cet ensemble de traits se trouve réuni par *C. pallidicaule*, et correspond aux conditions rencontrées dans une grande partie de son aire de culture.

### DES TRAITS RACINAIRES FIXES OU PLASTIQUES ?

Les syndromes que nous avons décrits s'organisent autour de traits racinaires comme le SRL ou la longueur moyenne des segments qui apparaissent comme des attributs intrinsèques, peu variables, des populations. Ces traits contribuent à la survie des plantes dans les conditions moyennes de leurs milieux d'origine. Mais la variabilité climatique est forte en milieu aride, et dans les agrosystèmes utilisant peu d'intrants elle est difficilement compensable (Jackson 1997). Les plantes de ces milieux doivent donc pouvoir répondre aux changements extérieurs par une certaine plasticité fonctionnelle de leurs traits physiologiques mais aussi architecturaux, notamment ceux définissant le système racinaire dont dépend toute l'alimentation en eau et en nutriments (Ingram and Malamy 2010). Nos résultats ont mis en

évidence de telles variations en réponse aux changements du milieu, notamment pour les traits contrôlant la topologie racinaire. La vitesse d'élongation de la racine principale, la longueur maximale d'enracinement, ainsi que le nombre de racines latérales, ont été modifiés par la disponibilité en ressource du milieu. L'accélération, sous l'effet d'un déficit hydrique, de la vitesse d'élongation de la racine principale chez l'écotype de *C. quinoa* de région aride lui permet ainsi d'accroître rapidement et significativement le volume de sol exploré. Devenant ensuite rapidement le support de nombreuses ramifications latérales, cette partie du système racinaire contribue à amplifier la capacité d'exploration du sol et, par-là, la disponibilité en ressources pour la plante. La priorité donnée à l'allongement de la racine principale en cas de déficit hydrique se traduit par une modification de l'indice topologique dans le sens d'un système racinaire plus *herringbone*. Le schéma topologique des racines est donc lui-même un trait plastique, illustrant ainsi la relation complexe entre déterminisme génétique et plasticité fonctionnelle (Ingram and Malamy 2010).

### PLASTICITÉ DE LA TOPOLOGIE RACINAIRE FACE AU DÉFICIT HYDRIQUE

Un cadre théorique d'analyse des variations de l'indice topologique des systèmes racinaires a été proposé par Taub et Goldberg (1996). Considérant à la fois les variations taxonomiques et celles dues aux changements du milieu, ces auteurs constatent qu'en règle générale le système racinaire des espèces monocotylédones est plus nettement *herringbone* que celui des dicotylédones mais qu'il est aussi moins plastique face aux variations du milieu. En réponse à une diminution de la disponibilité des ressources du sol, les premières conserveraient la même topologie racinaire tandis que les secondes auraient tendance à développer une topologie plus *herringbone*, comme l'illustre la figure 1. Ces auteurs postulent ensuite qu'au sein des dicotylédones, les espèces provenant d'habitats pauvres en ressources montreraient une plasticité de la topologie racinaire plus grande que les espèces de milieux plus riches.

Dans notre étude sur quatre populations de *Chenopodium* provenant d'habitats contrastés dans les Andes, nous avons trouvé que toutes présentaient une typologie nettement *herringbone*, conformément à ce qui est communément observé chez les chénopodiacées (Fitter 1987). Cette similarité des indices topologiques reflète la proximité phylogénétique des groupes étudiés (Fuentes-Bazan et al. 2012). Face à une réduction de la disponibilité de l'eau dans le sol, l'ensemble des populations a montré une augmentation des indices topologiques, qu'ils soient calculés par la méthode de Fitter et Stickland (1991) ou par celle de Glimskär

(2000) (Fig. 2). Cette augmentation statistiquement significative est apparue dès le traitement (T1), indiquant un ajustement de la topologie du système racinaire même dans le cas d'un déficit hydrique modéré. En revanche, et malgré un contraste important entre les milieux d'origine des deux écotypes de *C. quinoa*, aucune différence significative de plasticité topologique en relation avec la richesse en ressources et le degré d'aridité de leur milieu d'origine n'a pu être mise en évidence. Nos observations corroborent donc en grande partie mais pas entièrement le modèle théorique de Taub et Goldberg (1996), soit que les choix expérimentaux (e.g. période de croissance, intensité des déficits hydriques), soit que les caractéristiques mêmes des populations étudiées, taxonomiquement très proches entre elles, n'aient pas permis de discriminer un effet du milieu d'origine des populations sur la plasticité de leurs réponses racinaires.

En général, les relations topologiques observées chez des espèces annuelles sous différentes conditions de croissance (Arredondo & Jonhson 1991, Fitter 1991, Glimskär 2000, Paula et al. 2011, Roumet et al 2006), suggèrent une modification de la topologie des racines due à l'émergence accélérée de racines latérales (augmentation de la magnitude  $\mu$ ) dès que l'altitude dépasse 200 segments approximativement ( $\log(\alpha) = 2.4$ ).

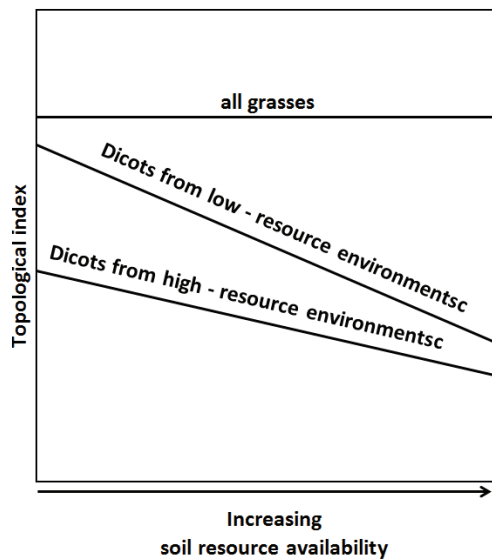


Figure 1. Schéma théorique des variations de topologie racinaire dans un gradient de disponibilité des ressources du sol. D'après Taub et Goldberg (1996).

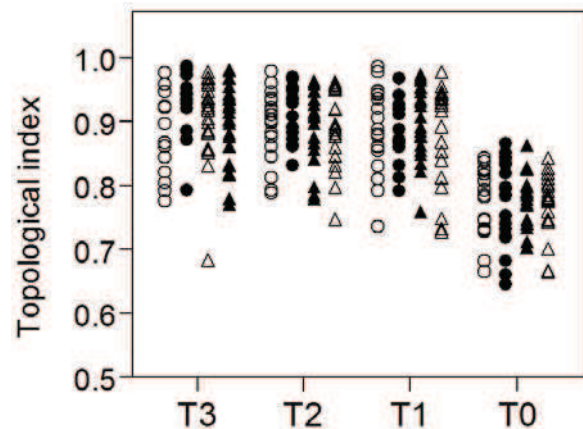


Figure 2. Variations de l'indice topologique TQI observées chez des populations de *Chenopodium quinoa* d'habitat aride ( $\Delta$ ), *C. quinoa* d'habitat humide ( $\blacktriangle$ ), *C. hircinum* ( $\bullet$ ) et *C. pallidicaule* ( $\circ$ ) soumises à un gradient de déficit hydrique décroissant de T3 à T0. (T0 est significativement inférieur aux trois autres traitements,  $df = 3$  ;  $F = 51,56$  ;  $P < 0,01$ )

S'ajoutant à l'effet de taille des plantes mentionné par Glimskär (2000), cet effet ontogénique dans la relation altitude / magnitude constitue une limitation à l'usage de l'indice topologique de pente (TI) proposé par Fitter et Stickland (1991). En effet, à taille égale, une plante à un stade de développement avancé aura un indice topologique plus faible (plus dichotomique) qu'une plante à un stade plus juvénile, indépendamment de tout effet de réponse au milieu. Ce qui doit être pris en compte dans l'interprétation des différences inter-spécifiques de réponse racinaire à la disponibilité en ressources du milieu.

Les résultats de notre étude suggèrent que cette plasticité architecturale du système racinaire reste contrainte par les caractéristiques génétiques des plantes, en particulier la taille de leurs graines et leur niveau de ploïdie (voir chapitre 1). C'est ce qu'indique la comparaison de *C. pallidicaule* avec deux espèces apparentées : chez cette espèce diploïde aux graines petites, la capacité d'exploration des racines reste limitée aux horizons relativement superficiels (50 cm au plus dans nos essais en rhizotrons) malgré une topologie racinaire nettement *herringbone*. Cette limitation n'est pas affectée par la présence d'une couche de 15 cm de sol sec, mais elle est très aggravée en cas de déficit plus sévère, l'élongation racinaire étant alors stoppée à 25 cm de profondeur quand, dans les mêmes conditions hydriques, les plantes de *C. quinoa* et *C. hircinum*, toutes deux tétraploïdes et à graines plus grosses, colonisent le sol jusqu'à 80 cm de profondeur.

Ces fortes différences observées dans le potentiel exploratoire du système racinaire en lien avec la taille des graines des espèces étudiées corroborent l'affirmation de Richards et al. (2007) pour qui la taille de l'embryon serait le trait le plus important pour la vigueur du système racinaire. Or, chez *C. quinoa* et *C. pallidicaule*, la taille des embryons est liée à celle des graines (Bruno 2006) et, d'une façon générale, la taille des graines est très dépendante du niveau de ploïdie des espèces (Evans and Dunstone 1970, Villar et al. 1998). Notre étude sur trois espèces de *Chenopodium* apparentées montre bien une association forte entre taille des graines, niveau de ploïdie et vigueur des racines pendant la phase d'installation des jeunes plantes. On peut signaler ici que, tout comme Villar et al. (1998) dans leur étude comparant 20 espèces d'*Aegilops*, nous n'avons pas trouvé de lien entre taille des graines et RGR.

### PERSPECTIVES

Une des difficultés majeures du travail sur les systèmes souterrains, c'est d'en avoir une connaissance fiable et non biaisée et un accès facile ! Depuis plus d'un siècle, les travaux sur la compréhension des systèmes racinaires s'enchaînent avec des approches très diverses à de multiples échelles. L'étude du compartiment racinaire dans son intégralité pose de nombreux problèmes et demande énormément de temps et d'énergie. Ainsi, au cours de ce travail, nous avons réalisé une analyse détaillée de l'architecture des systèmes racinaires basée en particulier sur les travaux de Fitter et à l'aide du logiciel (WinRhizo 2009). Cependant, à la fin de ce travail, force est de constater que de multiples contraintes logicielles ne rendent pas ce travail très facile et que bien des améliorations devront encore être faites avant d'avoir un outil opérationnel.

Une phase passionnante s'ouvre : celle de l'utilisation de l'ensemble des résultats expérimentaux de ce travail dans le cadre des modèles de représentation et de fonctionnement des systèmes racinaires. Un des objectifs de la modélisation est de combiner les règles morphogénétiques décrites au niveau de la racine pour produire des représentations quantitatives du système racinaire dans sa globalité. À cet égard, l'inclusion de processus stochastiques dans ces modèles est apparue comme un moyen économique de simuler des systèmes racinaires réalistes (Pagès 1999). Depuis les premiers modèles numériques de systèmes racinaires de Lungley (1973), se sont développés des applications très diverses couvrant l'étude hydro-architecturale (Doussan et al. 1998), les dynamiques de croissance (Leitner et al. 2010 ; Lynch 2005) et l'étude de la représentation de l'architecture (Pagès et al. 2004). Ce dernier auteur et ses collègues (Le Bot et al. 2010) ont développé le modèle Dart pour l'analyse de l'architecture racinaire qui peut être employé dans des protocoles semblables à ceux utilisés dans les deuxième et troisième chapitres de notre travail. Un aller-retour entre données et modèles permettra de valider ou non les hypothèses de ces modèles, d'en affiner certaines, et aussi d'évaluer les conséquences fonctionnelles des types d'architecture et de leur plasticité sur la capacité des espèces étudiées à répondre aux changements du milieu.

Après ce travail réalisé en conditions contrôlées, nous considérons important de contraster nos résultats avec des études qui devraient être réalisées *in situ* en conditions normales de culture afin de proposer des interprétations biologiques et écologiques plus pertinentes. Par ailleurs, ces travaux devraient se développer en lien étroit avec ceux conduits



dans la perspective de la « seconde révolution verte » évoquée par Lynch (2007). Il s'agira d'aborder la durabilité écologique des systèmes de production agricole en prenant en compte un certain nombre de traits fondamentaux des systèmes racinaires dont la plasticité permettra aux cultures de faire face, sans surcoût énergétique, aux modifications climatiques attendues en termes de tendance moyenne mais aussi d'augmentation des valeurs extrêmes.

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