MONTPELLIER SUPAGRO
Centre International d'Etudes Supérieures en Sciences Agronomiques

THESE

pour obtenir le grade de

DOCTEUR EN BIOLOGIE

ECOLE DOCTORALE SIBAGHE
(Systèmes Intégrés en Biologie, Agronomie, Géosciences, Hydrosciences, Environnement)

Control of shoot and root growth by water deficit in *Arabidopsis thaliana*: a parallel analysis using artificial and natural mapping populations

soutenance le 11 Juillet 2011

Marie Bouteillé

devant le JURY suivant:

M. Xavier DRAYE	Rapporteur
M. Jacques LE GOUIS	Rapporteur
Mme Evelyne COSTES	Examinatrice
Mme Dominique THIS	Examinatrice
M. Detlef WEIGEL	Co-directeur de thèse
M. Bertrand MULLER	Directeur de thèse

Quelle aventure! Ma thèse se termine, une page se tourne, et avec elle ce qui aura rythmé trois (un peu plus même. no comment...) années de vie bien remplie entre Montpellier et Tübingen. Même si cela doit être long, j'ai le plaisir et le devoir d'être exhaustive pour la gentillesse et la qualité des personnes que j'ai été amenée à rencontrer durant ces trois années.

Cette thèse a été partagée entre le laboratoire LEPSE à Montpellier, et l´Institut Max Planck de Tübingen en Allemagne. Je voudrais donc remercier mes directeurs et co-directeurs de thèse, Bertrand Muller et Detlef Weigel qui m´ont tant apporté, chacun à leur manière, au cours de ces années. Bertrand, merci pour cette aventure qui a commencé réellement en 2006 (déjà!), pour un stage de Master 2. Merci pour ta proximité, ta disponibilité, et ton enthousiasme, tu as toujours su répondre présent et me motiver quand je doutais. Merci pour ta grande curiosité scientifique, qui m´a donné envie d´explorer de nouvelles questions scientifiques chaque jour, au risque de me disperser.... Je voudrais évidemment remercier aussi Detlef, qui m´a fait confiance en m´ouvrant les portes de l´Institut Max Planck, et qui fait partie lui aussi des personnes qui donnent envie de se surpasser. J´ai particulièrement apprécié sa facon de travailler et d´être toujours en avance d´une idée (ou de 10...). Le cotoyer a été un honneur pour moi.

Je voudrais aussi exprimer toute ma reconnaissance aux membres du jury, pour l'honneur qu'ils me font en acceptant d'évaluer mon travail. Je souhaite remercier en particulier Xavier Draye et Jacques le Gouis qui ont eu la lourde tâche d'être les rapporteurs de cette thèse, et qui ont formulé des commentaires particulièrement enrichissants pour mon travail. Je souhaite aussi remercier Dominique This et Evelyne Costes d´avoir accepté d´évaluer ce travail, ainsi que les membres de mes comités de thèse, et notamment Olivier Loudet, qui a participé aux trois comités de thèse et qui m´a toujours encouragée, Mathieu Reymond, Patrice This et Catherine Roumet qui m´ont permis d´avancer tout au long de ces années.

Je suis au laboratoire LEPSE depuis maintenant six ans, et je voudrais remercier les personnes qui m´ont accompagné: Je pense en particulier à Christine, qui m´a d´abord permis de faire cette thèse en contactant Detlef à Tübingen, avec qui elle collaborait sur un autre projet, et qui m´a aussi beaucoup apporté, scientifiquement et humainement (la bonne ambiance du LEPSE lui doit beaucoup!). Je remercie aussi Denis, que j´ai souvent embêté de longues heures sur des questions de statistiques, d´écologie, de traitement de données, de scripts R à débuger, et mêmes sur des questions de macarons ou de cannelés..... Merci aussi à Olivier qui a été mon premier encadrant de stage au LEPSE, sur le Mais à l´époque. A Claude, qui m´a transmis sa passion de la génétique, de l´évolution des populations, et de la remise en contexte, historique, sociologique, politique, des questions scientifiques, et avec qui j´ai eu de nombreuses discussions particulièrement enrichissantes. Merci aussi à Francois et Thierry pour les

discussions qui m´ont permis d´avancer dans mon travail, à Angélique, ainsi qu´à Lydie et Jérémie qui ne sont plus au LEPSE maintenant, mais qui ont beaucoup apporté à ce laboratoire, de par leurs qualités scientifiques et leurs personnalités.
Au cours de mes stages de M1, de M2, et de ma thèse, j´ai constamment travaillé en équipe, en particulier avec les différents techniciens du laboratoire, que je voudrais remercier de tout coeur pour leur aide, et pour la qualité des manips que nous avons faites ensemble: Cris et Gaelle ont répondu présent pour toutes les nombreuses journées de remplissage, semis, suivi, récolte, le peignage de racines, les scans et les pesées des organes de 13000 plantes environ!!! Merci beaucoup à tous les deux, cette thèse est vraiment le fruit de notre travail commun! Merci aussi à Myriam pour son aide permanente sur les manips dans Phenopsis, et pour les debugages du weekend, merci à Jean-Jacques (un heureux retraité j´imagine), Joe, Nico, Alexis, Fred, Adeline, Romain, Benjamin, qui ont aussi sauvé quelques journées de manips en venant donner un coup de main.
Merci aux thesards, ex thésards et autres précaires de la fonction publique (:)) avec qui j´ai fait un bout de chemin, Juliette, Caro, Boris, Llorenc, Florent, Francois, Grégoire, Cécilio, Maryline, Justine, Alex, Joe, Adeline, Romain, et à ceux qui ne sont plus précaires, mais qui sont sympas quand même, Karine, Christina, Cathy, Nico, Vincent. Un grand merci à Seb aussi pour son soutien sans faille pendant toute ces années, pour sa formation privée QTL, mais aussi et surtout pour plein de supers moments passés à Montpellier et même à Tarbes! Chouettes souvenirs, vraiment!
Merci à Marie-Claude, Marie-Francoise (à Montpellier), et Hülya (à Tübingen) pour leur gentillesse et leur réactivité face à quelques complications administratives pas toujours simples à régler.

Les 18 mois passés à Tübingen ont été particulièrement enrichissants, et je tiens à remercier toutes les personnes avec lesquelles j´ai travaillé et qui m´ont acceuillie avec une grande gentillesse et un grand intêret: Detlef, Hülya, M.Mazouz, Roosa, Beth, Eunyoung, Sang-Tae, Yasushi, ainsi que le groupe d´informaticiens de Karsten Borgwardt et en particulier Oliver et Christoph qui ont pris en charge la partie "génétique d´association". Merci pour leur patience, même en évoluant dans deux mondes un peu parallèles on a fini par se comprendre, si si!
Cette parenthèse allemande m´a aussi permis de rencontrer des gens adorables avec qui j´ai passé des journées et soirées fantastiques, notamment pendant la coupe du monde, de grands moments.... Merci donc à Carmen et David (et á Alvaro que je ne connais pas encore!), à Helena, Nacho, Tobi, Vini, Schallum, Felippe, Marco, Roosa, Maricris, Korbinian, Yasushi. Merci aussi évidemment à mes fabuleuses colocs de Haußerstraße 30 UG, Svenja, Inga, Chrissi, qui sont réellement devenues des amies, et que j´espére revoir le plus tôt possible!

Je souhaite aussi remercier tous les gens qui m´entourent et qui ont participé à tout ca un peu malgré eux: toute ma famille, mes parents, mes grands parents, mon frère et sa petite famille, ma presque belle famille

aussi, et bien sûr tous les cops, ceux qui se sont embarqués dans la même galère :), Florie (qui termine en juin!), Ando (qui a fait sa thèse en même temps que moi et qui s´est envolée depuis aux Philippines), Yoan, mais aussi Fred, Guillaume, Seb, Cricri, Philou, Damien, Steph, Aurélie, Bastien, Mailo, Fred, Aurore, Mathieu, Clarky, et les copains du CKPDP, le 2ème Cricri, Isa, Marlène, Evelyne, qui ont suivi toute cette aventure, qui m´ont encouragé, remotivé, soutenu sans faillir, mais qui m´ont aussi permis de penser à autre chose, de prendre du recul et de la distance quand c´était nécéssaire.

Enfin, je voudrais remercier Benoît, que j´ai rencontré au LEPSE (c´est pas banal!) il y a 6 ans, et qui m´a accompagnée depuis tout au long de cette aventure. Notre histoire est particulièrement jolie, merci du fond du coeur.

Contents

Preface

With the ever-increasing human population, use of plant products is likely to be reinforced to face different types of demands. Food is and will remain the first destination for crop plants for human consumption of cereals, vegetables, and derived products, but also for feeding cattle animals. Moreover, plants will have to satisfy new demands such as biofuel or drugs or will have to render ecological services. For these reasons, plant production has to increase in the next decades on a worldwide basis and this will require an extended use of water. Moreover, the climatic changes going on in the biosphere will globally lead to an increased demand for water by agriculture due to increased temperature and evaporative demand. At the same time, competition for water will be increasing with other usages (drinkable water, industry, natural ecosystems). Therefore, one of the main challenges for plant research community in the coming years will be to help breeders to select water-efficient varieties and species and for that, to decipher the mechanisms that allow plants to adapt to changing environments. These advances will be made possible by using the existing genetic diversity within germplasm collections, or by re-introducing new sources of tolerance present within wild relatives that have evolved to adapt to a varieties of environments whereas northern crops have often been selected for productivity rather than for their resource use efficiency. For this issue, a single approach is unlikely to be appropriate, as a diversity of ideotypes will be necessary to continue to meet the demands of regional environments, economic and social constraints, and cultural preferences.

Water is one of the most important environmental factors for plant productivity and the scenarios for global environmental change suggest a future increase in the frequency and intensity of drought periods in many areas of the earth (IPCC, 2001, Parmesan *et al.,* 2006). Drought is the result of a difference between water supply and demand and both can be promoted by various climatic variables such as high temperatures, low precipitations, low air humidity, high irradiance or salinity. Drought can also be observed for plants that grow in soil that are not able to retain water, even if the evaporative demand is low and water supply is sufficient (Monneveux *et al.,* 1996). Drought scenarii can also be different according to regions of the world, and according to plant growing season. For example, Mediterranean regions are characterized by a very hot summer, with high levels of evaporative demand, and the plants of these regions are often exposed to drought conditions. But drought events can also be reported in continental regions, in which the temperature variation can be extreme. This

diversity of drought has led to the natural selection of various types of tolerance mechanisms at different level of plant organisation (molecule, cell, organ, whole plant) that alter physiological processes and can have consequences for growth, development and survival of plants (Hsiao *et al.,* 1973, Muller *et al.,* 2011). The timing, intensity and duration of stress episodes are pivotal to determine the effects produced by drought on plant morphology and physiology. Among the many biochemical and developmental processes that are affected by water stress, such as the decrease of photosynthesis (Bradford and Hsiao, 1982, Chaves *et al.,* 2003), changes in water fluxes within the plant (Olien and Lakso, 1986), reduction of both cell division and expansion (Hsiao and Acevedo, 1974, Tisné *et al.,* 2008), and accumulation of sugars and osmoticum (Wang *et al.,* 1995, Hummel *et al.,* 2010), have been proposed as candidate mechanisms involved in reduction of productivity (reviews by Chaves *et al.,* 2003). Some of these responses are often proposed to be mediated by hormonal signalling, in particular abscissic acid (ABA) (Davies and Zhang, 1991, Voisin *et al.,* 2006, Parent *et al.,* 2009). At the whole plant level, water deficit is known to affect plant leaf area, biomass accumulation, organ number and assimilate partitioning (Hsiao *et al.,* 1973, Cativelli *et al.,* 2008, Chaves 2002, Muller *et al.,* 2011). It also affects the trade-off between the different organ growth, or within the organs (Turner*,* 1997; Lei *et al.,* 2006). While shoot growth is early and strongly reduced by water deficit conditions, the response of root growth seems to be more complex and variable, and can be reduced, maintained or stimulated depending on cases (Poorter and Nagel 2000). Moreover, the relationship between these morphological modifications and the ability of plants to tolerate drought remains unclear. While an increased root growth can be favourable in some instances (Tuberosa *et al.,* 2006), the reverse has been also observed for instance when soil depth is limited (Bruce *et al.,* 2003). Tolerance to water deficit could thus reside in adequate trade-off between root growth allowing water uptake and shoot growth allowing adequate photosynthesis.

Since it was created in 1993, the laboratory of plant ecophysiology under environmental stresses (LEPSE, Montpellier) has been focusing on plant responses to drought. As part of the INRA (National Institute for Agronomical Research), its research was originally focused on main crops such as maize, pea and sunflower, revealing the first importance of growth and stomatal adjustments and clearly showing that these changes tend to prevent cellular dehydratation. Landmark papers on the effect of water deficit on shoot growth rate were released on important crop species such as maize (Salah *et al.,* 1997, Tardieu *et al.,* 1999), pea (Lecoeur *et al.,,* 1995), sunflower (Granier and Tardieu 1999), sorghum (Lafarge *et al.,* 1998), grapevine (Lebon *et al.,* 2006) and more recently on rice (Parent *et al.,* 2010). Noteworthy, only a few studies were performed on the impact of water deficit conditions on root growth (Freixes *et al.,* 2002). In the early 2000's, the model plant *Arabidopsis thaliana* was added to the panel of species studied as a model dicotyledone because genetic resources in crop species were much more limited and omic tools were emerging in this species. Moreover, because of its short life cycle and small size, it became clear that this species could be used at high throughput in small spaces allowing genetic analysis of plant response to drought (Granier *et al.,* 2002, Cookson *et al.,* 2005, Granier *et al.,* 2006). In parallel, genetics studies were performed to identify QTL of variable traits linked to plant response to

drought. In maize, QTL related to the variations of leaf elongation rate (Tardieu *et al.,* 2003, Reymond *et al.,* 2003, Tardieu and Tuberosa 2010), or flowering (Welcker *et al.,* 2007) were identified. The set-up of platforms for high-throughput phenotyping in Arabidopsis (Granier *et al.,* 2006) and maize (Sadok *et al.,* 2007) opened the door for genetic studies on large populations of genotypes in Arabidopsis. In Arabidopsis, QTL explaining the variation of traits related to plant growth under water deficit conditions were identified using RIL populations (Tisné *et al.,* 2008, Tisné *et al.,* 2010).

The Max Planck Institute (MPI) for Biology in Tübingen was refounded in 1948, as successor to the Kaiser Wilhelm Institute for Biology in Dahlem (Berlin). The virus research at the institute gave rise to an institute on virology, which was renamed to MPI for Developmental Biology in 1985. The department of molecular biology was newly established in 2001 and is one of the six departments of this institute. One team of this department is interested in elucidating the mechanisms that produce natural variation as a result of evolutionary processes in the wild. Recently, it has pioneered the use of next-generation sequencing for establishing a catalogue of genome wide polymorphism (www.1001genomes.org; Weigel and Mott, 2009; Cao *et al.,* 2011). One of its aims is to understand the evolutionary basis of adaptation to environmental constraints (Clark *et al.,* 2007).

The work presented in this thesis originates from early discussions between the two institutions with the idea to put together the phenotypic capacity of the LEPSE for drought responses with the powerful genetic tools developed at the MPI. This program was made possible by the KBBE FP6 ARABRAS project led by Marteen Koornneef and designed to find connections between genetic basis of Arabidopsis and brassica relatives responses to abiotic stresses (low nitrogen, low water). The objective of the present work that will be detailed below is the identification of the genetic basis of Arabidopsis responses to soil water deficit in terms of organ growth and biomass partitioning between roots and shoots. It combines the use of two types of genetic material, a population of RIL and a collection of accessions collected in a variety of sites throughout Eurasia. It combines the use of a high throughput phenotyping platform PHENOPSIS (Granier *et al.,* 2006) that has already proved efficiency in genetic analysis (Tisné *et al.,* 2008; 2011) with well-established techniques for QTL detection (El-Lithy *et al.,* 2004; Loudet *et al.,* 2005), innovative techniques to take relationship between variables into account as well as breakthrough techniques linked to genome wide genetic analysis using available single nucleotide polymorphisms (SNP) along the genome of these accessions (Cao *et al.,* 2011).

This manuscript is composed of six parts among which four are proposed as independent papers that are or will be submitted for publication in the coming months. Main concepts and general considerations of the topic are exposed in a first part of bibliography review. These bibliographic elements will in particular highlight states of the art and concepts within the various disciplines used in this study.

The first chapter tackles the problem of interconnection of genetic basis for root and shoot growth in the absence of water deficit (in hydroponics). It uses a population of recombinant inbred lines (Bay-0 x Shahdara, Loudet *et al.,* 2002) and proposes the use of various analytical tools (coordinates on principle component analysis axes, residuals from main trends) to identify QTL involved in the degree of coupling between root and shoot variables.

The second chapter shows how the global responses of plant growth and biomass partitioning to soil water deficit are conserved between both the Bay x Sha RIL population and the collection of 88 accessions originating from a large range of regions of the northern hemisphere. This chapter also challenges the idea that drought tolerance could be associated with one or more of the measured variables, in particular those related to biomass partitioning.

Chapter 3 aims at testing the hypothesis that drought tolerance in the wild is related to the climate of origin. We use available climatic data collected from databases and show after an extended analysis that no single variable is identified accounting for intra- or inter-region variation. However, we were able to derive a variable that could be best related to drought as the balance between precipitation and potential evapotranspiration. This variable was remarkably able to account with a unique relationship for drought tolerance within three distinct regions (Spain, Caucasus and Asia) where climatic gradients were the largest within the collection. We also show that flowering strategies (vernalization) are also related to tolerance.

The identification of loci responsible for these responses in both the accessions and the Bay x Sha RIL population are explored in chapter 4. Genome wide analysis points to few loci having most important effects and genes associated are listed and their possible role discussed. In parallel, the QTL analysis confirms the strong relationship between root and shoot growth loci, but specific regions controlling either shoot or root growth could be detected, more specifically under water deficit conditions, confirming the hypothesis that water deficit could uncouple root and shoot genetic dterminisms. A final part provides a conclusion to the whole study and proposes some perspectives that could be followed to further understand plant adaptation to water deficit.

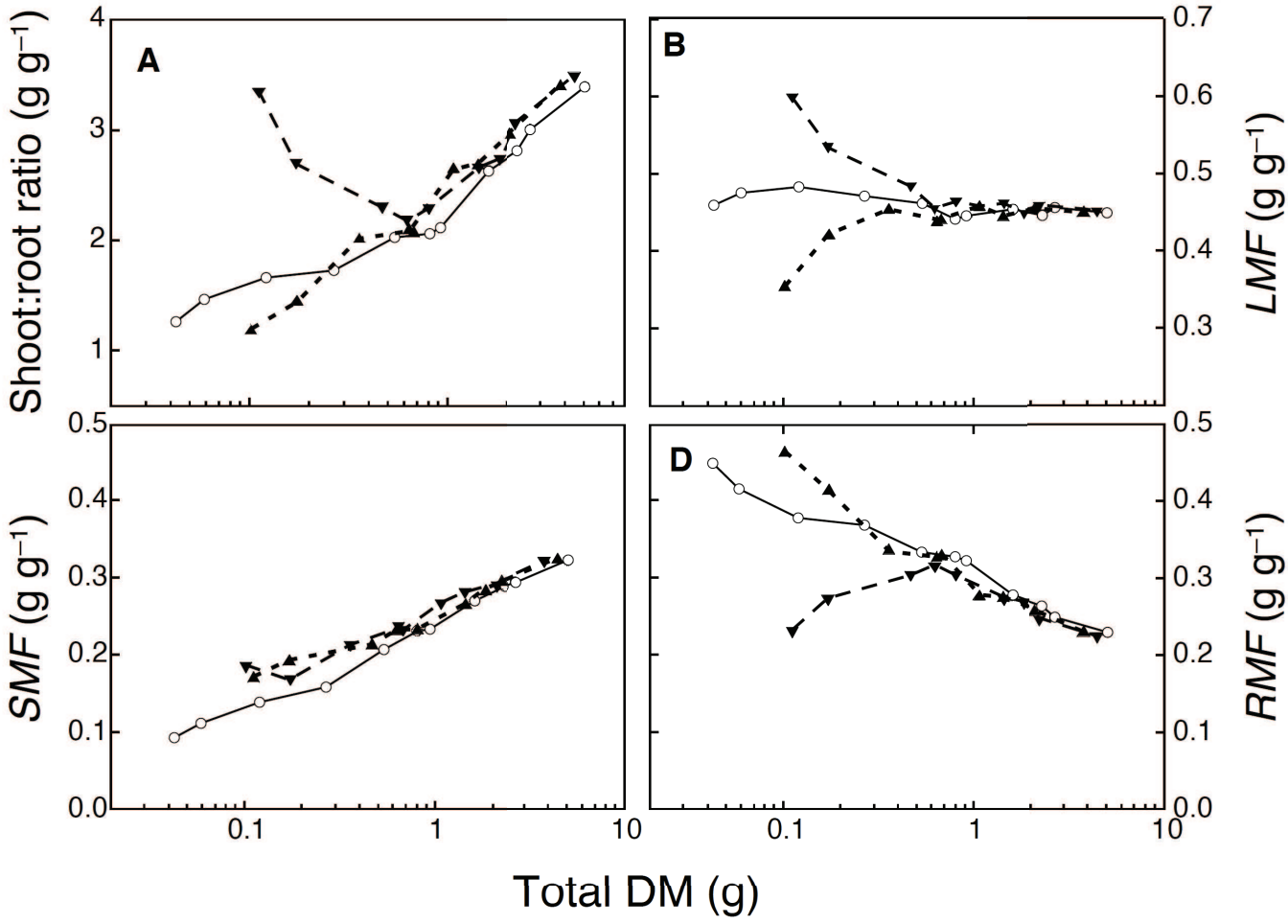


Fig. 1. Biomass allocation of leaf-pruned (▲), root-pruned (▼) and control plants (o) of Hordeum vulgare plotted as a function of total plant mass. (A) Shoot:root ratio; (B) leaf mass fraction; (C) stem mass fraction; (D) root mass fraction. (from Poorter and Nagel, 2000)

Context

1. Plant growth and biomass partitioning between shoots and roots

1.1 Coordination between root and shoot growth

Contrary to animals, almost all plants are able to produce the energy that they need to grow. Through photosynthesis reactions, leaves produce assimilates from the carbon dioxyde that is taken up with the ambient air through stomata. This source of carbohydrates is essential for plant growth. They are used at the shoot level but are also translocated to reach the other parts of the plant. Roots are heterotrophic organs for the carbon sources (Freixes *et al.,* 2002). They do not produce biomass by themselves, and their growth (defined as the accumulation of dry mass) is completely dependent on assimilates coming from the leaves. This is illustrated by experiments in which reducing irradiance, and then biomass production by leaves, affects the elongation rate of primary roots (Aguirrezabal *et al.,* 1994, in sunflower; Muller *et al.,* 1998 in maize). In the same way, defoliating a plant causes a rapid decrease in primary and secondary root elongation rate (Bingham *et al.,* 1996). A classical experiment on the regulation of biomass allocation is that of Brouwer (1962). He removed either half of the leaves or half of the roots, and observed, within one week of pruning, a remarkably restored initial proportion between root and shoot mass (Fig. 1). But its dependancy of roots towards the shoots is reciprocal, and root system has also essential functions for global plant growth. It provides anchorage in the soil, but is also crucial to extract water and nutrients stored in the soil. For instance, soil water deficit is known to have a strong impact on shoot growth (Tardieu *et al.,* 1999; Reymond *et al.,* 2003; Muller *et al.,* 2011). In the same way, Zhao *et al.* showed that sorghum plants grown under soil nitrogen deficiency had reduced levels of photosynthesis, lower stomatal conductance and lower shoot growth than control plants (Zhao *et al.,* 2005). A similar reduction in photosynthesis and biomass production was also observed under phosphorus deficiency (Chapin and McNaughton, 1989).

Thus, shoots and roots are connected and their respective growth is integrated and coordinated at the whole

plant level. The mechanisms allowing this coordination imply hormonal (Werner *et al.,* 2003, Teale *et al*., 2006) and metabolic signals (Freixes *et al.,* 2002) among others. Production of phytohormones such as auxins, cytokinins or abscissic acid is strictly controlled, and modifications of their relative abondance at different developmental stages or in response to environment drive the growth of the different organs. For instance, the ratio between auxins (mainly synthesized in leaves and translocated to root through phloemian vessels) and cytokinins in the root system has a strong impact on rhizogenesis, and this ratio varies over time during plant development or in response to environmental conditions, such as light intensities (Fett-Neto *et al.,* 2001; Laplaze *et al.,* 2007). Coordination of shoot and root growth in response to soil water deficit involves the synthesis of abscissic acid, another phytohormone, that is mainly translocated from the roots to the leaves in response to water constraints (Sobeih *et al.,* 2004, Tardieu *et al.,* 2011). Metabolic signals such as the quantity of soluble sugars can also help to adjust the coordination of root and shoot growth (Freixes *et al.,* 2002). The mechanisms which regulate the assimilate partitioning between roots and shoots are difficult to decipher (Wardlaw, 1990, Minchin and Lacointe, 2005). Diverse modelling approaches have been used to analyse the correlation between root/ shoot signalling and organ growth, through the evaluation of assimilates transport in phloemian vessels (Lacointe, 2000, Minchin and Lacointe, 2005), or through estimation of the sink strength of the organs (Ho, 1988, Drouet and Pagès, 2006; Christophe *et al.,* 2008). In ecology, allometric relationships between roots and shoots, especially as an estimator of plant fitness (Penning de Vries and Van Laar, 1982; Enquist and Niklas 2002) have been widely investigated.

1.2 Environmental conditions modify the biomass allocation patterns between shoots and roots

Plants are also able to modify their growth and to adjust allocation patterns between the organs in response to environment. When a resource is limited, an increased biomass allocation can be observed towards the organ responsible of the limiting resource acquisition. This theory has been called the " functional equilibrium theory ", formalized first by Brouwer in 1962, and assumes that the organ involved in the acquisition of a resource has priority over that resource. This theory is a cornerstone of many others in all domains of plant biology, from ecology and evolution to modelling and ecophysiology (Grime 1979; Shipley and Peters 1990; Lacointe, 2000). An implied assumption of this is that there are trade-offs in allocation between leaf, stem and root parts of plants (Thornley 1972; Bloom *et al.,* 1985). Plants shift their allocation towards shoots if the carbon gain of the shoot is impaired by a low level of above-ground resources, such as light and carbon dioxyde. Similarly, plants shift

allocation towards roots at a low level of below-ground resources, such as nutrients and water. These shifts could be seen as adaptive, as they enable the plant to capture more of those resources that most strongly limit plant growth. At low irradiance, shoots (leaves) retain more of the limiting amount of assimilates, leaving less carbon for root growth. At low nutrient and water availability, roots use relatively more of these resources, leaving less for the shoots. Consequently, leaf growth is limited by the supply of nutrients and water and less assimilates are incorporated above-ground. The excess assimilates are then transported to the root, enhancing root growth relative to that of shoots (Wison *et al.,* 1988; Ericsson, 1995; Reynolds and D'Antonio, 1996; Farrar and Gunn, 1998; Grechi *et al.,* 2007). This integration of environmental conditions at the whole plant level can also be illustrated by split root experiments. In that case, a part of the root system is submitted to a treatment, and the perception of this treatment will have effects not only on the treated part, but also on growth and development of the rest of the plant (Gansel *et al.,* 2001, Dong *et al.,* 2010, Girin *et al.,* 2010). Other examples of the integration of environmental signals at the whole plant level are the tight relationship that has been observed between root growth and PPFD (photosynthetic photon flux density) in sunflower (Aguirrezabal *et al.,* 1994) or between shoot growth and the light quality through red/far red ratio (Robin *et al.,* 1994, Christophe *et al.,* 2006).

1.3 Variations of growth and biomass allocation patterns during plant development

Despite many studies showing results in accordance with the functional equilibrium theory, its generality has been questioned (Coleman, McConnaughay & Ackerly 1994; Coleman & McConnaughay 1995; Muller, Schmid & Weiner 2000; Reich 2002). Specifically, much of the variation in biomass partitioning in a given environment could, in fact, be driven by differences in plant size, or developmental stage (Coleman *et al.,* 1994; Pigliucci *et al.,* 1996; Schlichting & Pigliucci, 1998; Cheplick, 2003; Valladares *et al.,* 2006). Indeed, biomass allocation patterns are not constant during plant life cycle (Troughton 1956). For example Goudriaan and Van Laar 1994 (on wheat) or Leblon and Guérif, 1992 (on rice) showed that the proportion of biomass allocated to roots was particularly variable, reaching 50% at the beginning of the vegetative phase and tends to 0 at flowering. Biomass production rate is maximal when the rosette is totally deployed, and biomass production per unit leaf area starts to decrease when the leaves are overlapping (Marcelis *et al.,* 1998). After the flowering, leaves become more and more senescent and biomass is reinvested in the developing seeds (Christophe *et al.,* 2008). The root system grows at its maximal rate just after plant emergence and stops its development after the apparition of the inflorescence (Christophe *et al.,* 2008). Therefore, the timing of the analysis of root/shoot allocation has to be considered.

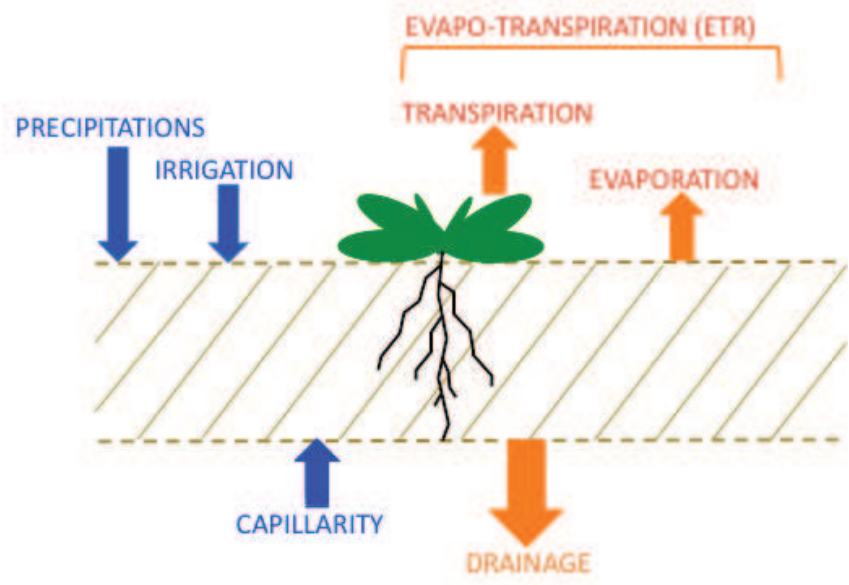


Fig. 2. Plant water balance (from Tardieu et al., 2006)

Plant water balance corresponds to the amount of water available for the plant. It results from water imput (blue arrows: precipitations, irrigations, and water rising by capillarity), and from water output (drainage, transpiration, evaporation).

2. Plant responses to water deficit

2.1 Water, drought and agriculture

Water is an essential component of plant life through several functions. It is involved in a large variety of biochemical reaction. Its movement into cells contribute to turgor maintenance. Its transport out of the plant (transpiration) contributes to temperature maintenance (cooling effect). The amount of water necessary to plant build-up is as high as 300-500 kg/kg of fresh plant material produced depending on species efficiencies. High yielding western agriculture uses massive amounts of water, in particular through irrigation. Debits of water for irrigation are estimated at 15% of the total water debits (industry, drinkable water, energy production). But since agriculture releases less water in the environment than the other activities, the net debit raises to almost 50%, and can reach 90% in summer, when water demand by crops is at its maximum (IFEN, French institute for environment, 2003). This high demand for water by agriculture faces a high level of competition for this resource in many regions of the world. In regions with chronic water deficit or inadequate water management, water deficit is considered as the main limiting factor for crop yield (Tardieu *et al.,* 2006).

The plant world is one of the components of the soil-plant-atmosphere continuum and water deficit is the result of an imbalance between water supply and demand in this continuum. It can then be the result of either a lack of water in the soil and/or a high evaporative demand by the atmosphere. The extractable water in the soil is the difference between the amount of water in the soil volume explored by the root system at full capacity, and the amount of water remaining at the permanent wilting point. This amount depends on rains, irrigations, and of water stored in the deep soil layers that can be released by capillarity. At the other end of the continuum, the evaporative demand of the atmosphere is a direct function of air temperature, windspeed, irradiance and relative air humidity, well approached by the Penman-Monteith equation (Beven, 1979; Fig. 2). 95% of water circulating within the plant only transfers the excess of temperature caused by the sun.

In this context, two types of strategies are classically engaged and combined by farmers. The first strategy consists in the choice of the species to be grown considering the position of the crop cycle during the year

(winter-spring or spring-summer) and of course the expected economic yield. In several regions of the world, drought is likely to be more probable in summer and dry seasons and focusing on spring-winter crops is thus assimilated to an escape strategy. In the wild several species use this strategy to complete their cycle before summer drought. Some crop species (as grapevine and alfalfa), are well adapted to drought because they can develop long root systems able to extract water from deep soil layers. Other species are known as drought tolerant (*eg* sunflower and sorghum) as they display high yield maintenance to water deficit associated with adequate maintenance of vegetative and reproductive growth and development. At the other end of the tolerance spectrum, species such as maize or pea are very sensitive to water stress (and thus require heavy irrigation) during their life cycle. Maize, in spite of its C4 metabolism that confers an elevated water use efficiency, is very sensitive to water deficit, especially around flowering (Claasen and Shaw, 1970; Zinselmeyer *et al.,* 1999). This paradox and the major role of maize in the worldwide production of cereals, justify that many scientific projects targeting drought tolerance have been performed on maize.

The choice of cultivars is also crucial as a large range of variation for drought tolerance exists in several species (sunflower, Chimenti *et al.,* 2002; maize, Bruce *et al.,* 2003). However, this range could have been narrowed during human selection for high yielding varieties grown under optimal conditions. Therefore, attemps to re-introduce sources of tolerance in ancestors or wild relatives are going on (for example in durum wheat, Ashraf *et al.,* 2010). Moreover, the concept of neo-domestication has been developed recently to reshape crop species based on current needs for tolerant crops (such as in sunflower, Nooryazdan *et al.,* 2011). Beside acting on genetic sources of tolerance, crop management is also a powerfull leverage for improving water use efficiency by actiong on sowing date, tillage, soil coverage (Debaecke and Aboudrare 2004)).

2.2- Plant phenotypic plasticity in response to water deficit

Environments are highly heterogeneous both in space and time, and organisms must either acclimate to, or escape from, adverse conditions. Phenotypic plasticity, or the capacity of a given genotype to render different phenotypes under different environmental conditions, is a means to cope with environmental heterogeneity that is particularly adequate for sessile organisms (Bradshaw, 1965; Sultan, 2000; Sultan, 2001; González & Gianoli, 2004; Saldaña *et al.,* 2005). Many studies have shown that plants are plastic for numerous ecologically important traits, ranging from morphology, physiology and anatomy, to developmental and reproductive timing,

breeding system and offspring developmental patterns (Sultan, 2000). In the last two decades phenotypic plasticity of plants has become a central issue of ecological and evolutionary research (Scheiner, 1993; Sultan, 1995, 2001; Pigliucci, 2001; Schlichting, 2002; DeWitt & Scheiner, 2004; van Kleunen & Fischer, 2005; Valladares *et al.,* 2006).

There is abundant evidence that plant species and populations may differ remarkably in the extent of their plastic responses to comparable environmental challenges (Schlichting & Levin, 1984; Valladares *et al.,* 2000, 2002a; Balaguer *et al.,* 2001; Sultan, 2001). Because phenotypic plasticity can be very advantageous for plants, the question arises of why plasticity is not always maximal. The fact that plasticity observed in nature is often lower than that expected suggests the existence of costs and limits of plasticity. The costs and limits of phenotypic plasticity are not as well understood as its benefits (DeWitt *et al.,* 1998; Givnish, 2002). The potential plastic response in a given trait may be large but the observed plasticity can be lowered by resource limitation or environmental stress (van Kleunen & Fischer, 2005).

In the specific case of water deficit, all plant processes (organogenesis, morphogenesis, and metabolism) display a considerable plasticity to deal with the constraint. Metabolic responses are observed as short-term responses to water deficit, whereas plant structure modifications result from long period of water deficit conditions. However, it is possible to group plant responses in four axes: (i) Limitation of water loss by stomata adjustments (Tanaka *et al.,* 2005), (ii) Limitation of water loss reducing the exchange area, the shape and the number of leaves (Reymond *et al.,* 2003), (iii) Increase of water absorption, increasing the soil volume explored by the roots (Turner *et al.,* 2001, Chaves *et al.,* 2002; Costa França *et al.,* 2000, Zlatev 2005), (iiii) Limitation of tissue dehydratation by osmotic adjustment (involving inorganic ions, carbohydrates, and organic acids) (Munns 1988; Save *et al.,* 1993; Nguyen *et al.,* 1997; Hummel *et al.,* 2010) or by the production of heat shock proteins that will protect the organites and the membranes (Burke *et al.,* 1985; Wang *et al.,* 2004).

Those responses appear in the order precised above following soil water deficit. Depending on the water deficit intensity, all or only a part of these responses may occur. The first response can take place very quickly (a few minutes), and is reversible, whereas the others need a few hours at least to be carried out and are generally irreversible when they affect plant structure (organ sizes or shapes). If soil dessication continues, the plant will die from tissue dehydratation. These responses, including temporary responses, induce adverse reactions as a decrease in photosynthetic activity, or as the loss of vegetative and reproductive organs that can make the biomass production and yield lower. At the whole plant level, the decrease of the rate of shoot growth is one of

the earliest phenotypic responses to water deficit (Hsiao 1973; Boyer 1970; Muller *et al.,* 2011). It occurs before stomata closure and photosynthesis reduction (Bogeat-Triboulot *et al.,* 2007) and well before cellular processes associated with tolerance to dehydration take place (Tardieu 1999).

Following the functional equilibrium theory, drought-stresses plants should increase root biomass allocation. Whereas shoot growth is well known to be early and strongly reduced by water deficit (Tisné *et al.,* 2010; Parent *et al.,* 2009), the response of root growth seems more complex and variable. Indeed, it has been reported to be reduced, unchanged or stimulated, depending on cases (*e.g.* Poorter and Nagel 2000). However, in all cases, root growth seems to be less affected than shoot growth (Spollen *et al.,* 1993; French and Turner, 1991, Shao *et al.,* 2008), which could lead to an increased root/shoot ratio. This is observed in many cases (Chartzoulakis *et al.,* 1993, Turner *et al.,* 1997 in wheat ; Asseng *et al.,* 1998, Bogeat-Triboulot in poplar ; Lei *et al.,* 2006, Hsiao *et al.,* 2000 in maize Padilla *et al.,* 2009, Erice *et al.,* 2010, Wu *et al.,* 2008, van den Boogaard *et al.,* 1996), but not in all the cases. Some studies report a constancy of the root/shoot ratio under water deficit conditions (Shone *et al.,* 1983, Osorio *et al.,* 1998, Heilmeier *et al.,* 2001), or even a root/shoot decrease under such conditions (Rice *et al.,* 1979, Hirai *et al.,* 1994, Asch *et al.,* 2004). This absence of consensus on the root/shoot ratio could be explain by the equal importance of shoot and root growth maintenance for plant to maintain water and mineral uptake by conserving root growth, and to maintain photosynthesis and biomass production at the shoot level.

Another trait reflecting the trade-off between leaf expansion and biomass allocation or production within leaves is the Specific leaf area (*ie* leaf area per unit dry mass, Specific leaf area). The Specific leaf area is often used as an indirect indicator of leaf thickness, and reported to be reduced under drought conditions (Marcelis *et al.,* 1998, Liu and Stützel 2004). Decrease in Specific leaf area in droughted plants could be due to the different sensitivity of photosynthesis and leaf area expansion to drought (Jensen *et al.,* 1996, Tardieu *et al.,* 1999, Hummel *et al.,* 2010). Reduction of Specific leaf area is assumed to be a way to improve water use efficiency (WUE) (Wright *et al.,* 1994, Craufurd *et al.,* 1999), because thicker leaves usually have a higher density of chlorophyll and proteins per unit leaf area and, hence, have a greater photosynthetic capacity per unit leaf area than thinner leaves. The root equivalent of Specific leaf area, the Specific root length, is an indirect indicator of root thickness. Specific root length illustrates the trade-off between long and thin roots (high Specific root length) or short and thick roots (low Specific root length) with the same biomass. High Specific root length has been shown to be favourable to exploit water in the deep soil layers while low Specific root length could contribute to root growing more easily in a compact drying soil (Yoshida 1982, Eissenstat 1991, Zheng *et al.,* 2000). Specific root length has been reported to be either decreased (Kage *et al.,* 2004 on cauliflower) or increased (Azhiri-Sigari *et al.,* 2000 on rice) in droughted plants. Nevertheless the correlation between modification of specific root

length and plant tolerance to drought is still under debate (Schwinning *et al.,* 2001, Vamerali *et al.,* 2003).

Besides, phenotypic plasticity is a source of ample phenotypic variation that may promote adaptive divergence and, thus, evolution and speciation (West-Eberhard, 2003).

3. How to connect phenotypic and genotypic variation? Quantitative genetics

3.1. Concepts

Trait variation can be continuous or discrete. Formal genetic was originally based on the study of traits for which the phenotypic variation is discrete, and that can be spread into classes without necessarily quantifying the phenotype by measurements (i.e., tall vs. short, large vs. small, etc...). That was the analysis frame of the green pea of Mendel, but this is also the case for example in all the studies of mutants recovered from forward genetic screens, which normally only consider individuals that are many standard deviations away from the population mean. These variations were explained by a monogenic factor, for which we compare the effect of two different alleles. The observation of this type of variation is related to the terms “wild-type” and “mutant”, designing the two phenotypic classes, or the two alleles. However, most of the traits of interest for breeders and agronomists do not follow this description. These traits present continue phenotypic variation, corresponding to a complex determinism, and then controlled by the action of several genetic and environmental factors (Gallais, 1989). Those traits are quantitative because the individuals cannot be arranged into classes, but their phenotype is measured. Precocity, enzyme activity, organ sizes and weights, or grain yield for example, are quantitative traits. The phenotypic trait is described by its distribution, often assimilated or brought down to normal distribution, and characterized by the mean and the variance measured in a sample of individuals. In parallel, each individual is characterized by its genotype, corresponding to the allele combination that it has at one or several loci. The whole factors that can affect the phenotypic value of a trait, in addition of its genotype is called environment (Johannsen, 1909; East, 1916).

Two main methods have been developed to identify genotype/phenotype relationships. The first one is called

linkage mapping, or *QTL mapping* (for Quantitative Trait Loci), and the first QTL analysis was done in the 20s, with the studies of Sax (Sax, 1923), which aimed at identifying genes influencing the seed mass with markers that were morphological at this time (seed pigmentation and shape). Since the 1990s, the discovery of molecular markers and the advances in rapid and cost-effective genotyping methods and the development of statistical methods for QTL mapping have revolutionized the field of mapping quantitative traits. The landmark paper by Lander and Botstein (1989) launched an avalanche of QTL studies (Mackay *et al.,* 2009). The second approach is called *linkage disequilibrium mapping*, or *association mapping* (Mackay and Powell, 2007). Both QTL and association mapping rely on the use of recombination events in populations that have fragmented the genome of each individual into small parts that can be associated with the measured variation of phenotypic traits. Considering this aspect, linkage mapping is a specific case of association mapping, but these methods differ in particular in the source of recombination. Both approaches were used in this study, and will be detailed in the next sections.

3.2. QTL mapping

The accuracy of any QTL analysis mapping depends on many elements (Carbonell and Asíns 1996). The type of segregating population, its size, the heritability of the trait, the number and contribution of each quantitative trait locus to the total genotypic variance, their interactions, their distribution over the genome, the number and distance between consecutive markers, the percentage of codominant markers, the reliability of the order of markers in the linkage map, the evaluation of the trait, and the statistical detection method influence the power and resolution of QTL mapping.

3.2.a. Segregating mapping populations

There are several types of experimental designs that are suitable for QTL analysis, depending on the mating system of the species. But the populations used for QTL studies have in common to be artificially created from a cross between parental lines (generally two) so that the recombination events that occur within the population are known. In autogamous species, QTL mapping studies make use of F2 or backcross progenies because they are the easiest and earliest to obtain (only two crosses). But these populations have the disadvantages not to be fixed as homozygous, and each individual plant is unique and cannot be multiplied. The F2 and backcross populations have therefore to be re-created and re-genotyped for each experiment, which is time and money

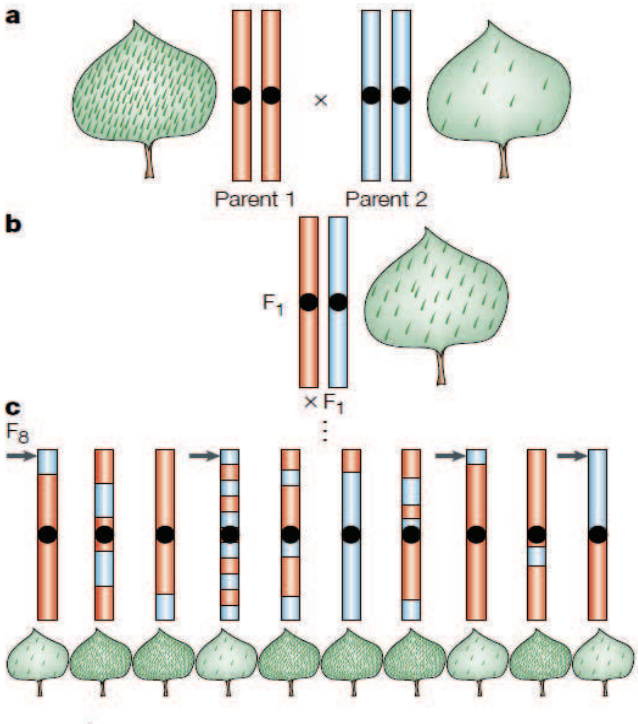


Fig. 3. Creation of Recombinant Inbred Lines (RIL) population and principles of mapping quantitative trait loci (adapted from Mauricio, 2001).

The basic strategy behind mapping quantitative trait loci is illustrated here for (**a**) the density of trichomes hat occur on a plant leaf. Inbred parents that differ in the density of trichomes are crossed to form an F1 population with an intermediate trichome density. (**b**) An F1 individual is selfed to form a population of F2 individuals. (**c**) Each F2 is selfed for six additional generations, ultimately forming several recombinant inbred lines (RIL). Each RIL is homozygous for a section of a parental chromosome. The RIL are scored for several genetic markers, as well as for the trichome density phenotype. In **c**, the arrow marks a section of chromosome that derives from the parent with low trichomes density. The leaves of all individuals that have inherited that section of chromosome from the parent with low trichome density also have low trichome density, indicating that this chromosomal region probably contains a QTL for this trait.

consuming. On the other hand, F2 and backcross populations, because they conserve many heterozygous loci, can be used to detect both additive and dominance genetic effects, the latter being only expressed when the QTL locus is heterozygous. By contrast, recombinant inbred lines (RIL) are fixed, homozygous individuals, obtained from successive selfing generations starting from F2. At each generation, the level of heterozygosity decreases, and after six generations, the proportion of the heterozygosity along the genome is theoretically 3.1 %. Moreover, at each generation, crossing-overs split up the parental genomes into fragments. Finally, each RIL corresponds to a specific combination of the parental alleles at each locus (Fig. 3, Mauricio 2001). Once the population has been genotyped, RIL can be multiplied, allowing repeated measurements of various traits in different conditions (Doerge, 2002). This "immortality" confers a real advantage, especially to study traits that have a low heritability (Knapp and Bridges, 1990; Lander and Botstein, 1989). But the low amount of heterozygosity makes it impossible to detect dominant effects.

The first populations created for the QTL analysis in Arabidopsis were F2/F3 families (Koornneef *et al.,* 1983; Chang *et al.,* 1988; Nam *et al.,* 1989). These types of populations are still used (Xiong *et al.,* 1999; Mei *et al.,* 2004). The two Arabidopsis RIL populations that have been used most frequently have been derived mainly from laboratory accessions, namely Ler/Col (Lister and Dean 1993) and Ler/Cvi (Alonso-Blanco *et al.,* 1998), and QTL for drought or ozone tolerance, flowering time, plant or seed size, seed dormancy and pathogen resistance have been identified (review in Alonso-Blanco and Koornneef 2000). The Bay/Sha RIL population (Loudet *et al.,* 2002), obtained from the cross of Bay-0, coming from fertile plains of Germany, and Shahdara, collected in the high mountains of Tadjikistan has been shown to display large variability of growth related traits, especially associated to root architecture (Loudet *et al.,* 2005), in response to various environmental conditions (Loudet *et al.,* 2003b; Reymond *et al.,* 2006). Moreover, recent studies showed that Central-Asian accessions, such as Shahdara represent an original material, genetically distant from globally unstructured European accessions such as Bay-0 (Innan *et al.,* 1997; Sharbel *et al.,* 2000). The Bay/Sha RIL population was chosen for this study, as the phenotypic variation resulting from such a cross is expected to reflect the adaptation to their specific habitat and the genetic distance between them.

3.2.b. Genetic linkage and QTL maps

The analysis of the segregation of parental alleles in a population requires the use of molecular markers. These markers should present several characteristics: They have to be polymorphic (reflect the the genomic diversity between the individuals), at least biallelic, codominant (none of the parental allele shoud mask the presence of the others), and neutral (no effect on the phenotype) (de Vienne and Santoni, 1998; Santoni *et al.,* 2000).

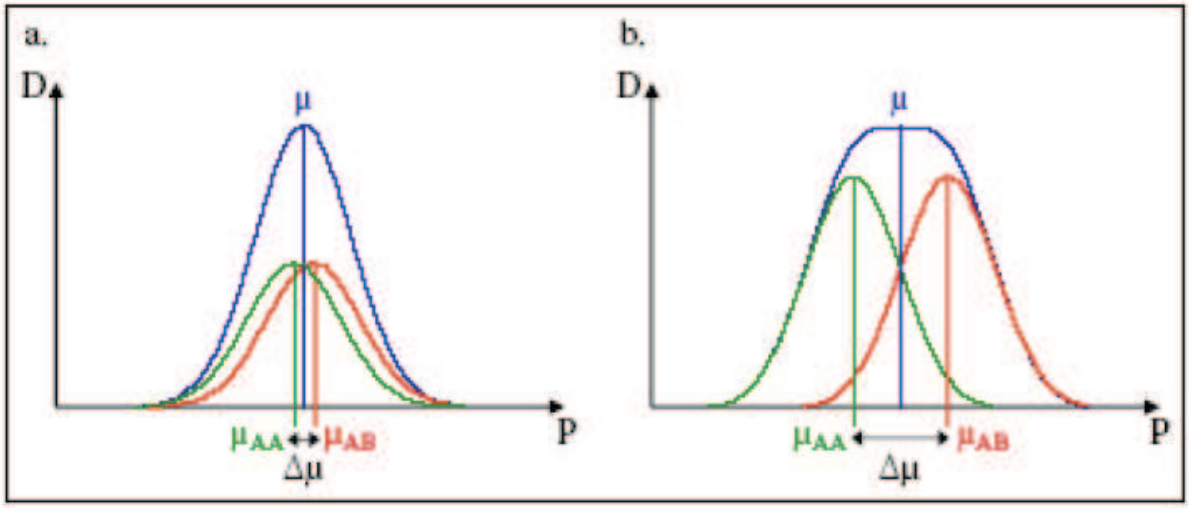


Fig. 4. Schematic representation of the absence (a) or presence (b) of a quantitative trait loci on the distribution of a quantitative trait (from Segura, 2006).

The QTL identified in the **b** case is due to the substitution of the A allele by the B allele for allelic classes AA and AB at one specific marker. μ: general mean value, μAB: mean of the allelic class AB, Δμ: Difference between the mean of AA and AB allelic classes (effect of the substitution of A by B allele). P: Phenotypic value, D: Density.

Several types of molecular markers exist (AFLP, RFLP, SNP, microsatellites), and their identification is based on different types of polymorphism (of length, of presence/absence of restriction sites).

The genetic map of the Bay/Sha RIL populations was initially constructed with 38 (Loudet *et al.,* 2002) and later 69 microsatellite markers (or SSR for simple sequence repeat). These markers consist in the repeat of a short sequence of nucleotides (one to five in general). Their polymorphism depends on the number of repeats of these short sequences in the different individuals. Characterizing a population of individuals with molecular markers allow to build genetic maps. The principle of genetic map building relies on the concept of genetic linkage. In the absence of recombination, the whole genetic information of one chromosome and the molecular markers associated, thereby physically linked, must be transmitted to the next generation as one block. During meiosis, crossing overs lead to recombinations between these different groups of linked markers, and markers of the same chromosome can be rearranged (Morgan, 1911). The number of these recombinations is as a first approximation proportional to the distance between the two loci on the chromosome. The estimation of the distance between two loci allows building a genetic map, which is a network covering the whole genome, and based on markers. The genetic map is termed saturated when each point of the genome is linked to at least one marker. The advantage of these genetic maps is to enable, for each individuals in the population, the estimation of the genotype at each point of the genome, knowing the genotype at the markers linked to this point.

3.2.c. Methods for QTL detection

QTL mapping consists in searching for statistical correlations between a phenotype and the polymorphism of markers in the whole population, *i.e* the analysis of phenotypic mean values of the different allelic classes (Fig. 4). The first QTL analyses, in the 80s, consisted in testing the effects of markers one by one, with a simple analysis of variance (Tanksley *et al.,* 1982; Edwards *et al.,* 1987; Paterson *et al.,* 1988). In that case, if the marker polymorphism has an effect on the mean phenotypic value, a significant difference between the allelic classes will be detected. But this method, with each marker tested one by one, does not enable determining whether a marker is linked to one or several QTL; does not enable to precisely determine the QTL position, and is not very powerful because of confounding effects of recombination events between the marker and the QTL (Lander and Botstein, 1989; Zheng *et al.,* 1994).

Other methods, termed *Interval Mapping* (Lander and Botstein, 1989), have been developed subsequently, taking into account genetic information between two adjacent markers, infered by probabilistic models. The most commonly used test is a *likelihood ratio test* (LRT, Lander and Botstein, 1989), producing a *logarithm of the*

odds ratio, or LOD score, which corresponds to the likelihood to have a QTL anywhere along the genome, even between the markers. With a fine-scale genetic marker map throughout the genome, *Interval mapping* can be performed at any position covered by markers to produce a continuous LRT statistical profile along chromosomes. The position with the significantly largest LRT statistic in a chromosome region is an estimate of QTL position. Many studies have shown that *interval mapping* method is more powerful than the *marker-by-marker* method, in particular when the number of markers is low (Zeng *et al.,* 1994; Rebai *et al.,* 1995). The main problem for interval mapping methods lies in the consideration of QTL one by one during the analysis, which could be a problem when many QTL control the variation of one trait (Haley and Knott, 1992; Lander and Botstein, 1989).

To take these multiple QTL into account, new methods based on a combination of interval mapping and multiple regression was developed, termed *Composite interval mapping* (Zheng *et al.,* 1994) or *Multiple-QTL model* (Jansen and Stam, 1994). This approach is performed in two steps: The markers that appear in a first run to be linked to a QTL (high LOD score) are used as cofactors in a second run of QTL detection. Decreasing the residual variance, this method increases the power of QTL detection, and enables to separate linked QTL (distant only from 20cM) compared to *Interval mapping* (van Ooijen, 1994; Utz and Melchinger, 1994). For mapping multiple QTL, Kao *et al.,* (1999) and Zeng *et al.,* (1999) developed a method that fits a multiple-QTL model including epistasis on a trait and simultaneously looks for the number, positions, and interaction of QTL. This method, called *Multiple Interval Mapping* (MIM), is based on maximum likelihood and combined with a model selection procedure and criterion. The multiple QTL model is tested again and again until obtaining the model explaining most of the variance of the trait. Compared with *Interval Mapping* and *Composite Interval Mapping*, *Multiple Interval Mapping* has a number of advantages, such as the improved statistical power in detecting multiple QTL (Zeng *et al.,* 2000), facilitation for analyzing QTL epistasis, and coherent estimation of overall QTL parameters. Even if some problems may arise, due to the complexity of automatic procedures in the model (Zheng *et al.,* 1999), this method has been successfully used in recent QTL studies (Kao *et al.,* 2004; Hao *et al.,* 2010).

3.2.d. Factors limiting relevance and statistical power of a QTL study

QTL studies could have different objectives: Understanding the genetic architecture of a specific trait, looking for markers to select favourable alleles, or cloning of genes implied in the determinism of a specific trait. But whatever the objective is, we have to optimize the QTL detection considering different factors that have an impact on QTL detection. First, QTL detection accuracy strongly depends on the number of individuals in the

population. The more individuals are used, the more the QTL detected will be accurate, and numerous (van Oiijen, 1992; Charcosset and Gallais, 1996; Charmet 2000). The studies that use the information of many markers to separate the effect of linked QTL profit from a large number of individuals, because this allows to increase the number of observed recombination events between QTL (Goffinet and Mangin, 1998). Noteworthy, the different types of QTL populations differ in the number of recombination events, with RIL populations therefore supporting more QTL than F2 ones (de Vienne, 1998). A second factor that can interfere with QTL detection power is the density of markers considered, and studies report that the ideal genetic marker density would be one marker every 20cM (Darvasi and Soller, 1994; Charmet 2000). However, if the genotyping means are limited, increasing the number of individuals genotyped would be more efficient for QTL detection power than increasing the number of markers (Charmet *et al.,* 2000). Finally, the third important factor to take into account for QTL detection is the heritability of the trait considered. By definition, traits that display high heritabilities are less affected by the environment (or genotype by environment interactions), leading to a higher part of the total variance being explained by genetics (QTL) (Zheng *et al.,* 1994; Charmet 2000; Asins 2002).

3.2.e. Landmark QTL studies

QTL mapping has historically been used as the main approach to map genes responsible for variation in ecologically and evolutionary significant traits (Lynch and Walsh 1998) and QTL studies have identified numerous loci that may be responsible for growth traits variation. Many studies that examine growth do so by contrasting growth in different abiotic environments (Maloof, 2003). QTL that have significantly different effects accross the environments will be associated with substantial genotype by enviroment (GxE) interaction effects. Such GxE interaction effects can be indicative of QTL that are specific to a particular environment, wheras a lack of GxE interaction can suggets that a QTL is a more general growth regulator.

Many QTL studies have examined plant growth or morphology characteristics in response to abiotic environment. For instance, QTL studies have examined root growth characteristics as they relate to growth under drought stress (Price *et al.,* 2002). In maize, one study examined the weight of adventitious roots and the length, diameter, and weight of primary roots, in hydroponics. QTL for each of these traits were compared to those of grain yield under well watered and water deficit conditions (Tuberosa *et al.,* 2002). Price and colleagues identified QTL that affect the weight, length and thickness of roots in drought stressed and watered rice (Price *et al.,* 2002). Shoot growth characteristics have also been widely studied. For instance, the shoot growth of Arabidopsis RIL in varying nitrogen conditions has been investigated (Loudet *et al.,* 2003). Some of all these QTL of interest have been cloned by map-based cloning in Arabidopsis, rice, and tomato for example, suggesting that QTL cloning could be a feasible way to investigate the genetic bases of growth variations in

response to environment (Frary *et al.,* 2000; Remington *et al.,* 2001; Maloof, 2003).

3.3. Association mapping

3.3.a. Natural populations and linkage disequilibrium

As for linkage mapping, the precision of association studies depends on the presence of recombination between the markers. But contrary to linkage mapping studies, which use populations in which the recombination is controlled and known, the association mapping studies take advantage of recombination that occurred during all the evolutionary history of the population of natural accessions. In the absence of physical link between two loci, they segregate randomly in the individuals of a population. Conversely, linkage disequilibrium refers to the non random segregation of loci. It is the correlation between polymorphisms [e.g., single nucleotide polymorphisms (SNP)] that is caused by their shared history of mutation and recombination (Kim *et al.,* 2007). Thus, the genotyped markers become proxies, or sentinels, for the functional variant because their genotypes are highly correlated with the genotypes of the functional variant. In a large, randomly mating population with loci segregating independently, but in the absence of selection, mutation, or migration, polymorphic loci will be in linkage equilibrium (Gupta *et al.,* 2005). In contrast, linkage, selection, and admixture will increase levels of linkage disequilibrium. The distance over which linkage disequilibrium persists will determine the number and density of markers, and experimental design needed to perform an association analysis.

Because allele frequency and recombination between sites affect linkage disequilibrium, most of the processes observed in population genetics are reflected in linkage disequilibrium patterns (Flint-Garcia *et al.,* 2003). Mutation provides the raw material for producing polymorphisms that will be in linkage disequilibrium. Recombination is the main phenomenon that weakens intra-chromosomal linkage disequilibrium, whereas inter-chromosomal linkage disequilibrium is broken down by independent assortment. Population size also plays an important role. In small populations, the effects of genetic drift result in the consistent loss of rare allelic combinations, which increase linkage disequilibrium levels. Population mating patterns and admixture can strongly influence linkage disequilibrium (Gupta *et al.,* 2005). Generally, linkage disequilibrium decays more rapidly in outcrossing species as compared to selfing species (Nordborg, 2000). This is because recombination is less effective in selfing species, where individuals are more likely to be homozygous, than in outcrossing species. But the linkage disequilibrium also depends on the population used (Storz and Kelly, 2008) and of the

genomic region in which it is measured (Barnaud *et al.,* 2006). Indeed, a region that is strongly conserved from generations to generations will display a larger linkage disequilibrium than others (Palaisa *et al.,* 2004).

Most of the linkage disequilibrium research in plants has been carried out in maize (Labate *et al.,* 2000; Remington *et al.,* 2001; Tenaillon *et al.,* 2001; Rafalski *et al.,* 2002) and Arabidopsis (Nordborg *et al.,* 2002 Kim *et al.,* 2007) and rice. The linkage disequilibrium pattern in Arabidopsis thaliana is a sharp contrast to the pattern in maize. As expected, linkage disequilibrium extends much farther in Arabidopsis because it is a highly selfing species (Nordborg, 2000). Hagenblad and Nordborg (2002) sequenced 14 short fragments from a 400kb region of the flowering time locus FRIGIDA. They found that linkage disequilibrium decayed within 250kb, equivalent to 1 cM. Analysis of 163 genome-wide SNPs in 76 accessions also revealed that linkage disequilibrium decayed within 250kb (Nordborg *et al.,* 2002) and even within 5kb in another population (Kim *et al.,* 2007). This extensive linkage disequilibrium may be due to the limited number of recombination events that have occurred over the past 200 years. As mentioned above, this extensive linkage disequilibrium in Arabidopsis has the advantage to decrease the number of markers necessary to map the genome of this species, but also decreases the resolution of the association detected is the number of marker is just sufficient to map these recombination events.

3.3.b. Association studies models

Using natural populations enable to capture most of the genetic diversity of a species. This makes the association studies very powerful to detect interesting allelic combinations (Yu *et al.,* 2006). Even with a large extent of linkage disequilibrium in *Arabidopsis thaliana*, association studies are likely to be more accurate than QTL studies, that face the same low number of recombination events. Association studies therefore represent a good choice when positional cloning is unfeasible (Neale and Savolainen, 2004). Depending on the attempts of the association study, it will focus only on a candidate region, or on the whole genome. The first association study of a quantitative trait based on a candidate gene was the analysis of flowering time and the *dwarf8* gene in maize (Thornsberry *et al.,* 2001), and other candidate gene analyses have been performed (Hagenblad and Nordborg 2002; Caicedo *et al.,* 2004). Recently, with the advances of the new techniques of sequencing, large markers densities can be determined for the whole genome, allowing genome-wide association studies (Aranzana *et al.,* 2005; Zhao *et al.,* 2007; Atwell *et al.,* 2007; Mariac *et al.,* 2011).

The statistical models used for association mapping do not dramatically differ from those of QTL studies, with the same equation: $P_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ (de Vienne and Causse, 1998), where P_{ij} is the phenotypic value measured in the

genotype j carrying the allele i, μ is the mean value of the population, α_i is the random effect of the allele i, and ε_{ij} is the residual error term. But contrary to the models for QTL studies, the allelic effects are here considered as fixed, that enable to test their significance by simple ANOVA or logistic regression (Thornsberry *et al.,* 2001). But two problems specifically related to association mapping have to be considered, the effect of the relatedness between individuals of natural populations (Pritchard *et al.,* 2000b), and the effect of multiple testing.

> Correction for population structure and of relatedness between individuals

Except in population genetics theory, randomly mating populations probably do not exist. Populations of natural accessions evolve under different selection pressures (mutation, migration, genetic drift and selection) from a common ancestor. Therefore, individuals of these populations cannot be considered as independent. In association mapping, complex patterns of genetic relatedness among individuals can be problematic when trying to map a phenotype whose variation is correlated with genetic relatedness. In such cases of genotype-phenotype covariance, many genetic markers across the genome will appear to be associated with the phenotype, when in fact these genetic markers simply capture the genetic relatedness among individuals. This problem is particularly apparent when trying to map traits that have been subject to adaptation to local environments that vary systematically with geography (such as temperature or growing season length), like flowering time or plant size (Aranzana et al., 2005; Flint-Garcia et al., 2005, Atwell et a*l.,* 2007) because variation in these phenotypes between populations is highly correlated with allele frequency differences between populations. This covariance can lead to spurious associations, and extremely high false positive rates (Lander and Schork, 1994; Myles *et al.,* 2009).

Two main corrections have been developed to take the population structure into account (Yu *et al.,* 2006). The first one considers the degree of genetic correlation between each individuals and an ancestral population, from which it would be derived. Then, all the individuals assigned to the same ancestral population would more likely share phenotypic and genetic variation. Following this idea, each individual can also be "attributed" to several ancestral populations. This was formalized in 2001 by the Structured association model of Thornsberry in 2001 (Thornsberry 2001). Many methods have been proposed to statistically infer this degree of population structure defining ancestry coefficients, based either on a bayesian method, as implemented in the STRUCTURE program (Pritchard *et al.,* 2000b) or on principal components analysis, in which each axis corresponds to an ancestral population (Pritchard *et al.,* 2000a). However, the degree of belonging to ancestral populations does not reflect the relatedness between individuals taken two by two (Yu *et al.,* 2006; Saidou *et al.,* 2009). This relatedness leads to alleles that are common to both individuals and inherited from a common ancestor. This second

dimension of population structure is refered as the definition of the level of relatedness between pairs of individuals, defining kinship matrix that could be integrated to the model (Saidou *et al.,* 2009).

> Correction for multiple testing

The multiple independant tests performed could lead to the detection of false positives simply associated by chance. Several methods have been proposed to calculate the number of false positives, and to include a correction term in the association model. The first one has been proposed by Bonferroni, and is calculated as: $\beta = \alpha/n$ (where α is the risk threshold, and n the number of tests). But this correction is very drastic, and could lead to an increased detection of false negatives. An elegant way to deal with the problem, that was recently advocated for ecological studies by Garcı´a (2003, 2004), is to control the proportion of significant results that are in fact type I errors ('false discoveries') instead of controlling the chance of making even a single type I error, as Bonferroni correction does. This new approach, called False discovery rate, was developed by Benjamini and Hochberg (1995).

FDR control provides a sensible solution: it offers an easily interpretable mechanism to control type I errors while simultaneously allowing type II errors to be reduced (Verhoeven *et al.,* 2005). Control of the false discovery rate is being widely adopted in genomic research. Genomewide associations studies necessitate the interpretation of hundreds or thousands of simultaneous tests, and minimizing the chance of making even a single type I error can keep the vast majority of true effects from being detected. FDR control can address a much wider range of multiple testing problems in evolution and ecology as well (García 2003, 2004), where the loss of power inherent to strict Bonferroni control does not do justice to the nature of many experiments. FDR control is more powerful and often is more relevant than Bonferroni´s correction. It is also flexible, and ease of interpretation is not affected by changing the significance threshold. Sensible biological interpretation of multiple testing results may therefore benefit more from FDR than from Bonferroni´s correction.

3.4. Combination of both QTL and Association mapping

Both association mapping and classical linkage mapping studies have been successful in the identification of genomic regions linked to important phenotypes (Loudet *et al.,* 2005, Atwell *et al.,* 2007). But they both have pro

Tab. 1. Advantages and drawbacks of methods for identifying genetic basis of complex traits in Arabidopsis thaliana (from Bergelson and Roux, 2010)

Methods	Starting year	Advantages	Drawbacks	
Traditional linkage mapping, that is, QTL mapping	1992	• No population structure effect • Identification of rare alleles • Few genetic markers required for a complete genome scan	• Coarse mapping • Limited genetic diversity • Not possible to distinguish between pleiotropic and physically close genes	Kowalski *et al.*, 1994
Association mapping with candidate genes	2002	• Fine mapping	• Requires detailed knowledge of the biochemistry and genetics of the trait under study • Approach is biased for previously identified genes	Ehrenreich *et al.*, 2009 Hagenblad and Nordborg, 2002
GWA mapping at the species scale	2005	• Fine mapping (blind approach) • Detection of common alleles	• False positives due to population structure • False negatives after controlling for population structure • Reduced power to detect rare alleles or weak-effect alleles • Genetic and allelic heterogeneity	Atwell *et al.*, 2010 Zhang *et al.*, 2007
Dual linkage–association mapping at the species scale (FIG. 2)	2007	• Fine mapping (blind approach) • Identification of false positives and false negatives	• Phenotyping of several thousands of individuals • Numerous traditional linkage mapping populations required • Genetic and allelic heterogeneity	Brachi *et al.*, 2010 Nemri *et al.*, 2010
GWA mapping in regional mapping populations	2010	• Fine mapping (blind approach) • Diminished population structure effect • Detection of genes involved in local adaptation	• Potential for limited phenotypic variation • Increased linkage disequilibrium: less precise than using a worldwide sample	Rosenberg *et al.*, 2010 Kim *et al.*, 2007
NAM at the species scale	Ongoing	• Fine mapping (blind approach) • Identification of false positives and false negatives • High-density genotyping of a small number of founders lines (<30)	• Importance of the crossing schemes and the number of founders • Phenotyping of several thousands of individuals • Genetic and allelic heterogeneity	Yu *et al.*, 2008 McMullen *et al.*, 2009

GWA, genome-wide association; NAM, nested association mapping; QTL, quantitative trait locus.

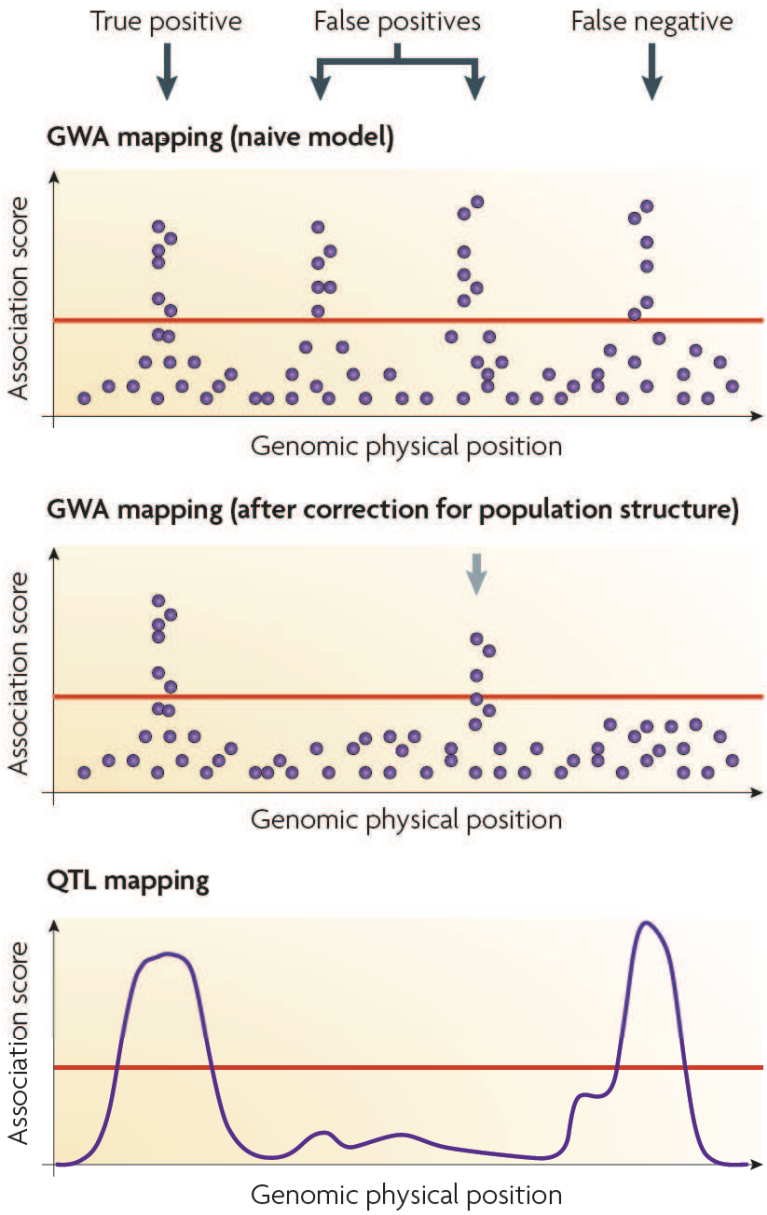


Fig. 5. Advantages of combining association and traditional linkage mapping methods. (from Bergelson and Roux, 2010).

Dual linkage– association mapping allows true positives and false negatives to be distinguished from false positives. True positives are causative SNPs that have been detected by genome-wide association (GWA) mapping and are overlapped by quantitative trait locus (QTL) regions. Population structure corrections highlight false positives that correspond to false phenotype–genotype associations. Because statistical methods that control for population structure only reduce (but do not abolish) the inflation of false positives, false positives may remain (grey arrow). In such cases, the remaining false positives are not validated by QTL regions, demonstrating the added value of QTL mapping in the detection of true positives. False negatives are causative SNPs that are lost as an artefact of population structure corrections but can be validated by QTL regions. The horizontal red line indicates the significance threshold for a phenotype–genotype association.

and cons, and one way to avoid these difficulties could be to combine both approaches. A very interesting paper has been recently published by J. Bergelson and F. Roux (Bergelson and Roux, 2010), and reviews the advantages and drawbacks of methods that can enable to identify genes associated to complex traits. The development of new mapping populations, such as Nested association napping in maize (Yu and Buckler 2006) or MAGIC lines (*Multiparent advanced generation inter-cross lines)* in Arabidopsis (Kover *et al.,* 2009) is discussed (Tab. 1). These new genetic material generally allow to combine the advantages of QTL analysis (control of recombination events, known population structure, easy statistical tests, rapidity of analysis) with the ones of association mapping (high allelic diversity, evolutionary considerations, ...). A second way of taking advantages of both approaches would be to use both QTL and association mapping successively or in parallel (Fig. 5).

4. Arabidopsis thaliana, a suited species to investigate genetic bases of plant tolerance to drought

In this study, we aimed at identifying the genetic basis of natural variation in drought tolerance. Therefore, the choice of the species studied was important. Ideally, it may combines both the advantages of a good model for genetic studies, and also display large natural variation of drought tolerance in diverse habitats.

Arabidopsis thaliana appeared to be well suited for our purpose. It is widely used as a model system in genetic studies for 30 years (Meinke *et al.*, 1998), because of specific characteristics that makes the species particularly appropriate for such analyses. Its genome size is very small (120 Mb) compared to other species such as rice (450Mb), or maize (2500Mb) (Barakat *et al.,* 1998) and concentrated on five chromosomes. This small genome size represents a great advantage for the construction of genetic map, because of limited number of markers will be sufficient to cover the entire genome. However, if the small genome size is an advantage for genetic studies, the small number of chromosomes could also represent a drawback, because it will lead to lower number of recombinations (Mauricio, 2001). The organization of the genome is also an asset for genetic studies, as it is composed predominantly of single-copy sequences and high level of information density (Pruitt and Meyerowitz, 1986; Szymanski, 2003). Since the choice of Arabidopsis as a model plant for genetic studies, the number of resources available for this species did not stop to increase. The genome of the reference accession, Col-0, was fully sequenced in 2000 (AGI, 2000). Several projects have followed (Clark *et al*., 2007) and are still running (1001 genomes project, Weigel and Mott, 2009; Cao *et al.,* 2011) to increase again the genomic data available

Tab. 2. Examples of ecological and evolutionary questions that can be addressed using model systems such as Arabidopsis thaliana (Mitchell-Olds, 2001)

• What genes are responsible for ecologically important variation? Why are they polymorphic? Are quantitative trait locus alleles young, deleterious and spatially restricted, or are they old widespread polymorphisms contributing to local adaptation?	Johanson *et al*., 2000
• Is plasticity adaptive? Do developmental responses to environmental conditions increase fitness? Have these responses been shaped by natural selection?	Dorn *et al*., 2000
• Can expression profiling identify candidate genes that influence insect resistance, drought tolerance, flowering time and other ecologically important traits in natural populations? Does natural selection promote adaptive evolution of these loci?	Schenk *et al*., 2000; Reymond *et al*., 2000
• How do polyploids differ ecologically and physiologically from their diploid relatives? How are these differences shaped by natural selection?	Nasrallah *et al*., 2000
• What is the genetic basis of speciation?	Lynch *et al*., 2000
• What is the relative importance of changes in gene regulation versus protein function within and among species?	Koch *et al*., 2001
• Do neutral molecular markers predict patterns of ecologically important variation in rare plants? What is the molecular basis of adaptively important polymorphisms in endangered species?	McKay *et al*., 2001

for this species. Large collections of mutants or EST, targeting specific known biological or physiological functions, have been developed and are now available in the stock centers (Bouche and Bouchez, 2001). The ever-increasing number of laboratories using this species also leads to the development of large databases such as TAIR (the Arabidopsis Information Resource), that include the complete genome sequence along with gene structure, gene product information, metabolism, gene expression, DNA and seed stocks, genome maps, genetic and physical markers that are crucial informations for geneticists. Biological characteristics of *Arabidopsis thaliana* make it also particularly appreciated for genetic studies. Its small size makes it easy to grow a large number of individuals in small spaces, and many experiments can be planned over the year because of its short generation time (around 6-8 weeks for early genotypes). The transformation with *Agrobacterium tumefaciens* is relatively easy in Arabidopsis, facilitating direct validation of specific gene functions in transgenic lines (Pitzsche *et al.,* 2011).

If the interests of *Arabidopsis thaliana* for genetic studies is now widely recognized, ecologists and evolutionary biologists have turned their attention to this species only much more recently and almost reluctantly. The reason for this is the perception that *Arabidopsis thaliana* is not particularly interesting ecologically and that it represents an oddity from an evolutionary standpoint (Pigliucci, 2002). Nevertheless, the crucifer *Arabidopsis thaliana* and its wild relatives provide a model system that has a vast array of molecular tools, genetic resources and biological information that can be used to address fundamental questions in ecology and evolution (Tab.2; Mitchell-Olds 2001). *Arabidopsis thaliana* is a widespread annual weed of rocky places and disturbed sites, native to Europe and central Asia and naturalized in North America, and thousands of accessions are now identified in the stock centers (Bevan *et al.,* 1999; Pigliucci *et al.,* 1998). Across this geographic range, it experiences a broad range of climatic conditions and selective pressures (Shindo *et al.,* 2007). This huge genetic variation has been exploited to analyse the genetic determinism of important adaptive traits, such as flowering time, plant and seed size, seed dormancy, pathogen resistance, and tolerance to abiotic stresses (for review, see Alonso-Blanco and Koornneef, 2000; Koornneef *et al.,* 2004). Considering flowering, *Arabidopsis thaliana* is a facultative long-day plant that varies among ecotypes as to its requirement for vernalization (over-wintering) (Hopkins *et al.,* 2008). In this species, environmental signals are processed by genetic pathways responding to day length, vernalization, ambient temperature and plant age, among others (Baurle and Dean, 2006). These pathways converge on a set of genes known as the 'floral pathway integrators', which ultimately will promote the transition to flowering (Kobayashi and Weigel, 2007). The interaction of these multiple pathways ensures a high degree of predictive power for the overall programme – allowing the plant to accurately determine what time of year it is and whether those conditions are suitable for flowering, according to climatic conditions (Amasino 2010). Interestingly, the genetic basis of vernalization requirement is not strictly conserved and utilizes distinct genetic components in different taxa (Alexandre and Hennig, 2008), indicating that this

environmental response may have evolved many times independently.

Moreover, *Arabidopsis thaliana* has a high frequency of self-pollination in the wild, hence individuals are homozygous at most loci. Such high rates of self-pollination may influence patterns of linkage disequilibrium, which helps to infer the evolutionary history of the species (Sharbel *et al.,* 2000; Beck *et al.,* 2007), but also provides the basis for association studies.

Chapter 1 / Disentangling the intertwined genetic basis of root and shoot growth in Arabidopsis

Marie Bouteillé[1], Gaëlle Rolland[1], Crispulo Balsera[1], Olivier Loudet[2] and Bertrand Muller[1]

[1] Institut de Biologie Intégrative des Plantes, Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, UMR759, INRA, Montpellier, France

[2] Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, F-78000 Versailles, France

Abstract

Root growth and architecture are major components of plant nutrient and water use efficiencies and these traits are the matter of extensive genetic analysis in several crop species. Because root growth relies on exported assimilate from the shoot, and changes in assimilate supply are known to alter root architecture, we hypothesized (i) that the genetic bases of these traits could be intertwined with the genetic bases of shoot growth and (ii) that the link could be either positive, with alleles favouring shoot growth also favouring root growth, or negative, because of competition for assimilates. We tested these hypotheses using a quantitative genetics approach in the model species Arabidopsis thaliana and the Bay-0 x Shahdara recombinant inbred lines population. In accordance with our hypothesis, root and shoot growth traits were strongly correlated and most root growth quantitative trait loci (QTLs) colocalized with shoot growth QTLs with positive alleles originating from either the same or the opposite parent. In order to identify regions that could be responsible for root growth independently of the shoot, we generated new variables either based on root to shoot ratios, residuals of root to shoot correlations or coordinates of principal component analysis. These variables showed high heritability allowing genetic analysis. They essentially all yielded similar results pointing towards two regions involved in the root – shoot balance. Using Heterogeneous Inbred Lines (a kind of near-isogenic lines) segregating in these two regions, we validated six main effect QTLs. Our study thus highlights the difficulty of disentangling intertwined genetic bases of root and shoot growth and show that this difficulty can be overcome by using simple mathematical tools.

Key words: Arabidopsis thaliana, root growth, shoot growth, QTL analysis, multivariate analysis, Heterogeneous Inbred Families

Introduction

Although roots are underground and therefore not easily visible, they are finally receiving an increasing attention, in particular in the context of a changing agriculture and climate. Their development, growth and architecture are thought to be major components of plant nutrient and water use efficiencies (Ochoa *et al.,* 2006; MacMillan *et al.,* 2006; Manschadi *et al.,* 2008). Thus, they are proposed to be one of the leverage for the next green revolution (Lynch, 2007). Moreover, roots can substantially contribute directly or indirectly to carbon sequestration (Deyn *et al.,* 2008) making them key actors in global earth carbon budget. Interspecific and intraspecific variation for root growth and architecture have been repeatedly reported in various species or genera opening the door to the design and breeding of crop or varieties carrying most useful root features adapted to various environmental conditions (de Dorlodot *et al.,* 2007). Reports are now convincingly accumulating showing that such strategy can bring substantial improvement of plant fitness and production (Sanguineti *et al.,* 2007; Hochholdinger and Tuberosa, 2007; Hammer *et al.,* 2009). Moreover, the partitioning of biomass between the root and the shoot is a key parameter strongly related to plant growth rate, life habitats and responses to environmental constraints such as nutrient deficiencies, drought or light (Poorter and Nagel, 2000; Poorter *et al.,* 2005).

Genetic analysis leading to the identification of regions (QTL) responsible for the variation of root variables have been conducted in a variety of species, the earliest reports being on rice (Price and Tomos, 1997) to more recently tree species (Kenis and Keulemans, 2007). QTL have thus been reported for total root biomass (Price and Tomos, 1997; Reiter *et al.,* 1991), root length (Zhang *et al.,* 2001), root branching (Robinson *et al.,* 1986; Chevalier *et al.,* 2003), proportion of shallow vs.deep roots (Liao *et al.,* 2004) or root angle (Giuliani *et al.,* 2005). QTL analysis in the model species Arabidopsis thaliana have also been engaged in a variety of mapping populations (Loudet *et al.,* 2002; El-Lithy *et al.,* 2004) as well as in other material such as advanced intercross RIL (Balasubramanian *et al.,* 2009; Kover *et al.,* 2009). Focused on root growth, such studies have pointed towards QTL involved in either constitutive (also called intrinsic) root variables (Loudet *et al.,* 2005) or QTL associated with root responses to the environment. The later concern a variety of root responses to low phosphate (Reymond *et al.,* 2006; Li *et al.,* 2009), low nitrogen (Rauh *et al.,* 2002), water deficit (Courtois *et al.,* 2000; Cui *et al.,* 2008), or osmotic stress (Xiong *et al.,* 2006; Gerald *et al.,* 2006). These distinct variables types (intrinsic and response) are thought to reflect the probable different nature of the molecular pathways involved (Malamy, 2005). Whether constitutive or environmentally determined, very few root QTLs have been conducted to cloning, all being in Arabidopsis (Mouchel *et al.,* 2004; Sergeeva *et al.,* 2006; Svistoonoff *et al.,* 2007).

Among genetic studies published so far on the mechanisms determining root system architecture or dimension, only a few have incorporated analysis of the aerial part as a possible co-variable of the root variables (Poorter *et al.,* 2005; Laperche *et al.,* 2006). However, roots, as sink organs, strongly rely on the continuous supply of assimilate from the shoot for both their growth and expansion, as well as for the establishment of their architecture. Indeed, changes in shoot biomass, shoot growth or intercepted irradiance can deeply modify root growth and architecture (Farrar and Jones, 1986; Aguirrezabal *et al.,* 1994; Freixes *et al.,* 2002). Intuitively, promoting root and shoot growth could be expected to be either favorable or not for plant growth. Favorable because a higher shoot growth is expected to increase carbon capture and energy production and defavorable because it may yield to competition for assimilates. Arguments for the occurrence of both situations have been reported. Root elongation rate is decreased by shoot pruning that reduces the source of carbon (Farrar and Jones, 1986). Similarly, root elongation rate is increased by increasing irradiance (Aguirrezabal *et al.,* 1996, Muller *et al.,* 1998) in association with higher sugar content in the root (Freixes *et al.,* 2002). By contrast, in some species, flushes of shoot growth strongly impair root growth when they occur (Thaler and Pagès, 1996) probably as a result of competition for assimilates (Thaler and Pagès, 1998). Both responses could contribute to the long depicted root and shoot growth co-ordination also called “functional equilibrium” describing how the growth of both organs rapidly respond to challenging external conditions (Brouwer 1962; Poorter and Nagel, 2000). At a much broader scale, root and shoot biomass partitioning is known to operate along a very narrow range when a large spectrum of species is considered (Enquist and Niklas 2002).

From these informations, we hypothesized that at least part of the basis for root growth variation could be related to shoot growth variation. To test this hypothesis, we conducted a series of experiments with Arabidopsis thaliana as a model species using a collection of recombinant inbred lines (RIL) derived from the Bay-0 x Shahdara cross (Loudet *et al.,* 2002) whose parents were expected to display contrasting root characteristics. The results of these experiments were also compared to what could be observed in a collection of accessions displaying widespread geographical origins. Then, a series of heterogeneous inbred families (HIF, equivalent to families of Near Isogenic Lines, Loudet *et al.,* 2005) were used to validate two of the QTL detected. In order to have easy access to the root system, all experiments were performed in hydroponics.

Materials and methods

Genetic material

For this study, we used three sets of genotypes. The first one is a sub-population of 165 RIL from the Bay-0 x Shahdara RIL population (Loudet *et al.,* 2002), chosen to capture maximum recombination. This population is genotyped with 69 microsatellites markers. Complete genetic and phenotypic information on this population is available at http://dbsgap.versailles.inra.fr/vnat/Documentation/33/DOC.html. A second set of genotypes was used for QTL validation. Heterogeneous Inbred Families (HIF) lines were derived from residual heterozygosity remaining in some of the F6 RIL at markers of interest (Loudet *et al.,* 2005). For each of these lines, 20 plants were individually genotyped at the segregating markers and two homozygous plants for each of the parental alleles (Bay or Sha) were selected and selfed to produce seeds for further phenotypic analysis. All this material was obtained from Versailles Biological Resource Centre (http://dbsgap.versailles.inra.fr/vnat/). Finally, a collection of 20 accessions that were the first one sequenced in a large SNP sequencing project (Clark *et al.,* 2007) was used. Seeds were obtained from the Max Planck Institute for Developmental Biology (Tübingen, Germany).

Plant growth conditions

Seeds were surface-sterilised for 15 minutes in a mixture of bleach in 50% (v/v) ethanol, rinsed once in ethanol and then 3 times in sterile water. Two seeds were laid down at the surface of small cones (bottom part of 0.5 ml Eppendorf cut at both ends) filled with nutritive media (agar 0.65% w/v + nutrient solution). Cones were stored in Petri plates at 4°C in darkness during 24 hours. Petri plates were installed in the growth chamber for 5 days to allow seed germination. Then, cones were transferred to the hydroponic system composed of 20 x 30 cm styrofoam plates (thickness 1.0 cm) pierced by 96 holes and adjusted to float on nutrient solution in 5L containers. The solution (one-tenth-strength modified Hoagland solution) was renewed every 3 days. All experiments were performed in a set of identical, 1m² growth cabinets, under the following climate: temperature was kept constant at 21°C days and night, relative air humidity was set at 80% in order to reach an air vapor pressure deficit of 0.6 kPa, light was 180 µmol.m-2.s-1 provided by a mixture of sodium and HQI lamps, during a 12h photoperiod. To avoid any unconsidered bias due to location within the growth cabinet, containers were randomly moved from one location to another every day.

Experiments

During experiment 1, only the parental lines Bay-0 and Shahdara were grown. Sixty cones per accession were used, from which 30 homogeneous plants were selected 12 days after sowing. Then, 6 plants of each accession were randomly chosen for harvest at 13, 17, 20, 24, 27 days after sowing. During experiment 2, the 165 RIL of the BayxSha population and the two parental lines were grown. 15 cones were used per RIL from which 8 homogeneous plants were selected 12 days after sowing. Four plants randomly selected among the 8 were then harvested at 20 and 24 days after sowing. The 15 cones of every RIL were shared out in three different containers to avoid possible block effect. To lighten the daily work load, experiment 2 was performed as 3 waves of sowing spaced by 3 days with 55-56 RIL at each date. Experiment 3 was devoted to phenotyping a collection of 20 accessions (Bay-0, Bor-4, Br-0, Bur-0, C24, Col-0, Cvi-0, Est-1, Fei-0, Got-7, Ler, Lov-5, Nfa-8, RRS-10, RRS-7, Shahdara, Tamm-2, Ts-1, Tsu-1, Van-0). The same experimental design was used than for experiment 2. Finally, experiments 4, 5, and 6 were dedicated to the culture of HIF. For each QTL to be validated, 2 independent HIF were available and used for phenotyping. On average, 80 plants of each HIF were cultivated, and at least 12 homogeneous plants per line were selected 16 days after sowing for the two harvests (at 20 and day 24 after sowing).

Variables measurements and data acquisition

At each date of harvest, all the replicate plants of each genotype were gently removed from the hydroponic system, and their shoot and root parts were separated. Each leave (blades) of the rosette was detached, spread out and stuck with double-sided adhesive on a sheet of paper. Total leaf area was determined as the sum of the areas of each leaf blade. Blades were then gathered for estimation of dry weight following 2 days at 80°C. In order to capture root architecture, root systems were gently spread at the surface of large (20 x 20 cm) Petri plates filled with water and a numerical image was taken at 800 dpi using a scanner in transmission mode. Images were later analysed using Image-J software and customized macros. After image capture, root systems were individually stored into 96 well plates each containing pre-weighed aluminium cell-cup to facilitate weighing. The plates were then oven dried for 2 days at 80°C and cups were weighed using a 5 digits balance to measure root dry weight.

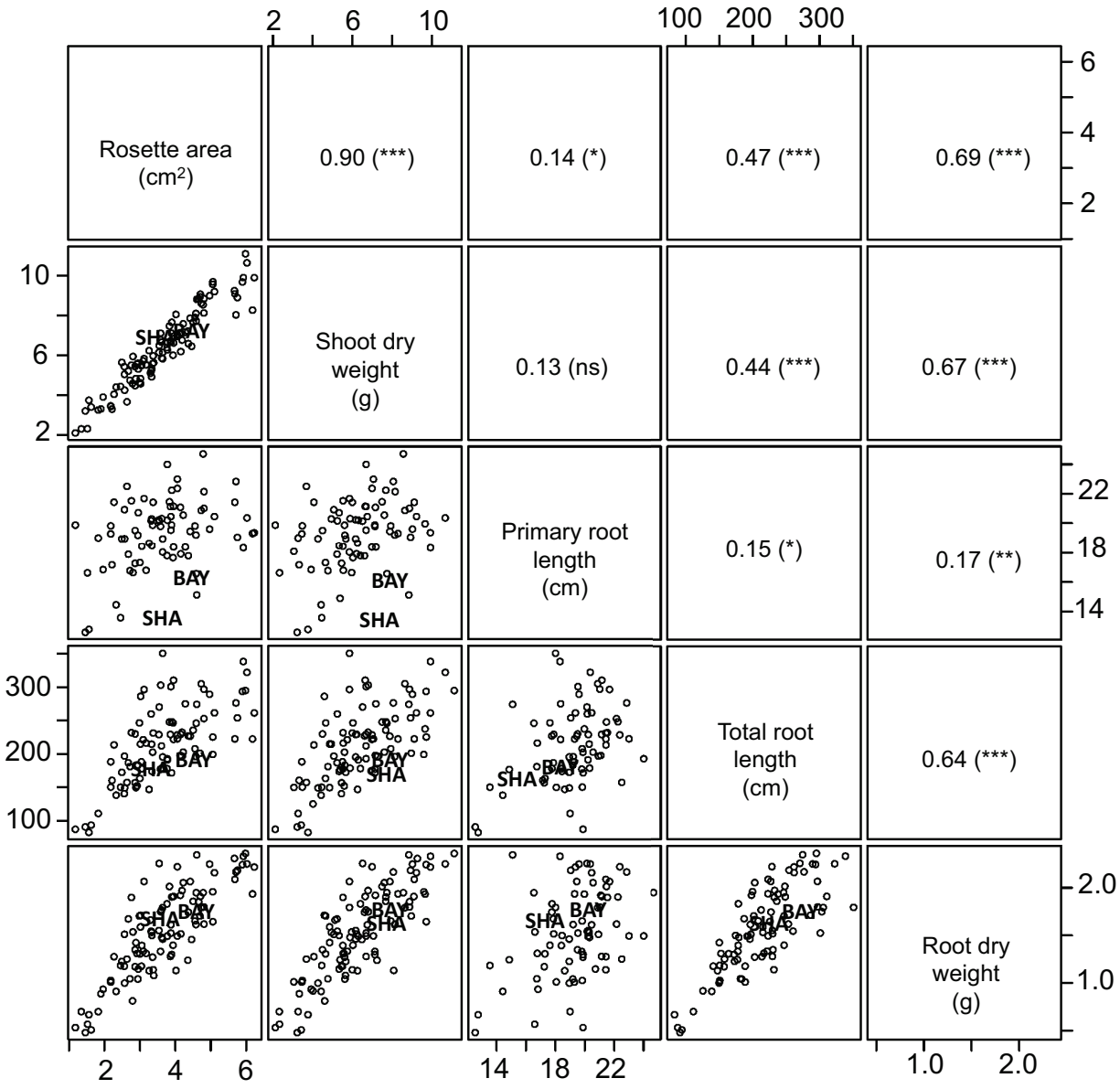


Figure 1. Correlation matrix between the different root and shoot growth variables at 24 days after sowing within the 165 individuals of the Bay-0 x Shahdara RIL population. Dots represent the mean values of each RIL (4 individuals), and Bay-0 and Shahdara parental lines are indicated. Pearson's coefficients (r) associated to correlations are shown with their p-value (***, p-value < 0.001, **, p-value<0.01, *, p-value<0.05, ns, p-value>0.05). Shoot and root dry weight are expressed in mg, rosette area in cm², total and primary root length in cm.

QTL detection and statistical analysis

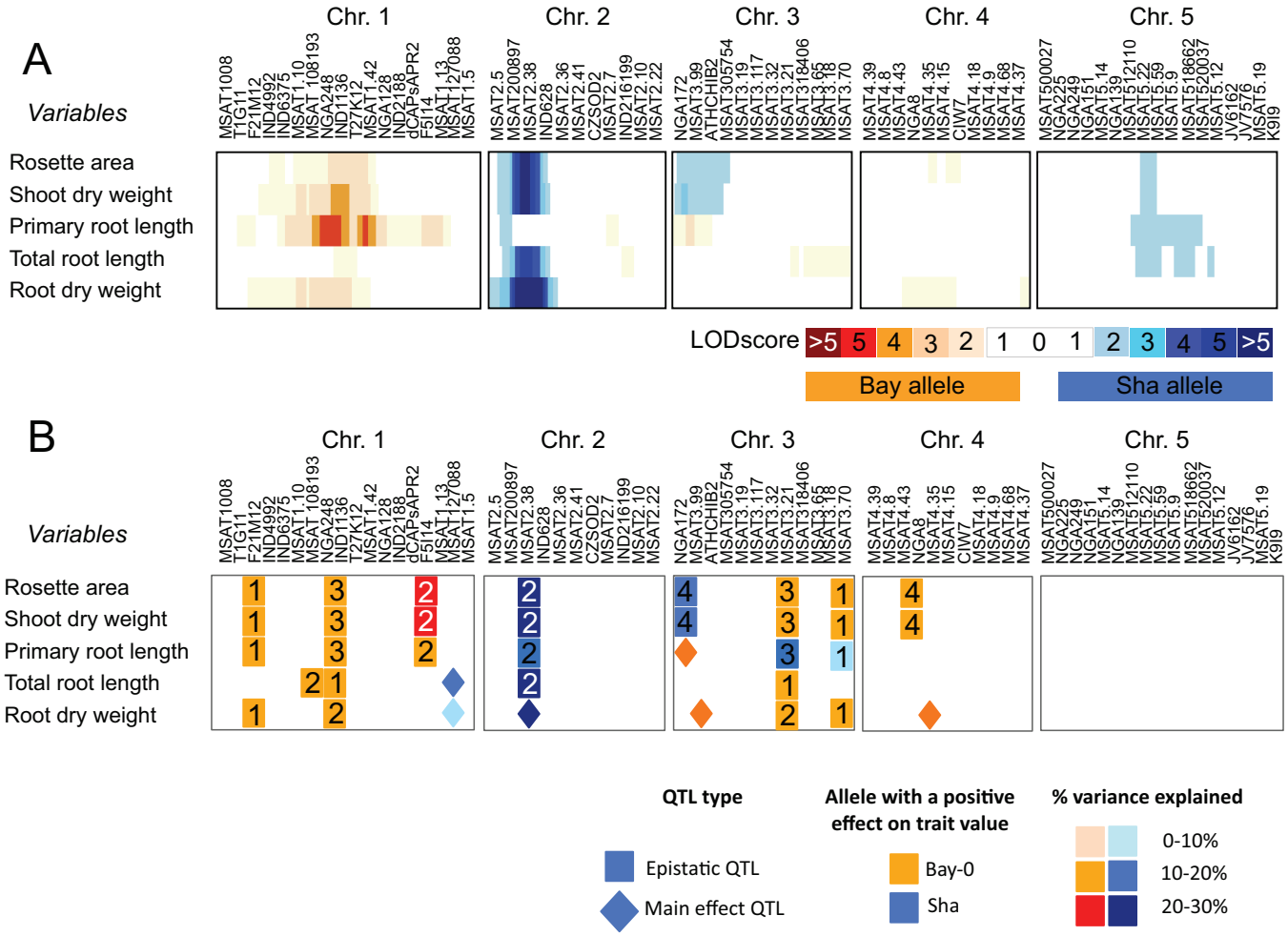
All statistical analyses were performed using the computer package SPSS 11.0.1 for Windows (SPSS) and the R software (R Development Core Team, 2007). Statistical differences between HIF lines were tested by t-test. Correlation were analysed using Pearson statistics. Normality of the distributions of each variable among the lines was verified by evaluating skewness. Heritability (broad sense) was estimated as the proportion of variance explained by between-line differences based on measurements of four plants per line, at each date of harvest. A first QTL detection using simple interval mapping (IM) was performed with the MapQTL5 software (MAPQTL®5, Kyazma BV, Wageningen, the Netherlands). Cofactors were then selected using the 'automatic cofactor selection' (ACS) chromosome per chromosome, and were used for Multiple QTL Mapping (MQM). The cofactors for which no QTL were detected (LOD score under a 95% LOD threshold (LOD < 2.4) estimated by permutation tests implemented in MapQTL5 using at least 1,000 permutations of the original dataset) were removed. The Epistat software was used to detect epistatic interactions between QTL (Chase *et al.,* 1997). Then, global QTL models combining main effects QTL and epistatic QTL were statistically tested using the GLM of the statistical package of SPSS 11.0.1 for Windows. The estimated additive effect, the percentage of variance explained by each individual QTL, and the total variance explained by the QTL model were obtained using the same package.

Results

Tight correlations between root and shoot growth variables in RILs

A large variability among the RILs was observed for each of the 5 variables with ample transgression from the parents (Figure 1 and Figure S2A). At 24 days after sowing (Figure 1), shoot dry weight varied from 2 to 10 mg, root dry weight varied from 0.5 to 2 mg while primary root length varied between 12 and 24 cm. A similar range of variation was observed at 20 days after sowing (Figure S2A).

Except the correlation between shoot dry weight and primary root length, which was not significant, all shoot and root variables were significantly and positively correlated one to another, with Pearson's coefficients ranging from 0.14 to 0.90, 24 days after sowing (Figure 1) (0.29 to 0.89 at 20 days after sowing, Figure S2A). Strong



C

	Heritabilities	R^2 QTL model
Rosette area	0.71	0.46
Shoot dry weight	0.66	0.50
Primary root length	0.59	0.47
Total root length	0.54	0.51
Root dry weight	0.60	0.50

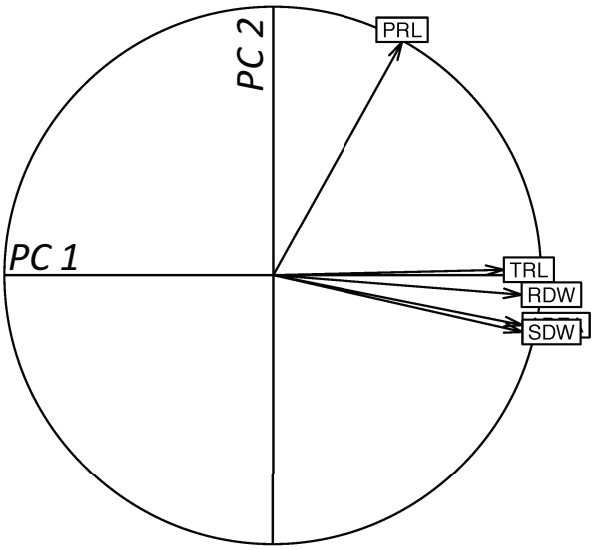
Figure 2. Genetic map of the QTL detected in the Bay-0 x Shahdara for shoot and root growth variables. A. Map of the LOD score values all along the genome using Interval Mapping analysis. A color code indicates the parental allele which increases the value of the variables at the marker (blue for Sha alleles, and red for Bay alleles). The LOD score value is shown as different color intensities. **B**. Map of the regions involved in models combining main effects and epistatic QTLs. A color code indicates both the allele which increases the value of the variable at one specific region and the percentage of variance explained by the QTL. Identical numbers are indicated in the two partners of the epistatic interaction. **C**. Broad-sense heritability and r^2 of the QTL models shown in B. Data are those obtained 24 days after sowing (the map at 20 days after sowing is shown as supplementary material).

correlations were observed in all cases between shoot dry weight and rosette area, indicative of a limited variation of the specific leaf area. Correlations between shoot and root variables were the tightest with root dry weight, slightly less tight with total root length and much weaker with primary root length. Correlations between shoot variables and both root dry weight and total root length were of the same strength at both dates whereas the strength of the correlations between shoot variables and primary root length strongly decreased between 20 and 24 days after sowing (Figure S2B). Correlations were strong between root dry weight and total root length and much weaker between these variables and primary root length. The ranking of the correlations based on their strength was essentially maintained at both dates (Figure S2B).

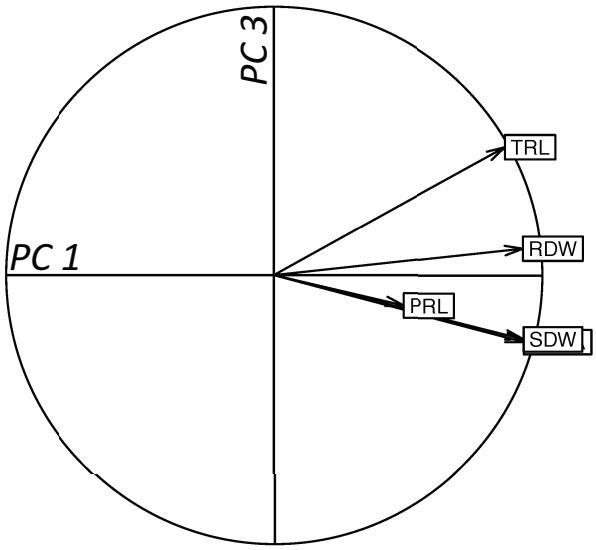
Correlations between shoot and root growth translated at the genetic level with common QTLs

Broad-sense heritability of root and shoot growth variables was high, ranging from 0.54 to 0.77, slightly higher for shoot than for root variables, and for the first date of harvest as compared to the second (Figure 2C at 24 days after sowing and Figure S3C at 20 days after sowing). A first detection of genomic regions involved in the control of these variables was performed using Interval Mapping (Figure 2A). However, despite the high heritability recorded for these variables, only few regions showing significant QTL (i.e. with LOD > 2.4) were detected. Two regions showed a significant effect on almost all variables. The most important region was located at the top of chromosome 2, with Sha alleles contributing positively to the variables with LOD score ranging from 3 to 5 for all variables except for primary root length. The middle of chromosome 1 was also important, with Bay alleles contributing positively to all variables but total root length with the strongest effect on primary root length. The middle of chromosome 5 was also involved in shoot and root variables with positive effects of Sha alleles. The top of chromosome 3 was involved for shoot variables only. Both the top of chromosome 2 and the middle of chromosome 5 were strongly involved in all roots and shoot variables at 20 days after sowing (Figure S3A). This first analysis thus revealed some similarities of LOD profiles for shoot variables, root variables, but also between shoot and root variables.

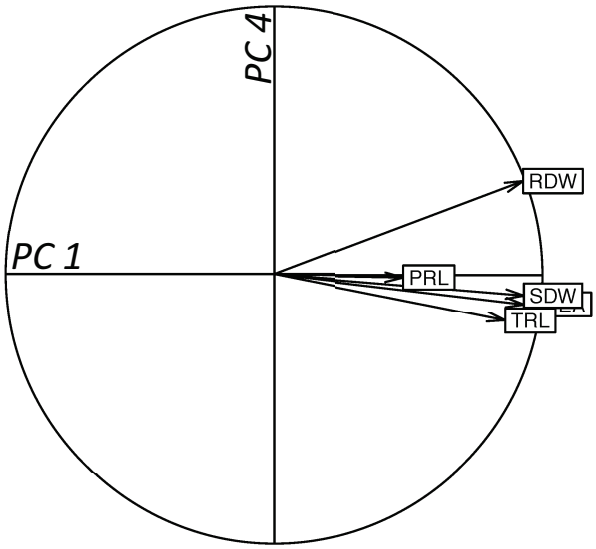
An analysis was performed to identify possible epistatic interactions between markers. These epistatic interactions were individually tested before they were included in a global model gathering epistatic and main effect QTLs (Figure 2B). The percentage of variance explained by the QTL models accounted for 46 to 51% of the phenotypic variance (Figure 2C), that corresponded to 60 to 90% of the genetic variance. This percentage was slightly higher at 24 days than at 20 days after sowing (Figure 2C, and Figure S3C), but similar markers were involved at both dates (Figure 2B and S3B). For all variables, genetic models were supported by both main



PC1: 71.4 PC2:16.8



PC1: 71.4 PC3: 7.4



PC1: 71.4 PC4: 3.4

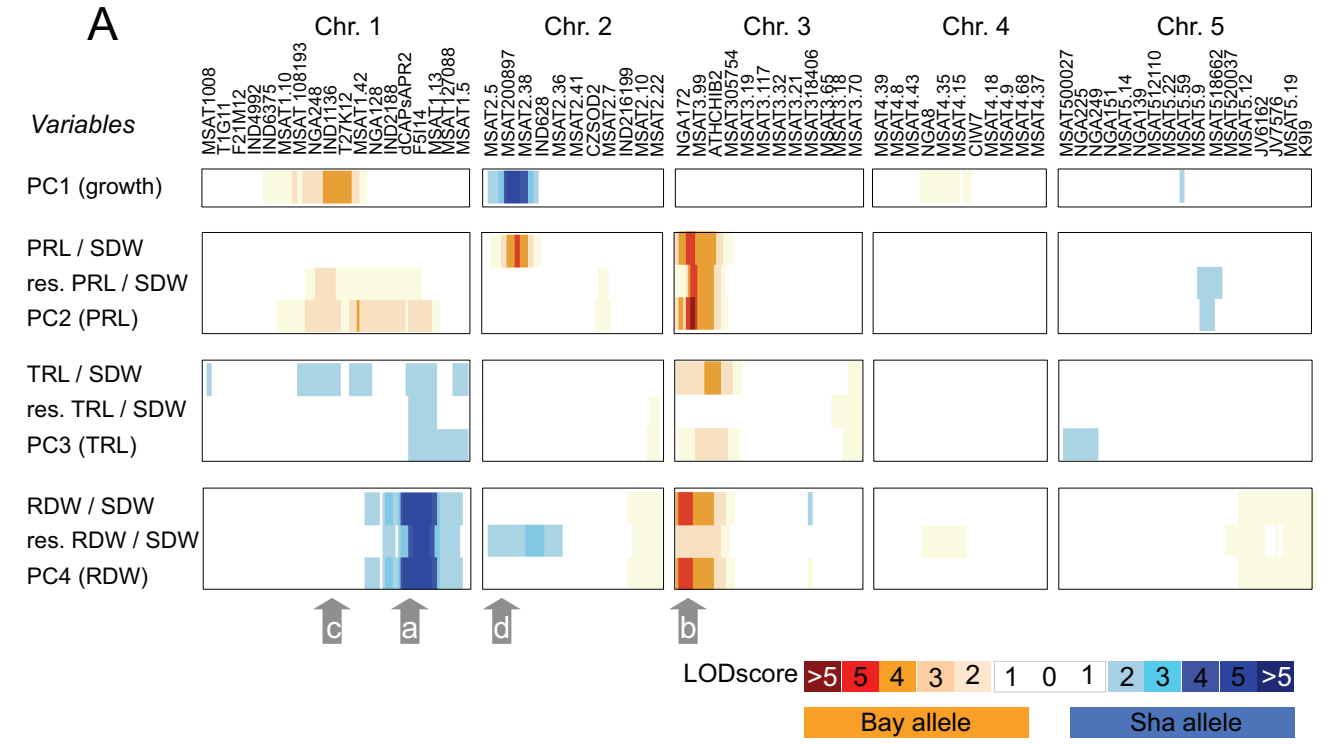
Figure 3. Principal component analysis based on root and shoot growth variables. As indicated at the bottom left of each circle, the first component (PC1) gathers 71.4% of the total variance whereas PC2, PC3 and PC4 gather 16.8, 7.4 and 3.4% of the total variance, respectively. The positions of the different variables, Rosette area (AREA), Shoot dry weight (SDW), total root length (TRL), primary root length (PRL), root dry weight (RDW) are represented. Data are those obtained 24 days after sowing.

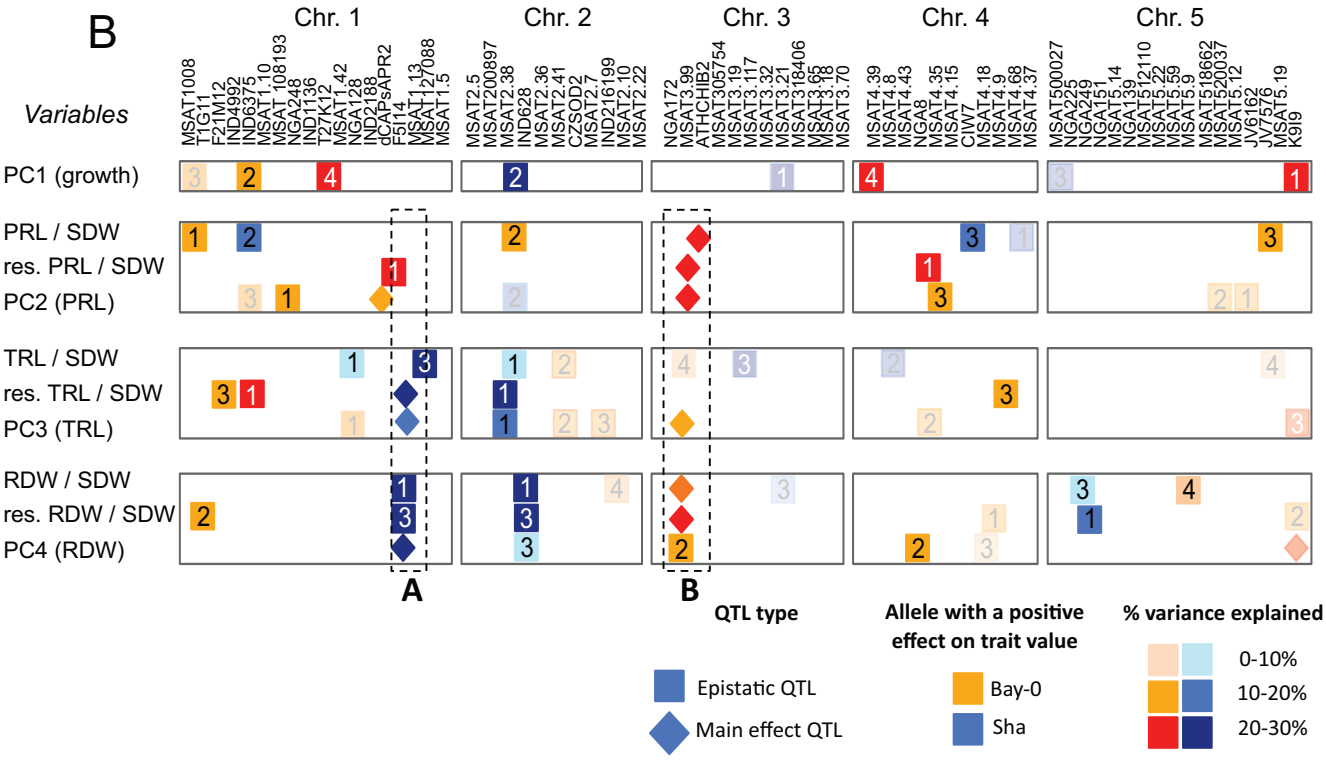
effect QTLs and epistatic interactions involving interactors in the five chromosomes. As for the interval mapping analysis, shoot and root growth variables were determined by similar genomic regions (Figure 2B and S3B). The first epistasis (squares numbered 1 on Figure 2B) between the top of chromosome 1 (F21M12 marker) and the bottom of chromosome 3 (MSAT3.70) explained between 10 and 20% of total variance of all root and shoot variables, with positive effect of Bay allele at both loci except for primary root length at MSAT3.70. The second epistasis (squares #2) involved the bottom of chromosome 1 (F5I14, positive effect of Bay allele) and the top of chromosome 2 (MSAT 2.38, positive effect of Sha allele), and explained 15 to 21% of the variance of all shoot variables as well as primary root length. A third epistasis was essentially associated with the middle of chromosome 1 (IND1136, positive effect of Bay allele) and the middle of chromosome 3 (MSAT3.21, positive effect of Bay alleles except for primary root length). Finally, an interaction between the top of chromosome 3 (squares #4, NGA172, positive effect of Sha allele) and the top of chromosome 4 (NGA8, positive effect of Bay allele), explained 10-15% of each variable, with an effect on shoot variables only. Interestingly, the region at the top of chromosome 3 also contained main effect QTLs controlling root variables with a positive effect of Bay allele. Analysis at 20 days after sowing (Figure S3B) pointed to essentially the same regions except that the third epistasis was not present. Even considering epistatic QTL models, very few QTLs specific for root growth variables were detected either at 24 days after sowing, or at 20 days after sowing (Figure S3B). Noteworthy, in many of the regions harbouring common QTLs for root and shoot variables, the same parental allele affected positively root and shoot variables except in the case of the bottom of chromosome 1 (although root and shoot QTL peaks were separated by 2 markers) and the top of chromosome 3, parental alleles had opposite effects on shoot and root variables. This feature was visible at both dates of harvest (Figure S3B).

Uncoupling root and shoot variables

In order to disentangle the intertwined genetic bases of root and shoot growth, three sets of variables were calculated. First, a principal component analysis (PCA) was performed using all five shoot and root variables on the whole RIL dataset at 24 days after sowing (Figure 3). The first principal component (PC1) captured most of the inertia of the data (71% of total variance) and was strongly related to all variables but primary root length. This component was thus considered as accounting for whole plant growth. The second one (PC2) explained 16.8% of the variance of the population, and was mainly driven by the primary root length that accounted for 66% of the variation of this PC. The third principal component (PC3) accounted for 7.4% of the total variance and was mainly driven by total root length (that accounted for 44% of the variation along this component). Finally, principal component 4 (PC4) accounted for only 3.4% of the total variance and was mainly accounted for by root dry weight (accounting for 37% of the variation). Four additional variables were thus calculated as the coordinates of each RIL on these 4 PC. The same analysis was performed with data at 20 days after sowing (not

Figure 4. Genetic map of the QTLs detected for root to shoot ratio, residuals of correlations between root variables and shoot dry weight and coordinates in the principal component analysis. A. Map of the LOD score values all along the genome using Interval Mapping analysis. A color code indicates the parental allele which increases the value of the variables at the marker (blue for Sha alleles, and red for Bay alleles). The LOD score value is shown as different color intensities. Arrows *a* to *d* refer to regions described in the text. **B**. Map of the regions involved in models combining main effects and epistatic QTLs. A color code indicates both the allele which increases the value of the variable at one specific region and the percentage of variance explained by the QTL. Identical numbers are indicated in the two partners of the epistatic interaction. A and B rectangles refer to regions controlling root related variable but not involved in global plant growth. Data are those obtained 24 days after sowing (the map at 20 days after sowing is shown as supplementary material). QTLs not retrieved in the map from 20 days after sowing plants are shown with a translucent color. **C**. Broad-sense heritability and r^2 of the QTL models shown in B.





C

	Heritabilities	R^2 QTL model
PC1 (growth)	0.68	0.67
PRL/SDW	0.51	0.46
residual (PRL/SDW)	0.46	0.43
PC2 (PRL)	0.57	0.55
TRL/SDW	0.42	0.36
residual (TRL/SDW)	0.46	0.44
PC3(TRL)	0.55	0.53
RDW/SDW	0.51	0.48
residual (RDW/SDW)	0.64	0.61
PC4 (RDW)	0.45	0.41

shown). Second, for each RIL, the orthogonal residuals of the correlations (Figure 1) between root variables (root dry weight, total root length, and primary root length) and shoot dry weight were calculated. These residuals are indicative of the deviation of root (or shoot) growth from the main trend linking both variables. RILs located above the main trend were thus having relatively higher root growth and lower shoot growth than the average trend. Finally, ratios of root variables (root dry weight, total root length or primary root length) to shoot dry weight were calculated. These calculated variables all displayed medium to high heritability ranging from 0.42 to 0.68 (Figure 4C and Figure S4C).

Identification of QTLs involved in the root-shoot balance

QTL detection was performed on residuals of root to shoot correlations, PC coordinates and root to shoot ratios. A first detection was performed using Interval Mapping (24 days after sowing, Figure 4A, and 20 days after sowing, Figure S4A). Four main regions were identified in this analysis (arrows a to d). The first principal component, corresponding to whole plant growth was mainly controlled by the middle of the first chromosome (c arrow, MSAT1.42) and by the top of chromosome 2 (arrow d, MSAT2.38). Among the root variables, only those related to primary root length showed association with these two QTLs controlling plant growth, with the same positive effect of Bay allele in c region, and opposite allelic effects in d region. Variables related to primary root length (PC2, residual of the correlation between primary root length and shoot dry weight, and the ratio between primary root length and shoot dry weight) were mainly controlled by the top of chromosome 3 (b arrow, AthCHIB2). This region was also associated with variables related to total root length and to root dry weight. For those variables related to total root length and root dry weight, another region at the bottom of chromosome 1 (a) was consistently involved. For the b region, a positive effect of the Bay allele was identified for all the root variables (primary root length, total root length, and root dry weight related variables). By contrast, the a region showed opposite allelic effect on either primary root length (positive effect of the Bay allele) or total root length and root dry weight (positive effect of Sha allele). Interestingly, among these four regions, the a and b regions were clearly detected at both 20 and 24 days while c and d regions were less clearly visible at 20 days after sowing (Figure S4A).

As with raw variables, several significant epistatic interactions ($P<0.05$; Table S3) were detected for each of these variables. QTLs models gathering main effect and epistatic QTLs individually explained 36 to 67% of total variance (Figure 4C and S4C). A major difference with the analysis from raw variables (Figure 2B) was that more regions, spread along the genome were involved, with some being involved in one specific variable, or at one date. Interestingly, we were able to detect some QTLs specific for plant growth (squares #4 in Figure 4B). Very few QTLs were associated with both whole plant growth and root related variables (eg MSAT2.38 and IND628 in

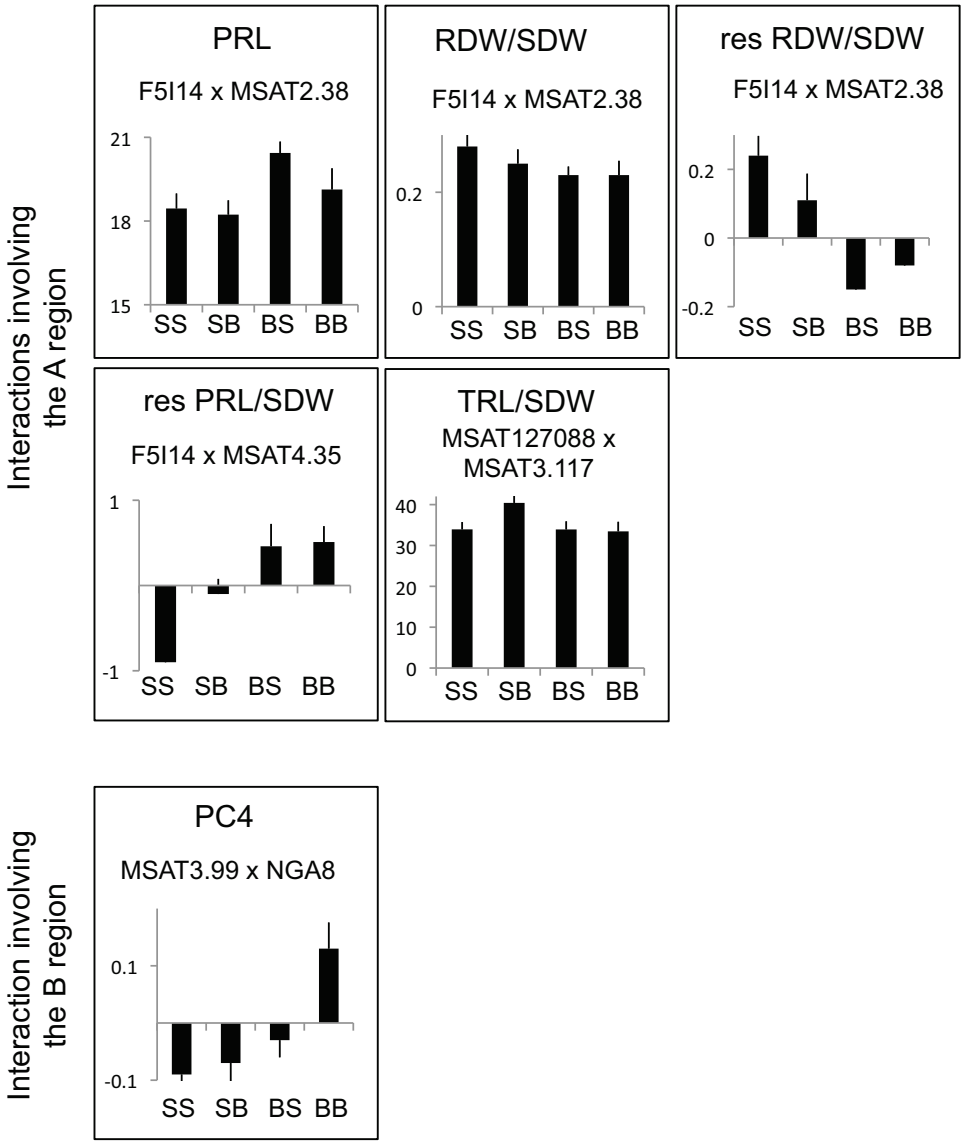


Figure 5. Mean values of root-related variables for the RILs in each of the four allelic classes for the 6 epistatic interactions involving the A and B regions : SS, SB, BS and BB refers to the RILs with the Sha allele at both markers indicated, the Sha allele at the first marker, and the Bay allele at the second, the Bay allele at the first marker, and the Sha allele at the second, and the Bay allele at both markers. Bars correspond to standard deviation.

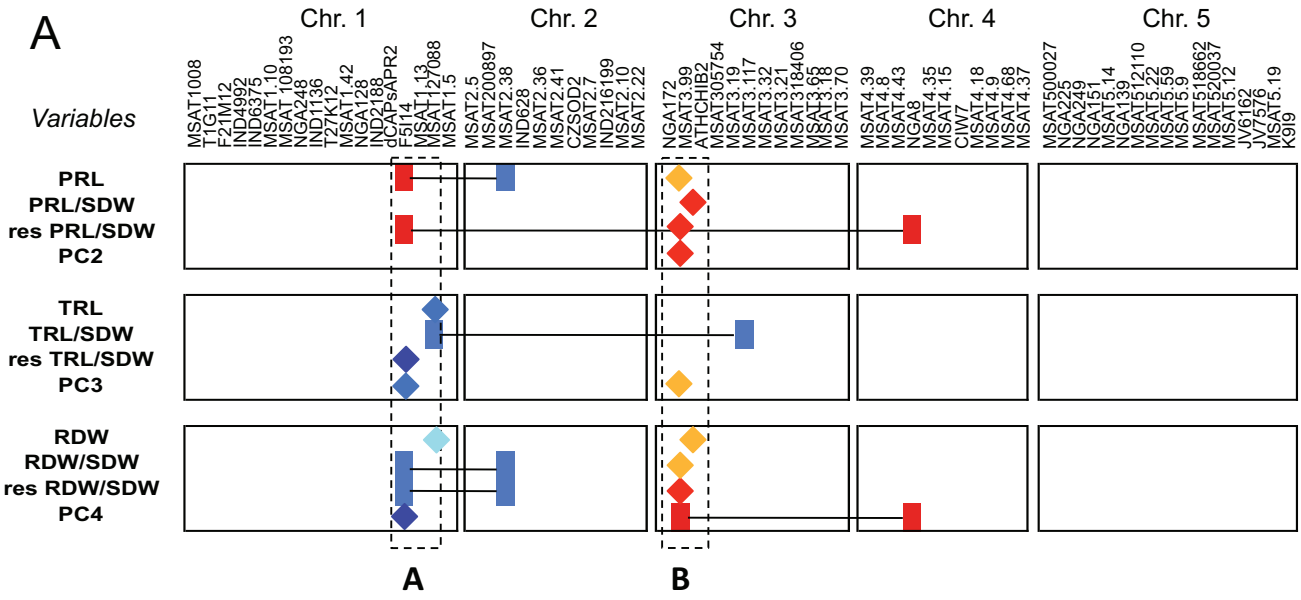
Figure 4B and S4B) suggesting that our analysis was successful to separate these components from whole plant growth. Indeed, we detected several QTLs associated with root-shoot balance and/or root specific variables only with no overlap with PC1 associated regions either at 24 days after sowing, or at 20 days after sowing. This was particularly the case for two regions labelled A and B on Figure 4B. These regions were already detected on the QTL analysis using raw data but they were then associated with both root and shoot QTLs. Moreover, root QTLs in these regions were clearly reinforced with a higher density of main-effect QTLs accounting for a higher proportion of the variance. We therefore focused our attention on these two regions.

Using HIF to validate the role of A and B QTLs, specifically controlling root-shoot balance and/or root specific variables

According to our analysis, A and B regions were involved in a total of 4 epistasis with other regions of the genome, among which one (F5I14 x MSAT2.38) affected 3 different variables. The barplots representing the mean value of variables for each allelic class is shown in Figure 5. The mean value of raw variables (primary root length, total root length, root dry weight and shoot dry weight) are indicated in Table S4. Region A (F5I14-MSAT127088) was involved in an interaction with the top of chromosome 2 (MSAT2.38) to control 3 variables (primary root length, see Figure 2B, the root dry weight to shoot dry weight ratio and the residual of the correlation between root dry weight and shoot dry weight correlation, see Figure 4B). Region A was also associated with MSAT4.35 to control the residual of the correlation between primary root length and shoot dry weight, and with MSAT3.117 to control the ratio between total root length and shoot dry weight. Region B (MSAT3.99) was associated with NGA8 to control principal component 4. The main effect QTLs and the epistatic interactions involving the A and B regions are shown on the genetic map on Figure 6A.

In order to validate A and B main effect QTLs, we used a set of Heterogeneous Inbred Families (HIF) generated from residual heterozygosity detected at the F6 generation of the RIL. We therefore compared "sisters" HIFs generated from the same RIL, but carrying either the Sha or Bay allele at the fixed region. Because the A and B QTLs were partly epistatic, we needed to consider the allele at the interactor (Figure 6B). We used two HIF segregating at the A region (HIF083 segregating at both F5I14 and MSAT1.13 and HIF107 segregating at MSAT1.13 only, Figure 6B) and two other HIF segregating in the B region (HIF004 segregating from ATCHIB2 to MSAT3.117 and HIF338 segregating from NGA172 to MSAT305754. Shoot dry weight, root dry weight, primary root length, total root length were measured in the different HIFs and ratios of root variables to shoot dry weight were computed (Figure 6C).

For both HIF083 and HIF107, a highly significant ($p<0.001$) positive effect of Bay allele on primary root length



C

		HIF 083	HIF 107	HIF 004	HIF 338
% changes in variables by Bay allele	SDW	ns	ns	ns	ns
	PRL	11 ***	7.5 ***	12 *	7.6 **
	PRL/SDW	ns	ns	16 *	28 **
	TRL	ns	ns	ns	ns
	TRL/SDW	ns	ns	13 **	12 **
	RDW	ns	ns	18 *	ns
	RDW/SDW	ns	ns	19 **	20 *
	Individuals	45	51	75	72
	Experiments	2	2	3	3

Figure 6. Validation of the role of A and B regions using heterogeneous Inbred Families (HIF). **A.** Synthesis of the epistatic interactions involving either the bottom of chromosome 1 (A region) and/or the top of the chromosome 3 (B region) for root related variables. The markers involved in the interactions are colored in blue or red depending on the allele that increases the value of the trait, Sha or bay respectively. **B.** Genetic map of the RILs that were selfed to produce HIFs from residuals of heterozygosity in F6 generation of the RILs. Blue and red is for Sha and Bay alleles respectively. Yellow show heterozygous regions in RILs in F6. These regions are then fixed in the HIF progeny. **C.** Validation of the presence of two root QTLs on chromosome 1 and 3 using the four HIFs generated. The percentage of change of the variable induced by Bay allele compared to the Sha allele at the segregating region is indicated with the *t-test* p-value associated. Data shown gather harvests performed from 20 to 24 days after sowing. The number of individuals and experiments analyzed is indicated.

was found, that increased the variable by 7,5 - 11%. It confirmed the effect of the F5I14 marker (A region) on this variable (Figure 2). This F5I14 marker was often detected in interaction with MSAT2.38. Indeed, in the RIL population (Figure 5), the effect of the interaction was maximal with Bay allele at F5I14 and Sha allele at MSAT 2.38, and lower but still visible with Bay alleles at both F5I14 and MSAT2.38. In that case, both HIF083 and HIF107 had a Bay allele at the interactor (MSAT2.38), so only the effect of the A region alone can be confirmed (Figure 6B).. No other QTL was validated using HIF083 and HIF107 although QTLs had been detected at this region with raw variables (as main effect QTL) and with composite variables both as main effect and epistatic QTLs (Figures 2B and 4B). In particular the epistatic interaction controlling the root dry weight to shoot dry weight ratio was not confirmed maybe because of the absence of the favourable allele (Sha) at the interactor MSAT2.38 in both HIF083 and HIF107 (Figure 6A and 6B).

The two HIF used to validate the QTLs at the top of chromosome 3 originated from RIL displaying partly overlapping heterozygous regions with the HIF004 and HIF338 lines representing the most distal and proximal portion of the NGA172 – MSAT3.117 region respectively. Both lines validated the presence of QTLs in this region. Four main effect QTLs were confirmed for primary root length, the primary root length to shoot dry weight ratio, the root dry weight and the root dry weight to shoot dry weight ratio with a positive effect of Bay ranging from 7.6 % (primary root length) to 28% (ratio between primary root length and shoot dry weight). No effect on total root length was confirmed in line with the lack of QTL for that variable in that region (Figure 2). The QTL responsible for shoot dry weight and rosette area variation at NGA172 was not confirmed using those lines. This marker interacts with NGA8 on chromosome 4, and the two HIF004 and HIF338 did not have the favourable allele (Bay) at this marker. The QTLs effects were almost similar for HIF004 and HIF338. A major difference between the two HIFs was the lack of confirmation of the QTL for root dry weight using the HIF338 which could indicate that the causal locus for this variable is located between MSAT305754 and MSTA3.19, a region that segregates only in HIF004. Finally, there was a difference in the response of the two HIF for the primary root length to shoot dry weight ratio, with a stronger effect of the QTL on HIF338.

Discussion

Coupling of root and shoot growth is translated at the genetic level

At the interspecific level, a strong coupling between root and shoot dimensions has been reported and conceptualised (Enquist and Niklas, 2002) suggesting that the diversification of biomass allocation strategies in plants has occurred within a narrow developmental window. Our results suggest that similar constraints also limit allocation patterns at the intraspecific level. Indeed, root to shoot biomass ratio only varied in a very narrow range among the set of 20 Arabidopsis accessions when grown under the same environmental conditions, despite their wide geographical and climatic origins. This is in line with recent findings showing no correlation between root-to-shoot ratio and climatic feature of the habitats of a range of Arabidopsis populations (Montesinos-Navarro *et al.,* 2010). Interestingly, the strong shoot - root linkage was partly loosen using a population of RIL that displayed root-to-shoot biomass ratio varying by a much larger factor associated with a large transgression of parental values. These results suggest that rules determining allometry in a plant are strong but can be partly dissected using adequate genetic material. Biomass allocation in a plant strongly depends on environmental conditions (Poorter and Nagel, 2000) with classically reported root-to-shoot increases under low nutrient (McConnaughay and Coleman, 1998) or low water (Hummel *et al.,* 2010). Moreover, determinism of intrinsic and environmentally-related variation of root growth and architecture are likely to differ (Malamy *et al.,* 2005). In the present study, plants were grown in the absence of stress and results are thus likely to highlight intrinsic rules of root system development and biomass allocation. It will however be interesting to evaluate how the main results found here withstand environmental variation.

Correlation between root and shoot variables were clearly translated at the genetic level with an essentially common set of main effect or epistatic QTL. Similar findings have been reported in previous reports in which roots and shoots parts were both considered. Some common QTL for shoot weight and root length, number and weight have been reported in maize (Hund *et al.,* 2004) as well as in rice (Kamoshita *et al.,* 2002; Cui *et al.,* 2008). In Arabidopsis grown under a range of N sources, some QTL overlapped between root and shoot dimensions or biomass (Rauh *et al.,* 2002). In winter wheat, shoot and root biomass QTL were partly overlapping under the strong influence of the dwarfing gene *Rht*-B1 (Laperche *et al.,* 2006). By contrast, some studies report little or no correlation and no QTL overlap between shoot and root variables but these correspond most often to nutrient limiting situations. For instance, under low phosphate conditions, correlation between shoot dry weight and seminal root length in maize was moderate (Zhu *et al.,* 2006). In Arabidopsis, low nitrate conditions led to a lack of common QTL between root and shoot variables (Rauh *et al.,* 2002). In our work, the strength of the correlation and the degree of overlap between genetic models was higher than ever reported before. A possibility is that the hydroponic culture systems favoured a strong coupling between root and shoot, because the liquid medium did not mechanically impede root growth as would a solid substrate. Favouring this

hypothesis, our own results show that root - shoot coupling is looser with plants grown on solid substrate (see Chapter 4 of this document).

A large proportion of epistatic QTL

Very few of the regions identified pointed to significant, main effect QTL and QTL models considering epistasis were much more successful in accounting for the genetic variance of all variables measured. Epistasis is clearly acknowledged as being the rule when complex variables are considered (Carlborg and Haley, 2004; Cooper *et al.,* 2009). For instance, recent studies suggest that epistatic effects are more important than additive effects for fitness traits, (Malmberg, 2005; Mei *et al.,* 2005), flowering time (Juenger *et al.,* 2005) and metabolism (Rowe *et al.,* 2008). In several instances, epistasis has been resolved at the gene level, including for growth-related traits (Kroymann and Mitchell-Olds, 2005; Bikard *et al.,* 2009; Vlad *et al.,* 2010). Our QTL analysis on raw variables highlighted several epistatic interactions in which the same interactors were essentially involved in the control of different variables. These epistatic interactions controlling whole plant growth were not in the scope of the present study and clearly need further attention. Recently, a two-way epistasis was shown to be responsible for leaf growth maintenance under water deficit in Arabidopsis (Tisné *et al.,* 2010).

Different relationships between shoot and root variables are consistent with carbon partitioning rules governing root - shoot balance

A consistent outcome of our study is that the strength of correlations between root and shoot variables and the degree of overlap between shoot and root QTL model depend on the root variables considered. The loosest correlations and overlaps were found between shoot variables and primary root length. If we follow the rationale that root growth relies on exported assimilates from the shoot (*i.e.* the source in a source – sink terminology), our results suggest that primary root length is under loose source limitation. This view fits well with recurrent findings that primary root elongation rate (by contrast with that of lateral roots) is unaltered when assimilate supply is modified (Farrar and Jones, 1986; Muller *et al.,* 1998; Freixes *et al.,* 2002). In other words, primary root is a first-priority sink in the root system that generally experience much looser response to environmental constraints such as carbon (Farrar and Jones, 1986; Bingham and Stevenson, 1993) or minerals (Lopez-Bucio *et al.,* 2002).

Another noticeable feature of both the correlation analysis and QTL results is that shoot-root correlations were stronger with root dry weight than with total root length. Because total root length is mainly determined by lateral root, this is indicative that some genetic variation exists for Specific Root Length (*i.e.* the root length to dry weight ratio) that could buffer variation in assimilate supply (Robinson, 1988; Ryser and Eek, 2000; Aguirrezabal *et al.,* 1994; Ostonen *et al.,* 2007).

This interpretation of the results leads to the idea that genetic correlations found between root and shoot variables are linked to genetic determinism of assimilate partitioning. Such determinism could be associated with functional and/or structural variations in the sink itself (*e.g.* metabolic rates in root meristems or root meristem sizes, Muller *et al.,* 1998), in the source part of plant (*e.g.* phloem loading rates or photosynthetic capacities) or along the path (*e.g.* sieve tube size or number, Wardlaw, 1990). The main effect QTL identified at the top of chromosome 3 and validated (see below) offers the possibility to further explore this hypothesis.

Using composite variables to disentangle intertwined root and shoot variables and identify root QTLs

Multivariate analysis such as principle component analysis for genetic analysis has previously been used to simplify datasets and generate composite variables. For instance, it was used to identify QTL associated with complex variables such as leaf shape in Arabidopsis (Langlade *et al.,* 2005), in Brassica oleracea (Sebastian *et al.,* 2002), or grain shape in wheat (Iwata *et al.,* 2010). In the present study, principle component analysis was used in order to extract components that show orthogonality to main trend, clearly driven by plant size (synthesized by PC1). PC2, 3 and 4 allowed to uncouple root variables (primary root length, total root length and root dry weight respectively) from plant size. The coordinates along these axes, ratios and residuals of correlation are by construction different mean to account for a variation to a main trend and were therefore used in combination to strengthen the analysis.

Consistent with the initial assumption, the two main regions associated with PC1 were also the two main regions responsible for the control of all (root and shoot) raw variables with the same alleles favouring growth of organs. Moreover, the main QTL regions involved in the genetic control of root-related variables (bottom of chromosome 1 and top of chromosome 3) were independent of PC1 suggesting that our analysis successfully separated root

and shoot component of plant growth. The role of these two regions was further examined by using HIF. The role of the first region was validated for primary root length determinism with the same allelic effect than in the QTL analysis but did not validated the role of this regions for other root related variables. One explanation could be linked to the absence of the favourable allele (Sha) at the epistatic site of this QTL (MSAT2.38) in both HIF (083 and 107) used that may have lowered the detection power. The second region analysed yielded more clear-cut results, possibly associated with a higher density of main effects QTL and the strongest epistatic QTL for root related variables. In contrast with the region at the bottom of chromosome 1, this could compensate the lack of the favourable epistatic partner in the HIF. Except for the root dry weight, similar effects were detected in both HIF 004 and 338 suggesting they point to the overlapping hererozygous region between them as being responsible for the QTL.

The two regions highlighted co-locate with previously reported root QTL. The QTL localised in the lower half of chromosome 1 localized close to a root growth QTL previously identified as being related to a cell wall invertase gene (Sergeeva *et al.,* 2006). It also co-locates with QTL for both lateral root length and density in the same population, grown in Petri plates on agar media (Loudet *et al.,* 2005). The top of chromosome 3 was previously shown to control lateral root length (Loudet *et al.,* 2005) as well as osmotic stress response of roots (Loudet O., unpublished data) and other growth related loci in another RIL set involving the Shahdara accession (El Lithy *et al.,* 2004).

Acknowledgments

This work was funded by ERAPGFP/06.034B – ANR-06-003-03 program ARABRAS and by ANR-GPLA-06-002 program DNV. MB thanks D Weigel and the Max Planck Institute for Developmental Biology in Tübingen for contributing to her PhD fellowship and EGIDE/PHC - Procope for travels. C Granier and S Tisne are acknowledged for a critical reading of an earlier draft of the manuscript.

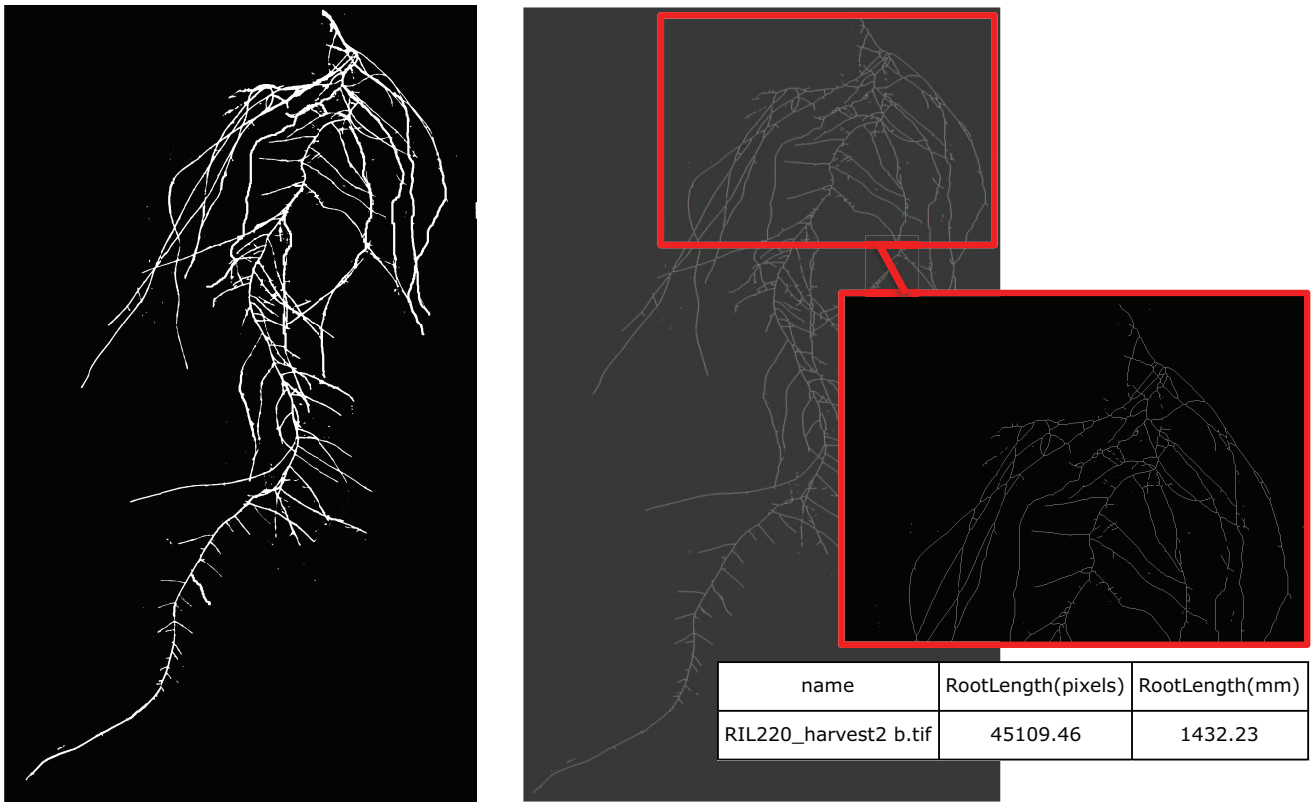
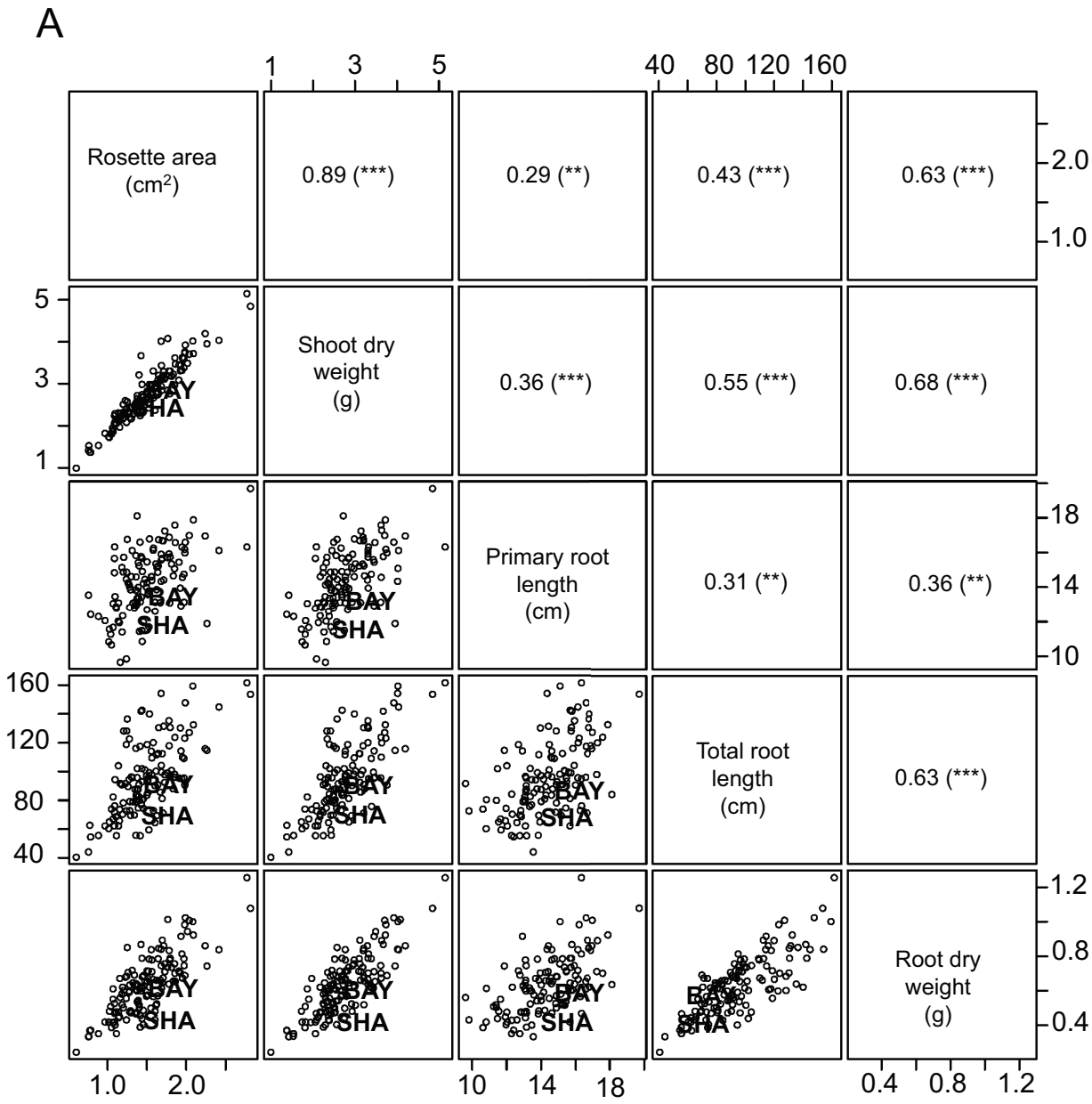


Figure S1. Root length measurements using a macro developped on Image J by Volker Backer (Montpellier Rio Imaging), and available at http://bioweb.supagro.inra.fr/phenopsis/MacroImageJ.php.



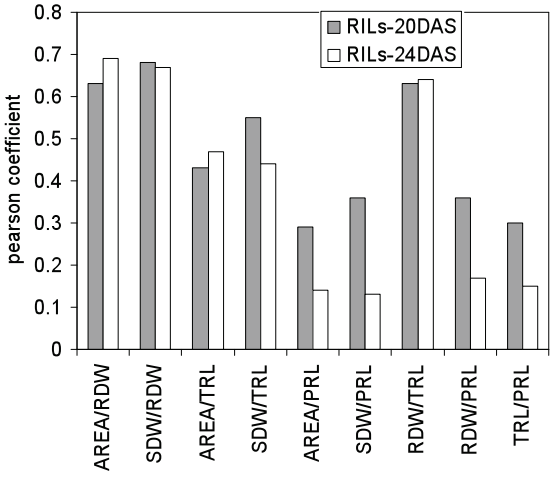
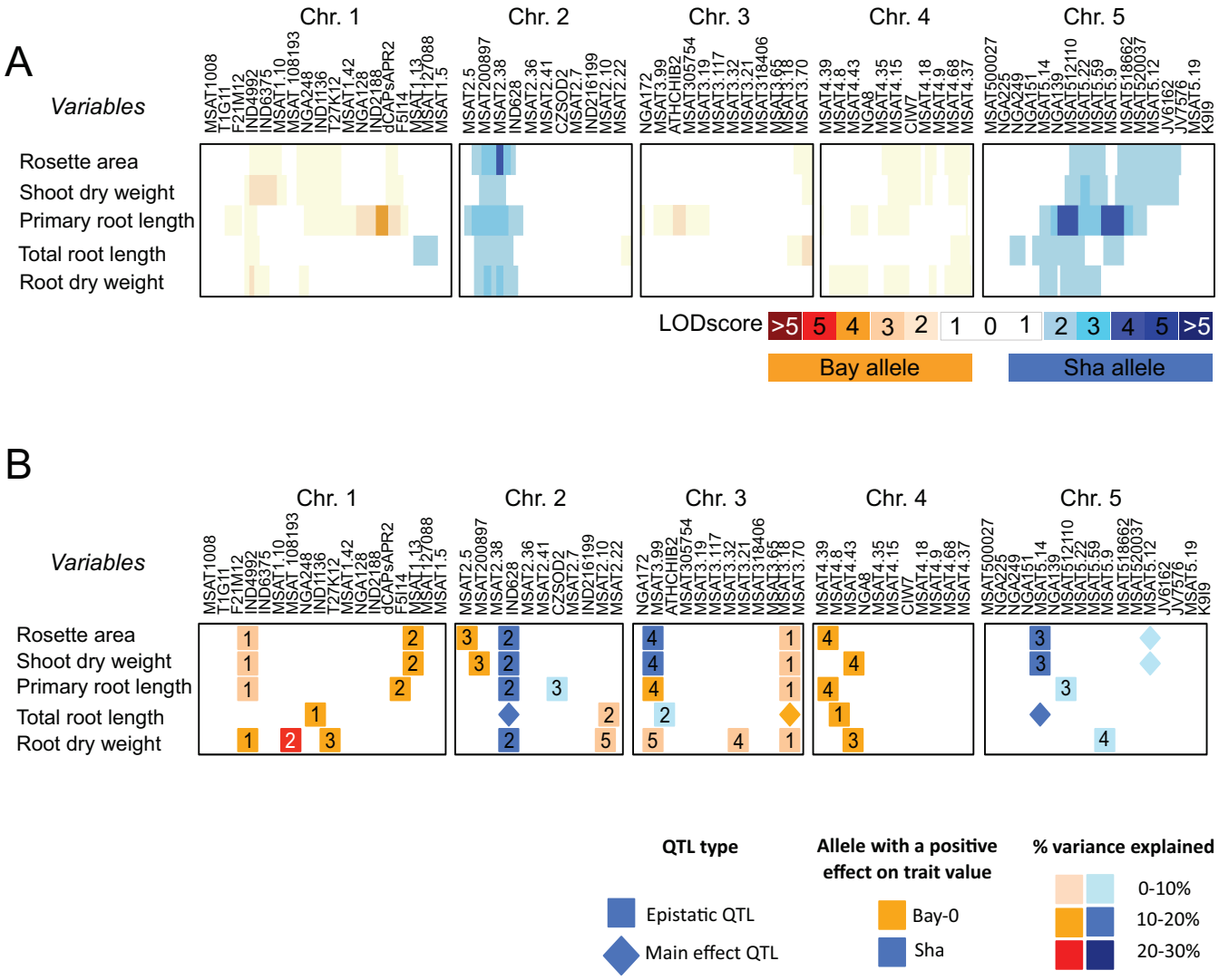


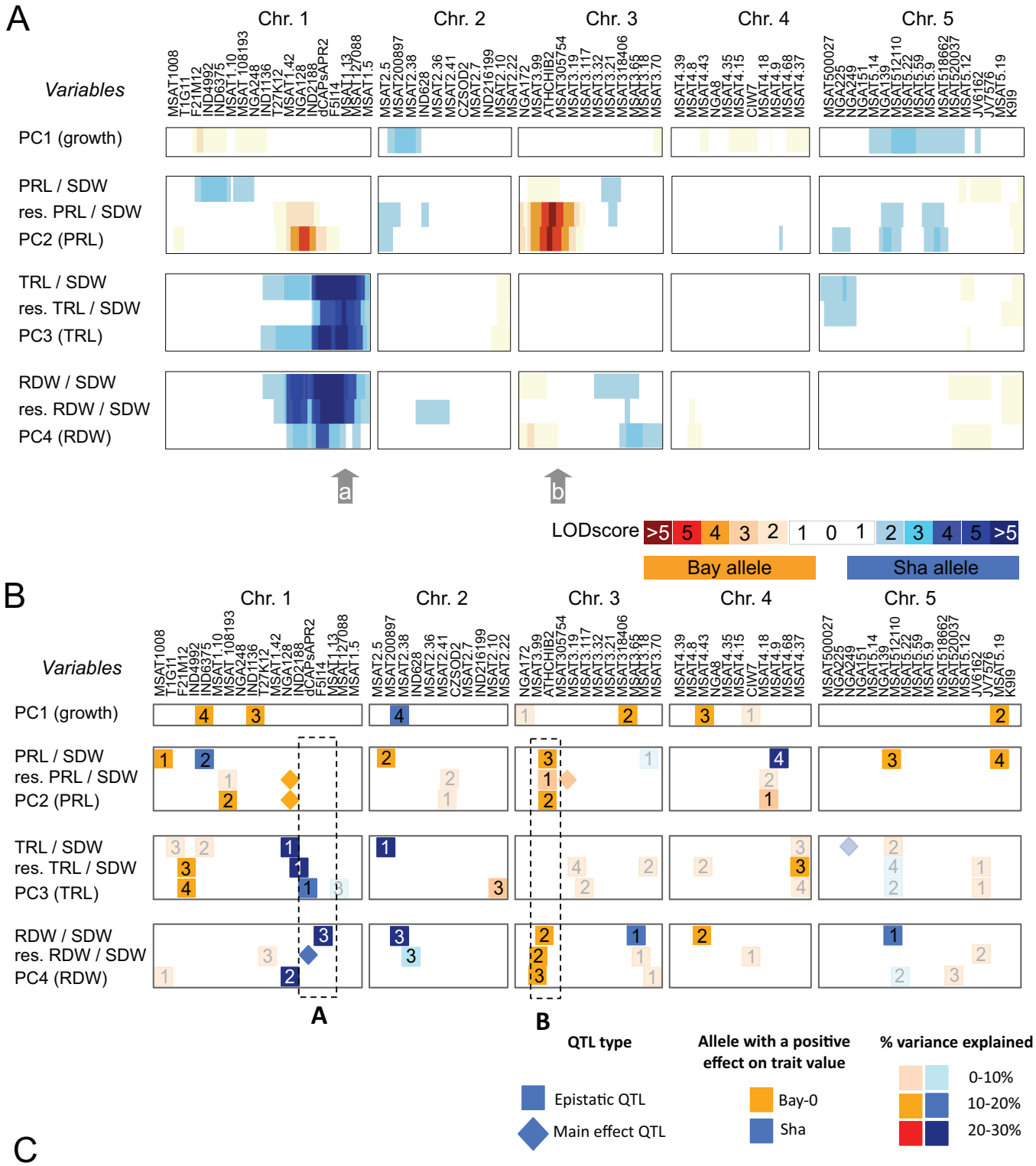
Figure S2 A. Correlation matrix between the different root and shoot growth variables within the 165 individuals of the Bay-0 x Shahdara RIL population. Data are those obtained 20 days after sowing. Dots represent the mean values of each RIL (4 individuals), and Bay-0 and Shahdara parental lines are indicated. Pearson's coefficients (r) associated to correlations are shown with their p-value (***, p-value < 0.001, **, p-value<0.01, *, p-value<0.05, ns, p-value>0.05). Shoot and root dry weight are expressed in mg, rosette area in cm², total and primary root length in cm. **B.** Pearson coefficients for all correlations among the Bay-0 x Shahdara RIL population at both 20 and 24 days after sowing (DAS).



C

	Heritabilities	R^2 QTL model
Rosette area	0.77	0.41
Shoot dry weight	0.73	0.38
Primary root length	0.61	0.42
Total root length	0.75	0.46
Root dry weight	0.66	0.41

Figure S3. Genetic map of the QTL detected in the Bay-0 x Shahdara for shoot and root growth variables. Data are those obtained at 20 days after sowing. **A**. Map of the LOD score values all along the genome using Interval Mapping analysis. A color code indicates the parental allele which increases the value of the variables at the marker (blue for Sha alleles, and red for Bay alleles). The LOD score value is shown as different color intensities. **B**. Map of the regions involved in models combining main effects and epistatic QTLs. A color code indicates both the allele which increases the value of the variable at one specific region and the percentage of variance explained by the QTL. Identical numbers are indicated in the two partners of the epistatic interaction. **C**. Broad-sense heritability and r^2 of the QTL models shown in B.



C

	Heritabilities	R^2 QTL model
PC1 (growth)	0.46	0.43
PRL/SDW	0.44	0.37
residual (PRL/SDW)	0.48	0.42
PC2 (PRL)	0.45	0.40
TRL/SDW	0.49	0.44
residual (TRL/SDW)	0.61	0.54
PC3(TRL)	0.56	0.48
RDW/SDW	0.51	0.46
residual (RDW/SDW)	0.54	0.51
PC4 (RDW)	0.43	0.37

Figure S4. Genetic map of the QTLs detected for root to shoot ratio, residuals of correlations between root variables and shoot dry weight and coordinates in the principal component analysis. Data are those obtained 20 days after sowing. **A**. Map of the LOD score values all along the genome using Interval Mapping analysis. A color code indicates the parental allele which increases the value of the variables at the marker (blue for Sha alleles, and red for Bay alleles). The LOD score value is shown as different color intensities. Arrows *a* and *b* refer to regions described in the text. **B**. Map of the regions involved in models combining main effects and epistatic QTLs. A color code indicates both the allele which increases the value of the variable at one specific region and the percentage of variance explained by the QTL. Identical numbers are indicated in the two partners of the epistatic interaction. A and B rectangles refer to regions controlling root related variable but not involved in global plant growth. QTLs not retrieved in the map from 24 days after sowing plants are shown with a translucent color. **C**. Broad-sense heritability and r^2 of the QTL models shown in B.

Variable	R^2 QTL model	Markers	p-value	% explained variance	Add.
AREA 20	R^2= 0.413				
		MSAT5.12	0.011	0.06	0.2
		NGA172 * MSAT4.35	0.002	0.14	+ x -
		MSAT2.5 * MSAT512110	0.006	0.11	- x +
		MSAT3.70 * F21M12	0.036	0.07	- x -
		IND628 * F5I14	0.000	0.19	+ x -
SDW 20	R^2= 0.378				
		MSAT5.12	0.014	0.06	0.14
		NGA172 * MSAT4.35	0.021	0.09	+ x -
		MSAT2.5 * MSAT512110	0.003	0.13	- x +
		MSAT3.70 * F21M12	0.009	0.11	- x -
		IND628 * F5I14	0.002	0.13	+ x -
PRL 20	R^2= 0.416				
		NGA172 * MSAT4.35	0.000	0.16	- x -
		MSAT3.70 * F21M12	0.024	0.08	- x -
		IND628 * F5I14	0.000	0.19	+ x -
		MSAT512110 * CZSOD2	0.038	0.07	+ x +
TRL 20	R^2= 0.457				
		T27K12 * MSAT4.8	0.010	0.10	- x -
		MSAT3.70	0.001	0.10	-14.04
		ATHCHIB2 * MSAT2.10	0.041	0.07	+ x -
		IND628	0.000	0.13	+16.86
		NGA249	0.031	0.05	+10.20
RDW 20	R^2= 0.409				
		IND628 * MSAT108193	0.000	0.18	+ x -
		T1G11 * MSAT3.70	0.012	0.11	- x -
		T27K12 * MSAT4.43	0.012	0.11	- x -
		MSAT5.59 * MSAT3.21	0.046	0.07	+ x -
		MSAT3.99 * MSAT2.10	0.041	0.08	- x -
AREA 24	R^2= 0.457				
		IND1136 * MSAT3.21	0.011	0.13	- x -
		F21M12 * MSAT3.70	0.002	0.17	- x -
		NGA172 * NGA8	0.012	0.12	+ x -
		F5I14 * MSAT2.38	0.000	0.21	- x +
SDW 24	R^2= 0.503				
		IND1136 * MSAT3.21	0.000	0.19	- x -
		F21M12 * MSAT3.70	0.001	0.18	- x -
		NGA172 * NGA8	0.006	0.14	+ x -
		F5I14 * MSAT2.38	0.000	0.22	- x +
PRL 24	R^2= 0.473				
		IND1136 * MSAT3.21	0.001	0.17	- x +
		MSAT3.99	0.001	0.16	-1.22
		F5I14 * MSAT2.38	0.002	0.15	- x +
		MSAT3.70 * F21M12	0.041	0.09	+ x -
TRL 24	R^2= 0.508				
		MSAT108193 * MSAT2.38	0.000	0.33	- x +
		T27K12 * MSAT3.21	0.002	0.16	- x -
		MSAT127088	0.012	0.12	+33.4
RDW 24	R^2= 0.504				
		F21M12 * MSAT3.70	0.004	0.14	- x -
		MSAT3.21 * IND1136	0.002	0.15	- x -
		MSAT4.35	0.001	0.16	-0.45
		MSAT3.99	0.002	0.15	- x +
		MSAT127088	0.000	0.29	+0.6

Table S1. QTL models for the shoot and root growth variables. AREA, SDW, PRL, TRL and RDW refer to rosette area, shoot dry weight, primary root length, total root length, and root dry weight respectively. Models are shown for both data at 20 and 24 days after sowing. The percentage of variance explained by the QTL model (R^2 QTL model), the markers involved as main effect or epistasic, the p-value of the t-test, the percentage of variance explained by each term of the model, and the corresponding additive effect are indicated.

Variable	R2 QTL model	Markers	p-value	% variance explained	Add.
PC 1 (growth) 20	R^2 = 0.428				
		NGA172 * MSAT4.35	0.02	0.10	- x -
		MSAT3.65 * MSAT5.12	0.01	0.13	- x -
		T27K12 * MSAT4.8	0.00	0.16	- x -
		IND628 * MSAT1.10	0.00	0.13	+ x -
PRL/SDW 20	R^2 = 0.366				
		F21M12*MSAT3.70	0.01	0.16	+ x +
		MSAT1.10*IND628	0.00	0.19	+ x +
		ATHCHIB2*MSAT5.14	0.00	0.19	- x +
		MSAT4.18*JV6162	0.01	0.12	- x -
resPRL 20	R^2 = 0.421				
		ATHCHIB2 * IND1136	0.02	0.10	- x -
		MSAT2.41 * MSAT4.15	0.01	0.11	- x -
		dCAPsAPR2	0.00	0.19	-0.02
		MSAT3.19	0.02	0.06	-0.10
PC 2 (PRL) 20	R^2 = 0.399				
		MSAT4.15 * MSAT2.41	0.00	0.10	- x -
		IND1136 * MSAT305754	0.00	0.18	- x -
		IND2188	0.00	0.18	-0.04
TRL/SDW 20	R^2 = 0.437				
		F5I14*IND628	0.00	0.27	+ x +
		MSAT1.10*MSAT512110	0.01	0.15	- x -
		F21M12*MSAT4.68	0.01	0.19	- x -
		NGA249	0.00	0.17	0.70
resTRL20	R^2 = 0.537				
		MSAT1.13 * MSAT518662	0.00	0.37	+ x -
		MSAT4.43 * MSAT3.65	0.00	0.23	- x -
		IND6375 * MSAT4.68	0.00	0.17	- x -
		MSAT3.117 * NGA139	0.02	0.14	- x +
PC 3 (TRL) 20	R^2 = 0.482				
		MSAT518662 * MSAT1.13	0.00	0.18	+ x -
		MSAT3.117 * NGA139	0.01	0.12	- x +
		MSAT1.5 * MSAT2.22	0.02	0.09	+ x -
		MSAT4.15 * IND216199	0.03	0.14	- x +
RDW/SDW 20	R^2= 0.456				
		MSAT318406 * NGA225	0.000	0.20	+ x +
		ATHCHIB2 * MSAT4.8	0.002	0.12	- x -
		MSAT1.13 * MSAT2.38	0.000	0.28	+ x +
resRDW 20	R^2 = 0.506				
		dCAPsAPR2	0.00	0.15	0.05
		MSAT4.15 * MSAT3.65	0.00	0.15	- x -
		MSAT3.99 * MSAT520037	0.00	0.17	- x -
		NGA128 * MSAT2.36	0.01	0.10	- x +
PC 4 (RDW) 20	R^2 = 0.371				
		MSAT3.70 * T1G11	0.01	0.13	- x -
		NGA151 * IND2188	0.00	0.22	+ x +
		MSAT3.99 * MSAT5.59	0.03	0.11	- x -

Table S2. QTL models for three types of calculated variables. Ratio between root variables (Root dry weight (RDW), Total root length (TRL), and Primary root length (PRL)) and Shoot dry weight (SDW), PCA coordinates on principal components 2, 3 and 4 (that are accounted for by primary root length, total root length and root dry weight respectively), and residuals of the correlations between root variables and shoot dry weight (SDW), at 20 days after sowing. The percentage of variance explained by the QTL model (R^2 QTL model), the markers involved as main effect or epistasic, the p-value of the t-test, the percentage of variance explained by each term of the model, and the corresponding additive effect are indicated.

Variable	R2 QTL model	Markers	p-value	% variance explained	Add.
PC 1 (growth) 24	R^2 = 0.669				
		K9I9 * MSAT3.21	0.01	0.21	- x +
		IND6375 * IND628	0.00	0.32	- x +
		MSAT100008 * MSAT500027	0.02	0.14	- x +
		T27K12 * MSAT4.39	0.00	0.28	- x -
PRL/SDW 24	R^2 = 0.458				
		MSAT1008 * MSAT4.37	0.00	0.15	- x +
		IND6375 * IND628	0.02	0.18	+ x -
		ATHCHIB2	0.01	0.24	-0.35
		MSAT4.18*JV7576	0.02	0.17	+ x -
resPRL 24	R^2 = 0.427				
		F5I14 * MSAT4.35	0.01	0.21	- x -
		ATHCHIB2	0.00	0.27	-0.03
PC 2 (PRL) 24	R^2 = 0.549				
		NGA248 * JV6162	0.03	0.12	- x -
		MSAT518662 * IND628	0.02	0.13	- x +
		MSAT4.15 * IND6375	0.02	0.15	- x -
		ATHCHIB2	0.00	0.24	-0.030
		dCAPsAPR2	0.00	0.16	-0.004
TRL/SDW 24	R^2 = 0.36.2				
		NGA128*IND628	0.01	0.09	+ x +
		CZSOD2*MSAT4.43	0.02	0.11	- x +
		MSAT127088 * MSAT3.117	0.01	0.13	+ x +
		MSAT3.99*JV7576	0.02	0.06	- x -
resTRL 24	R^2 = 0.435				
		IND6375 * MSAT2.38	0.00	0.23	- x +
		F5I14	0.01	0.18	+0.1
		F21M12 * MSAT4.9	0.04	0.13	- x -
PC 3 (TRL) 24	R^2 = 0.528				
		MSAT2.38 * IND6375	0.01	0.14	+ x -
		CZSOD2 * NGA8	0.00	0.19	- x -
		K9I9 * IND216199	0.00	0.20	- x -
		ATHCHIB2	0.00	0.19	-0.05
		MSAT1.13	0.00	0.19	+0.04
RDW/SDW 24	R^2= 0.481				
		MSAT1.13 * IND628	0.000	0.25	+ x +
		MSAT3.99	0.000	0.25	-0.05
		MSAT3.21 * NGA225	0.043	0.09	+ x +
		MSAT2.10 * MSAT5.22	0.029	0.10	- x -
res RDW 24	R^2 = 0.613				
		MSAT3.99	0.00	0.34	-0.02
		MSAT4.9 * NGA249	0.01	0.15	- x +
		T1G11 * K9I9	0.02	0.16	- x -
		MSAT1.13 * IND628	0.00	0.22	+ x +
PC 4 (RDW) 24	R^2 = 0.411				
		F5I14	0.00	0.28	+0.04
		K9I9	0.00	0.28	-0.07
		MSAT3.99 * NGA8	0.00	0.20	- x -
		IND628 * MSAT4.18	0.02	0.10	+ x -

Table S3. QTL models for three types of calculated variables. Ratio between root variables (Root dry weight (RDW), Total root length (TRL), and Primary root length (PRL)) and Shoot dry weight (SDW), PCA coordinates on principal components 2, 3 and 4 (that are accounted for by primary root length, total root length and root dry weight respectively), and residuals of the correlations between root variables and shoot dry weight (SDW), at 24 days after sowing. The percentage of variance explained by the QTL model (R^2 QTL model), the markers involved as main effect or epistasic, the p-value of the t-test, the percentage of variance explained by each term of the model, and the corresponding additive effect are indicated.

Chapter 2 /A trans-population analysis of growth and biomass partitioning response to soil water deficit in Arabidopsis thaliana.

Marie Bouteillé, Denis Vile, and Bertrand Muller

Institut de Biologie Intégrative des Plantes, Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, UMR759, INRA, Montpellier, France

Abstract

Drought is a common environmental threat experienced by plants worldwide that is expected to become more acute in the next decades, due to concomitant increase of temperatures, and competition for water usage for purposes other than agriculture. In the plant, drought is known to severely reduce the expansion of leaves but the impact on root growth is debated since it has been reported to be reduced, unchanged or stimulated. Beyond this Root/shoot biomass partitioning issue, the response to soil water deficit of the partitioning of biomass within roots and shoots evaluated through the Specific leaf area (leaf area per mass) and Specific root length (root length per mass) have also been debated since they could contribute to improving water use efficiency and soil exploration. Moreover, it is not known if and to what extend high Root/shoot ratio, dense leaves or thin roots are beneficial or not to drought tolerance.

In this study, we evaluated the effect of severe soil water deficit conditions on plant growth, and biomass partitioning both between and within shoot and roots. We used Standardized Major Axis (SMA) analysis to account for ontogenic effect that may bias the analysis. We used two sources of genetic variation, a Recombinant Inbred Lines population (RIL, Bay x Sha) and a collection of 100 natural accessions. As expected, shoot expansion was strongly affected by water deficit, while root response was lower, leading to average positive increase in Root/shoot ratio. Moreover, under water deficit, Specific leaf area strongly decreased while Specific root length remained unchanged. Interestingly, the shoot response to soil water deficit (used as an evaluation of drought tolerance) was well correlated with both Specific leaf area in optimal conditions and its response to soil water deficit. Moreover, the later was much steeper in accessions as compared to the RIL suggesting natural combinations of alleles allow responses that are partly lost in artificial genetic combinations. Tolerance to soil water deficit (in term of maintenance of dry weight accumulation) was thus on average a combination of small size, high Root/shoot ratio, high Specific leaf area as well as a capacity to strongly decrease it upon water deficit, thereby maintaining dry weight accumulation despite reduced leaf area.

Key words: Arabidopsis, water deficit, root growth, shoot growth, Root/shoot, Specific leaf area, Specific root length, Standardized major axis correlation, multiple regression

Introduction

Soil water deficit is a common environmental stress experienced by plants worldwide. In the near future, increased temperatures will also contribute to higher evaporative demands (Qaderi *et al.,* 2006) and water deficit will to continue to be a major abiotic factor affecting global crop yields and ecosystem production (Parmesan, 2006). Understanding the basis of adaptation in the wild is a major avenue of research for possibly orienting breeding programs of crop plants towards potentially more tolerant genotypes. In natural ecosystems, plants use various strategies to cope with drought, depending on factors such as species, genotypes, natural habitat, strength and timing of drought periods. For example, annual species that live in dry habitats classically develop drought escape strategies that consist in early flowering, allowing completion of life cycle before drought becomes too severe (Turner *et al.,* 2001; Xu *et al.,* 2005; Sherrard *et al.,* 2006). In parallel to this escape strategy, plants can also develop avoidance strategies that rely on decreasing water loss through transpiration, and/or increasing water uptake at the root level. Reduction of leaf expansion, rapid stomatal closure, and increased cuticular resistance are examples of ways to avoid water loss by transpiration. At the root level, water uptake can be enhanced through the development of a large and deep root system (Costa França *et al.,* 2000; Turner *et al.,* 2001; Chaves *et al.,* 2002; Zlatev 2005). At the cellular level, maintaining adequate cell turgor by osmotic adjustment (involving inorganic ions, carbohydrates, and organic acids) while preventing disruptions in cellular metabolism (Munns 1988; Save *et al.,* 1993; Nguyen *et al.,* 1997; Hummel *et al.,* 2010) and controlling cell-wall extensibilty (Lockardt *et al.,* 1968; Touchette *et al.,* 2006; Muller *et al.,* 2007) can also allow growth maintenance under limited water availability.

Among these modifications, the decrease of shoot growth rate is observed very early during the establishment of soil water deficit (Boyer 1970; Hsiao 1973; Muller *et al.,* 2011). It occurs before stomatal closure and photosynthesis reduction (Bogeat-Triboulot *et al.,* 2007) and well before cellular processes associated with tolerance to dehydration take place (Tardieu *et al.,* 1999). Because access to the root system is more complex, experiments taking it into account are rarer. Moreover, root growth and development has also been extensively studied in hydroponics or Petri plates, especially in the model plant Arabidopsis thaliana (Loudet *et al.,* 2005; chapter 1). Attempts to mimic water deficits in such conditions have been done, essentially using osmotic agents such as PEG or mannitol (van der Weele, 2003). However, the several drawbacks of these protocols (anoxia, rapid shock rather than slowly establishing stress, toxicity of the molecules) make them strongly debated (Spollen *et al.,* 2000) and it is therefore highly desirable to use more realistic conditions. Even under realistic conditions, there is no consensus on the root system response to water deficit. Indeed, it has been reported to be reduced, unchanged or stimulated, depending on cases (e.g. Poorter and Nagel 2000). However, in most

reported cases, root growth seems to be less affected than shoot growth (French and Turner, 1991; Spollen *et al.,* 1993; Shao *et al.,* 2008). As a result, an increased Root/shoot ratio was frequently observed in droughted plants (Turner *et al.,* 1997 in wheat; Asseng *et al.,* 1998 in wheat; Hsiao *et al.,* 2000 in maize; Lei *et al.,* 2006 and Bogeat-Triboulot *et al.,* 2007 in poplar).

As roots are heterotrophic organs, their growth depends on carbon capture in shoots through photosynthesis. Therefore, a tight relationship between the root and shoot biomass has been frequently reported (see chapter 1 and Enquist and Niklas 2002 for a trans-species generalization). Brouwer´s pruning experiments also illustrate the remarkable capacity of the plant to re-adjust the Root/shoot ratio in just a few days after it has been artificially modified (Brouwer 1962, Poorter and Nagel 2000, Farrar and Gunn 1998). How the tight Root/shoot relationship is affected under water deficit and how the shoot response relates to the root response are not known. Intuitively, a larger shoot growth reduction could lead to more carbon being available for root growth under water deficit (Hummel *et al.,* 2010). By contrast, because roots are strongly dependent on shoot for their growth, stronger shoot reduction could lead to stronger root reduction.

Another issue that we aimed to address in this study was the potential role of biomass growth and partitioning traits in improving drought tolerance. Biomass partitioning variables are particularly important since they usually constitute a trade-off between different strategies. The Root/shoot ratio is a first variable of interest. Whether genotypes having high or low Root/shoot ratio under optimal conditions is related to drought tolerance is not known. Intuitively, high Root/shoot could help the plant to forage the soil volume and have a better access to the water resource. However, this strategy can be cost full in term of assimilate. Another trait reflecting the trade-off between leaf expansion and biomass allocation or production within leaves is the Specific leaf area (*ie* leaf area per unit dry mass, Specific leaf area). The Specific leaf area is often used as an indirect indicator of leaf thickness, and reported to be reduced under drought conditions (Marcelis *et al.,* 1998, Liu and Stützel 2004). Decrease in Specific leaf area in droughted plants could be due to the different sensitivity of photosynthesis and leaf area expansion to drought (Jensen *et al.,* 1996, Tardieu *et al.,* 1999, Hummel *et al.,* 2010). Reduction of Specific leaf area is assumed to be a way to improve water use efficiency (WUE) (Wright *et al.,* 1994, Craufurd *et al.,* 1999), because thicker leaves usually have a higher density of chlorophyll and proteins per unit leaf area and, hence, have a greater photosynthetic capacity per unit leaf area than thinner leaves. The root equivalent of Specific leaf area, the Specific root length, is an indirect indicator of root thickness. Specific root length illustrates the trade-off between long and thin roots (high Specific root length) or short and thick roots (low Specific root length) with the same biomass. High Specific root length has been shown to be favourable to exploit water in the deep soil layers while low Specific root length could contribute to root growing more easily in a compact drying

soil (Yoshida 1982, Eissenstat 1991, Zheng *et al.,* 2000). Specific root length has been reported to be either decreased (Kage *et al.,* 2004 on cauliflower) or increased (Azhiri-Sigari *et al.,* 2000 on rice) in droughted plants. Nevertheless the correlation between modification of Specific root length and plant tolerance to drought is still on debate (Schwinning *et al.,* 2001, Vamerali *et al.,* 2003).

In this study, we have addressed the above questions in the model species Arabidopsis thaliana by combining two sources of genetic variation. The first source is natural, provided through a collection of 100 Arabidopsis accessions collected along the northern hemisphere essentially in Eurasia. The second is artificial, provided through a collection of recombinant inbred lines obtained from the cross between a European (Bay-0) and an Asian (Shahdara) accession (Loudet *et al.,* 2002). The experiments were performed using the Phenopsis platform (Granier *et al.,* 2006) at two levels of soil water content, corresponding to well-watered and severe soil water deficit. Growth variables refer to dry mass and size of roots and shoots, whereas biomass partitioning variables correspond to calculated ratio (Root/shoot ratio, Specific leaf area, Specific root length). Correlative relationships under both environmental conditions were used to characterize the water deficit response of the relationships between variables in these two sets of genotypes.

Materials and methods

Plant material

Two sets of genotypes were used in this study: The first one corresponds to a sub-population of 130 Recombinant Inbred Lines from the Bay-0 x Shahdara core-collection (Loudet *et al.,* 2002) genotyped with 69 microsatellites markers was selected to capture maximum recombination. This material was obtained from Versailles Biological Resource Centre http://dbsgap.versailles.inra.fr/vnat/. Complete genetic and phenotypic information on this population are available at http://dbsgap.versailles.inra.fr/vnat/Documentation/33/DOC.html.

The second set of genotypes corresponded to a collection of 100 accessions. This collection included 20 accessions that had been selected to represent the entire genetic diversity of the species along with 88 accessions that represented 8 geographic regions from the Eurasian range of the species. The two sets had 8 common accessions (see Appendix 1). The parental lines of the RIL population, Bay-0 and Shahdara are also

present in these two collections of accessions. The 20 accessions were the first ones sequenced in a large SNP sequencing project and were chosen to capture most of the common sequence variation of the worldwide Arabidopsis population (Clark *et al.,* 2007). The 88 accessions were collected in specific regions in Europe and Asia that are know to have been species refugees during the last glaciation (Sharbel *et al.,* 2000; Schmid *et al.,* 2006). Those accessions were also chosen as a starting point of an ambitious sequencing program of hundreds Arabidopsis accessions (www/1001genomes.org, Weigel and Mott, 2009). Seeds of these collections were obtained from the Max Planck Institute for Developmental Biology (Tübingen, Germany).

Plant growth conditions

All the experiments were performed in the PHENOPSIS automated phenotyping platform (Granier *et al.,* 2006). In each experiment, all micro-meteorological conditions were kept constant during the whole growing period. Day-length was maintained at 10 h to prevent early flowering, and light was provided by HQI lamps with additional cool white fluorescent tubes. Photosynthetic photon flux density (PPFD) was measured continuously at the plant level, using a photosynthetic sensor (LI-190SB, Li-Cor, Lincoln, NE, USA) and set to 180 µmol/m²/s in all cases. Air temperature and relative humidity were measured every 20 s (HMP35A Vaisala Oy, Helsinki, Finland) and set to 20-21°C (day and night) and 75% respectively. All measurements of temperature, PPFD and relative humidity were averaged and stored every 600 s in a data logger (Campbell Scientific, LTD-CR10Wiring Panel, Shepshed, Leicestershire, UK) and automatically sent to a database (http://bioweb.supagro.inra.fr/phenopsis/).

Seeds were sown in 200 mL conical pots (9 cm height and 4.5 cm diameter) filled with a mixture (1:1, v/v) of a loamy soil and organic compost. Soil water content was determined before sowing and set to 0.35 g(H_20). g(dry soil)$^{-1}$. Subsequent changes in pot weight were attributed to a change in soil water status. Soil water content was adjusted daily automatically with the automaton in the PHENOPSIS platform to two different values, 0.35 g H_2O g^{-1} dry soil corresponding to well-watered (WW), and 0.18 g H_2O g^{-1} dry soil corresponding to water deficit (WD) conditions (Granier *et al.,* 2002). These values correspond to a predawn leaf water potential of -0.3 MPa, and -1.1 MPa respectively (Granier *et al.,* 2006; Hummel *et al.,* 2010).

Experiments and treatments

Plants were grown in four independent experiments. During the first two experiments, RILs were grown first at optimal soil water content (RIL WW experiment) or under soil water deficit conditions (RIL WD experiment). In the next 2 experiments, accessions were grown at optimal soil water content (Acc WW experiment) and under soil water deficit conditions (Acc WD experiment). In all cases, germination occurred within 3-6 days after sowing. Pots were maintained at a soil water status of 0.35 g H_2O g^{-1} dry soil corresponding to well-watered conditions during the first 15 days after germination, and the soil water status was either maintained at this value (RIL WW and Acc. WW experiments) or reduced down to 0.18 (RIL WD and Acc. WD experiments). This value was reached within 3-4 days. Then soil water content was kept at this value by automatic irrigation twice a day. Each RIL and accession was grown in 3 and 5 pots respectively, randomly located within the same number of blocks. The lack of block effect was later tested using ANOVA. After 2 weeks, each pot was thinned to 1 to 3 homogeneous plants per pot, depending on plant size to avoid overlapping.

Variables measurements and calculation

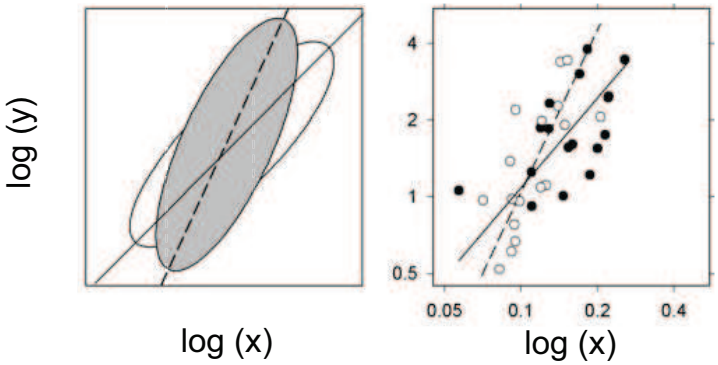
Plants were then harvested 10 days after the onset of water deficit corresponding to 28-31 days after sowing and 25 days after germination. 6 to 9 individual plants per genotype were collected and individually measured. At this time, a run of images of the rosette was taken by the automaton. Photos were further used to estimate projected Rosette area of each individual plant using Image-J software and customized macros. At harvest, all pots corresponding to one genotype were gathered, plants were gently removed from the pot, and rosettes were separated from the root system. Rosettes were then stored in paper bags for further measurements of Shoot dry weight after the tissues had been dried down after 2 days at 80°C. In order to capture root biomass and dimension, the root system was cleaned from every soil particles, and, spread at the surface of large (20 x 20 cm) Petri plates filled with water and a numerical image was taken at 600 dpi using a scanner in transmission mode. Total root length and Primary root length were measured on those images, using Image-J software and customized macros. After image capture, root systems were individually stored into 96 well plates each containing pre-weighed aluminium cell-cup to facilitate weighing of dry material. The plates were then oven dried for 2 days at 80°C and the cups were weighed to measure Root dry weight. All weights were measured using a 5 digits balance. Root/shoot ratio was calculated as the ratio between Root dry weight and Shoot dry weight, whereas Specific leaf area and Specific root length were calculated as the ratio between rosette area and Shoot dry weight and between Total root length and Root dry weight, respectively. The response to water deficit was expressed as the ratio between the value in water deficit condition to the value in well-watered conditions (and

Box 1. Allometric relationships

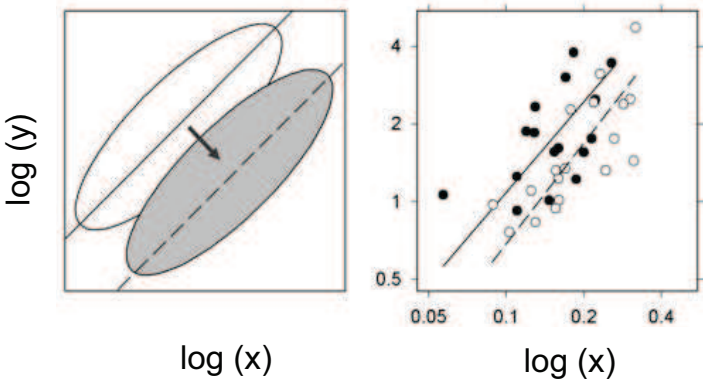
"Allometry is the study of variations of shapes and processes according to size effects (Niklas, 1994). The aim is to establish a functional relationship (in its mathematical sense) and its significance between one biological variable (or more) and the size of one organism (or of one part of this organism).The most widely used allometric equation for biology is the power function: $Y = b.\ X^a$; where Y is the variable of interest, and X the size variable. The a and b parameters describing the relationship between X and Y are called "exponent" and "allometric coefficient", respectively (Müller et al., 2000). The previous equation becomes linear after a logarithmic transformation: log Y = log b + a.logX. Then, the allometric coefficient (a) becomes the slope of the relationship between X and Y, and log b corresponds to the intercept." *(from Vile D. thesis, 2005)*

How to use of Standardized Major Axis analyses in allometric studies?

1. Test for differences in **slopes** between black and white points



2. Test for differences in **elevation**s between black and white points



Two different tests in Standardized Major Axis regressions. A collection of accessions is grown in two different environmental conditions (Condition 1, black points, and condition 2, white points). Two tests can be performed, the first is to test differences in slopes between black and white points (**1**), the second is to test differences in elevation between black and white points (**2**). A difference between slopes means that the relationship between the X variable and the Y variable depends on the value of the variables, whereas a difference between the elevations means that the environmental condition modifies the allometric coefficient between both variables, whatever their values.

called ‘response ratio’ hereafter).

Statistical analysis

All the statistical analyses were performed using R software (R Development Core Team, 2009). One-way analysis of variance was used to examine differences for each set of genotypes, between variables under water deficit and well-watered conditions. Standardized major axis (SMA) regression was used to describe the relationship between each possible pairwise combination of variables. This method is appropriate when the purpose of the study is to describe how variables are related (i.e. scaling relationships) (Warton *et al.,* 2006). Our aim was to estimate the line best describing the bivariate scatter of two variables, and SMA regression is considered to estimate lines with greater precision than major axis regression (Warton *et al.,* 2006; see Box. 1). Using log transformed variables, regression describes the best-fit scaling relationship between pairs of traits. When comparing the cloud of points that describe the pairwise relationship of traits from the different plant types, several outcomes are possible: (1) the slope of the line of best fit may differ between plant types; (2) if the slopes do not differ (are homogeneous), the clouds of points may completely overlap, or may be shifted along the common slope relative to each other, and/or may be shifted in one dimension only, resulting in a difference in elevation. SMA slopes were fitted for each combination of set of genotype/water treatment, and differences between slopes and/or elevations were tested. SMA regression analyses were performed using *smatr* software (Falster *et al.,* 2006), with significance tested at $\alpha = 0.05$. Multiple regression models were used to quantify the combined effects of growth and biomass partitioning variables on shoot response to water deficit. Broad sense heritabilities were calculated fro each variable as the ratio of genetic variance over total variance.

Results

Substantial genetically determined variation could be reproducibly observed

To evaluate the consistency of the dataset and of the responses recorded, the accessions Bay-0 and Shahdara that were always present in all experiments were compared (Fig. 1A). The values recorded both under well-watered and water deficit conditions were not significantly different for each variable either under well-watered conditions or under water deficit conditions in the different experiments. Shahdara displayed a greater shoot and

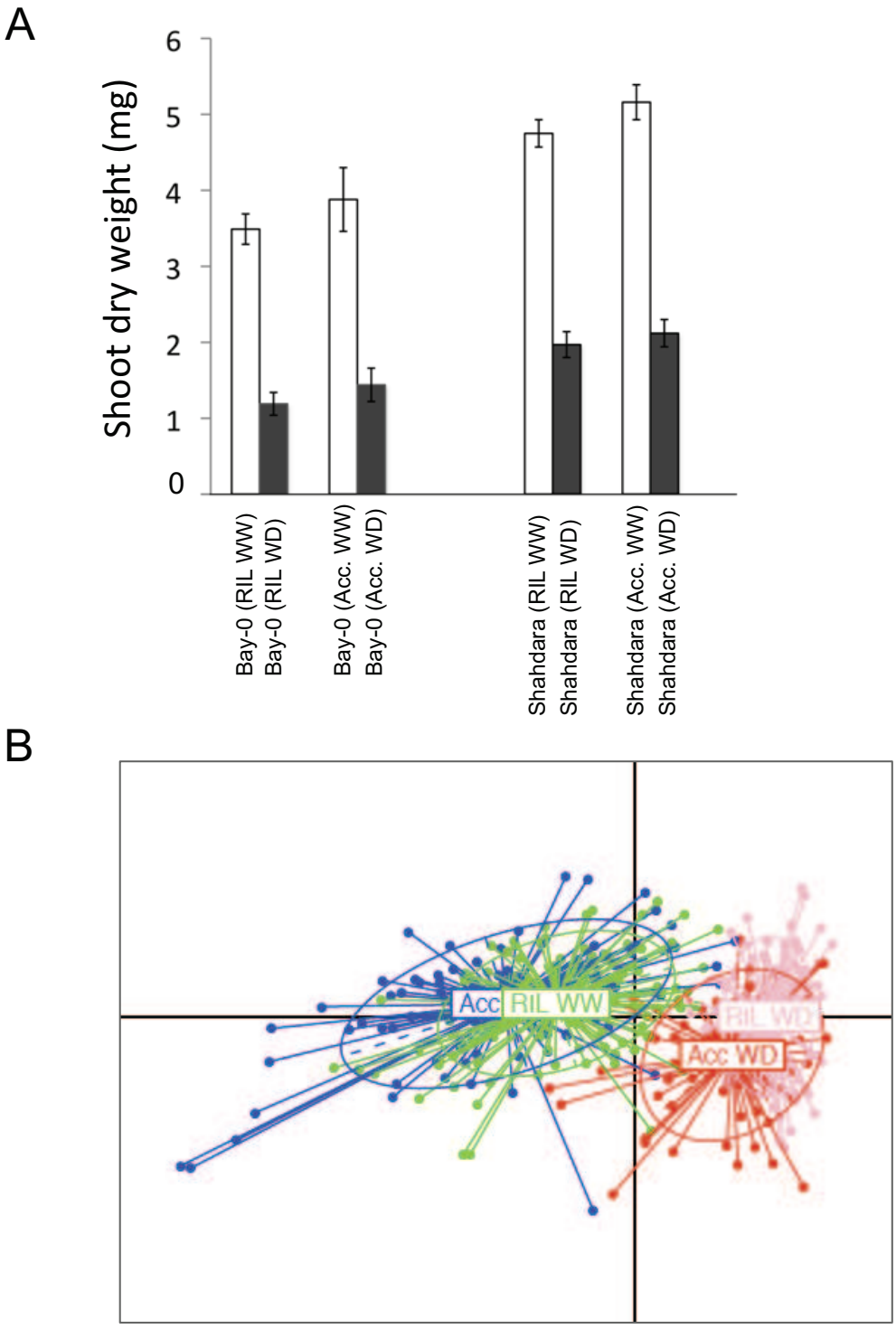


Fig. 1. Reproductibility of experiments. **A.** Shoot dry weight of Bay-0 and Shahdara lines in the four experiments (RIL WW, RIL WD, Acc. WW and Acc. WD). Bars represent the mean value of 9 individual values. Error bars represent standard deviation. **B.** Results of a PCA performed on the whole dataset, including for the four experiments shoot and root dry weight, rosette area, total and primary root length, as well as Root/shoot ratio, Specific leaf area and Specific root length. Each point represent a genotype. Blue and green points represent accessions and RIL under well-watered conditions, respectively. Red and pink points represent accessions and RIL under water deficit conditions, respectively.

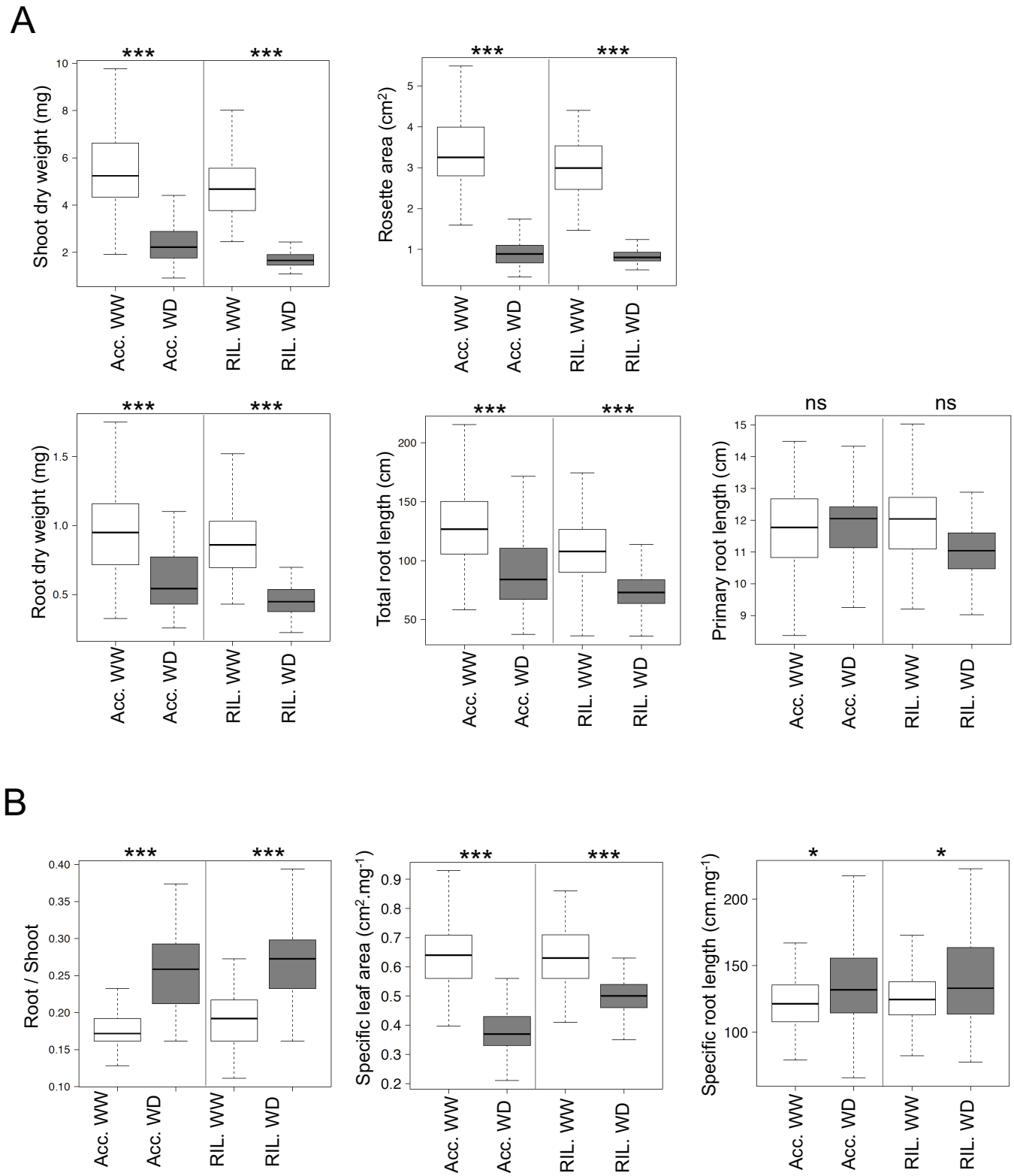


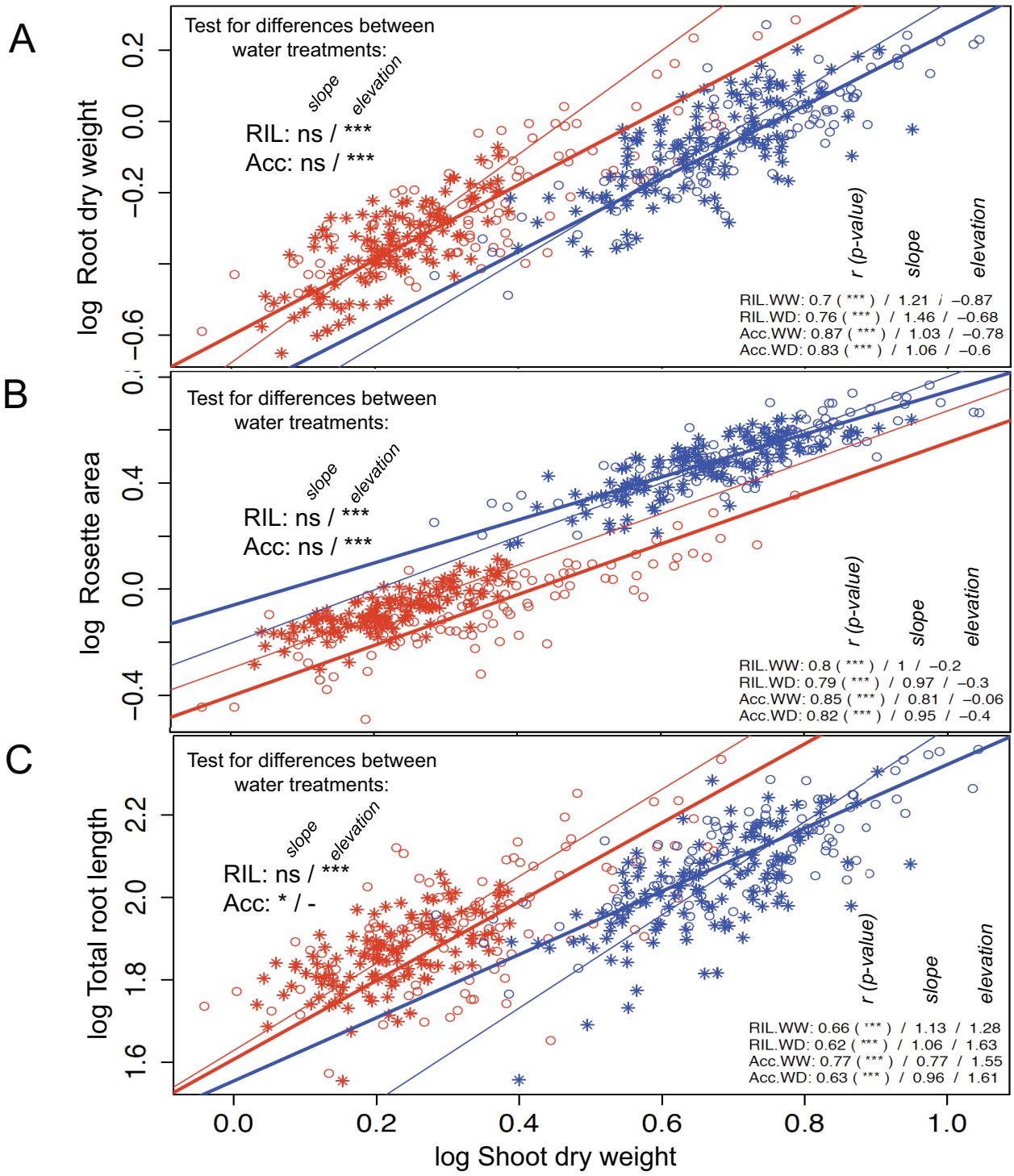
Fig. 2. Value of growth (**A**. Shoot dry weight, Rosette area, Root dry weight, Total root length, Primary root length), and biomass partitioning (**B.** Root/Shoot ratio, Specific leaf area, and Specific root length) variables in the two sets of genotypes (RIL and accessions), for the two levels of soil water status (white boxes: well-watered (WW), grey boxes: water deficit (WD)). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. A one-way ANOVA was performed to analyze the effect of water treatments for each set of genotypes (*:p-value<0.05; ***:p-value<0.001; ns: non significant).

root biomass compared to Bay-0 (Fig. 1A only shows Shoot dry weight measurements). In order to provide a broad view on the experiments, the whole dataset (Shoot and Root dry weight, Rosette area, Total and Primary root length, as well as Root/shoot ratio, Specific root length and Specific leaf area) was subjected to a Principal Component Analysis (Fig. 1B). The first two axes of the PCA accounted for 72 and 18% of total variance respectively. The first axis was driven by water deficit that affected biomass and size consistently. The centroids of genotypes were very similar between the two sets of genotypes (RIL, Accession) subjected to the same water treatment but the spread of the accessions was slightly higher than for RIL.

The part of the total variance attributable to genetic variance was estimated by calculating broad sense heritabilities of each variable (not shown). These heritabilities were high, ranging from 0.49 (Specific root length in water deficit conditions), to 0.71 (rosette area in optimal conditions) depending on variable and water treatment. Heritability was higher for shoot than for root variables, maybe due to a slightly higher error term for root variables.

Soil water deficit affected root and shoot growth differently, leading to modifications of biomass allocation patterns

Soil water deficit treatment had very consistent effects on all variables measured in the two sets of genotypes (Fig. 2). After almost 10 days of water deficit, Shoot dry weight and Rosette area were on average reduced by about 2/3 and ¾ respectively (Fig. 2A). Root dry weight and Total root length were on average reduced by 1/3 to ½ whereas Primary root length was on average not affected. For these biomass and dimension variables, there was a slight but consistent tendency towards a lower variation in the RILs exposed to soil water deficit as compared to the accessions. In line with this, a ranking analysis detected that the 10 least sensitive genotypes (showing the lowest reduction of the variable) were essentially composed of accessions. The response of biomass allocation patterns to water deficit, within shoots and roots as well as *between* shoots and roots is shown in Fig. 2B. In both RIL and accessions, soil water deficit increased on average the root / shoot ratio by about 1/3. It also decreased the Specific leaf area but this effect was two times more pronounced in the accessions (-41%) than in the RIL population (-16%). Finally, the Specific root length (*ie* the root length per unit root biomass) was on average slightly increased (+10%), indicating that roots were on average thinner and/or less dense under water deficit.


A
Test for differences between water treatments:
slope
elevation
RIL: ns / ***
Acc: ns / ***
log Root dry weight
r (p-value)
slope
elevation
RIL.WW: 0.7 (***) / 1.21 / −0.87
RIL.WD: 0.76 (***) / 1.46 / −0.68
Acc.WW: 0.87 (***) / 1.03 / −0.78
Acc.WD: 0.83 (***) / 1.06 / −0.6
B
Test for differences between water treatments:
slope
elevation
RIL: ns / ***
Acc: ns / ***
log Rosette area
r (p-value)
slope
elevation
RIL.WW: 0.8 (***) / 1 / −0.2
RIL.WD: 0.79 (***) / 0.97 / −0.3
Acc.WW: 0.85 (***) / 0.81 / −0.06
Acc.WD: 0.82 (***) / 0.95 / −0.4
C
Test for differences between water treatments:
slope
elevation
RIL: ns / ***
Acc: * / -
log Total root length
r (p-value)
slope
elevation
RIL.WW: 0.66 (***) / 1.13 / 1.28
RIL.WD: 0.62 (***) / 1.06 / 1.63
Acc.WW: 0.77 (***) / 0.77 / 1.55
Acc.WD: 0.63 (***) / 0.96 / 1.61
log Shoot dry weight

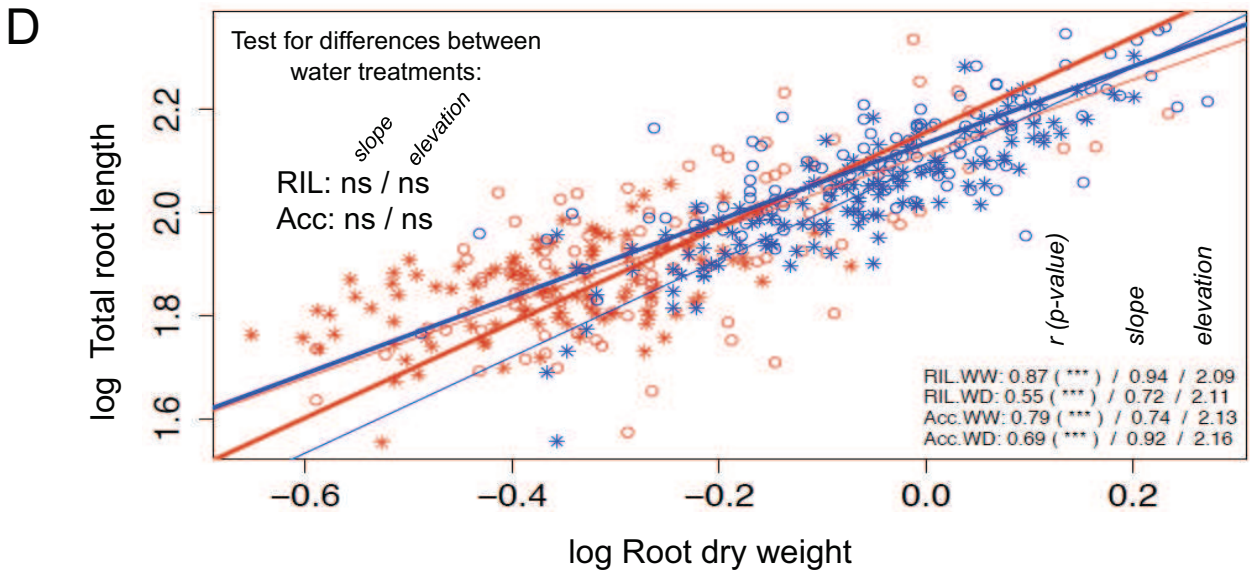


Fig. 3. Standardized major axis regressions (SMA) of Root dry weight (**A**), Rosette area (**B**), and Total root length (**C**) according to Shoot dry weight, and Total root length according to Root dry weight (**D**). This analysis was performed for both sets of genotypes (asterix: RIL, circles: accessions) in well-watered (WW, blue) and in water deficit (WD, red) conditions. The thin lines correspond to regression lines for the RIL population, and the thick line to regression lines for the collection of accessions. Values are log transformed. The bottom-right legend indicates for both sets of genotypes (RIL and accessions), for both water treatments (WW and WD): (From the left to the right) the Pearson´s coefficient (r) , the associated p-value (***:p-value<0.001; ns: non significant), the slope and elevation of the correlation. The top-left legend indicates the water deficit effect on the slopes of the correlations and, if the slopes are not different, between the elevations of the correlations.

Soil water deficit consistently affected the allometric relationships between roots and shoot, within the shoot but not within the root

We aimed at evaluating how water deficit affected the allometric relationships and if this effect was similar on the different sets of genotypes. Standardized major axis (SMA) regressions were performed on log transformed data (see a recent example of the use of SMA for allometric analysis in Liu *et al*, 2010). This technique is particularly useful to take into account ontogenic effects that are not seen when ratio are simply compared between two situations. Strong and significant correlations were detected between Shoot dry Weight and Root dry Weight (Fig. 3A), as well as between Shoot dry Weight and Rosette area (Fig. 3B) in both RILs and accessions, with an average Pearson coefficient of 0.8. Correlations between Shoot dry weight and Total root length were weaker but significant, with Pearson coefficients around 0.6 (Fig. 3C). The correlation between Root dry weight and Total root length was significant in both water treatments (Fig. 3D). For the four sets of correlations (with one exception), the slopes were close to 1, indicating that the partitioning of biomass towards roots did not depend on plant size. Only in the case of the Shoot dry weight/Root dry weight correlation for the RIL, the slopes were higher than 1 in RIL (1.21 and 1.46 in well-watered and water-deficit respectively) indicating that in that case, the proportion of biomass allocated to roots increased with plant size.

Slopes were not significantly affected by water deficit, except for the Total root length *vs.* Shoot dry weight correlation in the accessions, for which the slope increased under water deficit conditions. Thus, in all other cases, test for differences in elevation were performed, and a strong effect of water deficit on these elevations was observed. Consistent with the mean variations observed (Fig. 2), water deficit modified the equilibrium between root and shoot growth, favoring the root system, both in terms of dry weight and length (Fig. 3). Indeed, the elevations of the correlations between Shoot dry weight and Root dry weight and Shoot dry weight / Total root length increased under water deficit conditions, whereas it decreased for the Rosette area / Shoot dry weight correlation. Water deficit also modified the allocation patterns within the shoot, by changing the relationship between Rosette area and Shoot dry Weight illustrated by a decreased elevation under water deficit conditions. Interestingly the vertical shift of the correlation was significantly different between both genetic sources with a steeper reduction in the accessions than in the RILs. By contrast, water deficit had no effect on the Specific root length since the root length vs root biomass correlations remained unaffected both in slope and elevation. Noteworthy, as observed on Fig. 2, the variability of biomass and dimension variables under water deficit was larger for accessions than for RIL (visible in Fig. 3B).

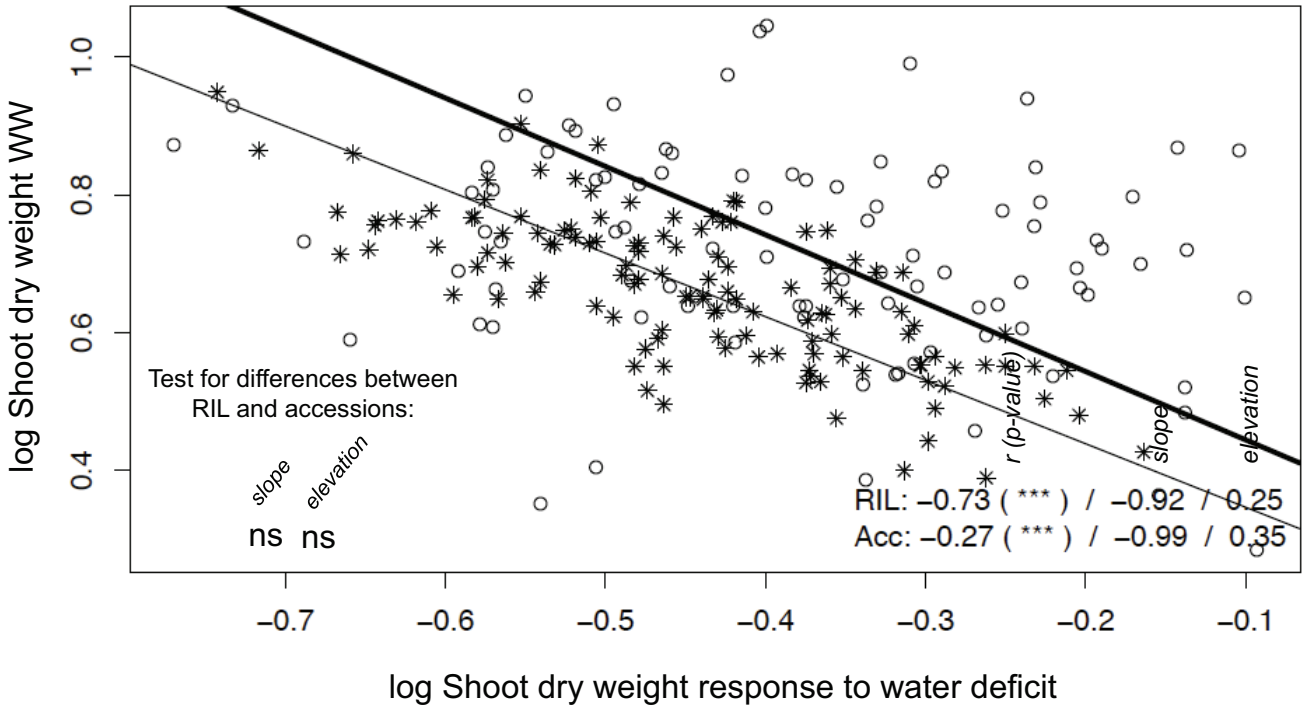


Fig. 4. Standardized major axis regressions (SMA) of the Shoot dry weight response to water deficit (*ie*, ratio of Shoot dry weight under water deficit conditions over Shoot dry weight under well-watered conditions) and the Shoot dry weight under well-watered conditions in both sets of genotypes (asterix: RIL, circles: accessions). The thin line corresponds to regression lines for the RIL population, and the thick line to regression line for the collection of accessions. Values are log transformed. The bottom-right legend indicates for both sets of genotypes (RIL and Acc): (From the left to the right) the Pearson´s coefficient (r) , the p-value associated (***:p-value<0.001; ns:non significant), and the slope and elevation of the correlation. The bottom-left legend indicates the effect of the set of genotypes on slope and elevation of the correlation.

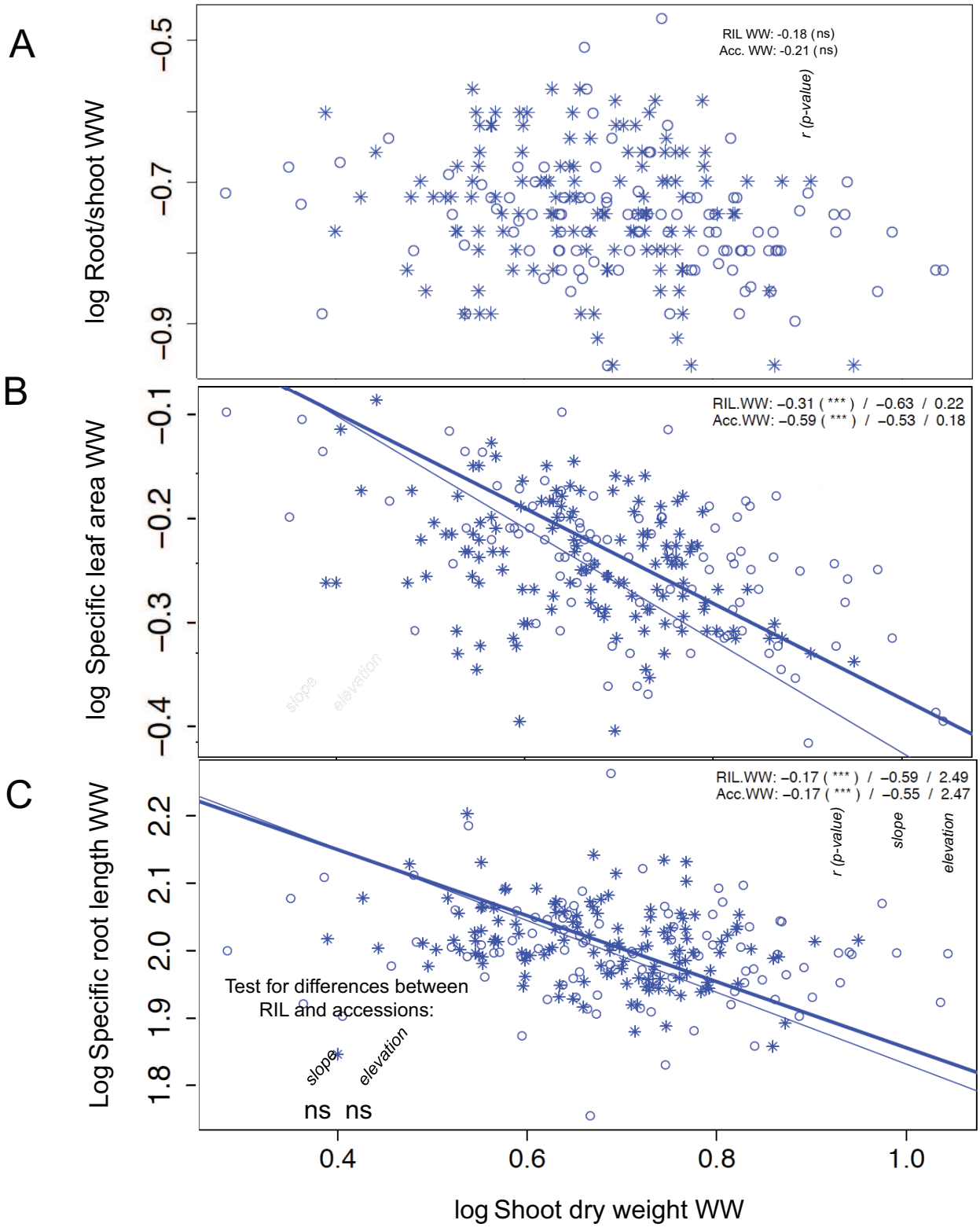


Fig. 5. Standardized major axis regressions (SMA) of the Shoot dry weight and the Root/Shoot (**A**), the Specific leaf area (**B**) or the Specific root length (**C**) in both sets of genotypes (asterix: RIL, circles: Accessions) in "well-watered" (WW) conditions. When the correlation is significant, thin lines correspond to regression lines for the RIL population, and thick lines to regression lines for the collection of accessions. Values are log transformed. The top-right legend indicates for both sets of genotypes (RIL and accessions): (From the left to the right) the Pearson´s coefficient (r) , the p-value associated (*:p-value<0.05; ***:p-value<0.001; ns: non significant), the slope and elevation of the correlation. The bottom-left legend indicates the effect of the set of genotypes on slope and elevation of the correlation.

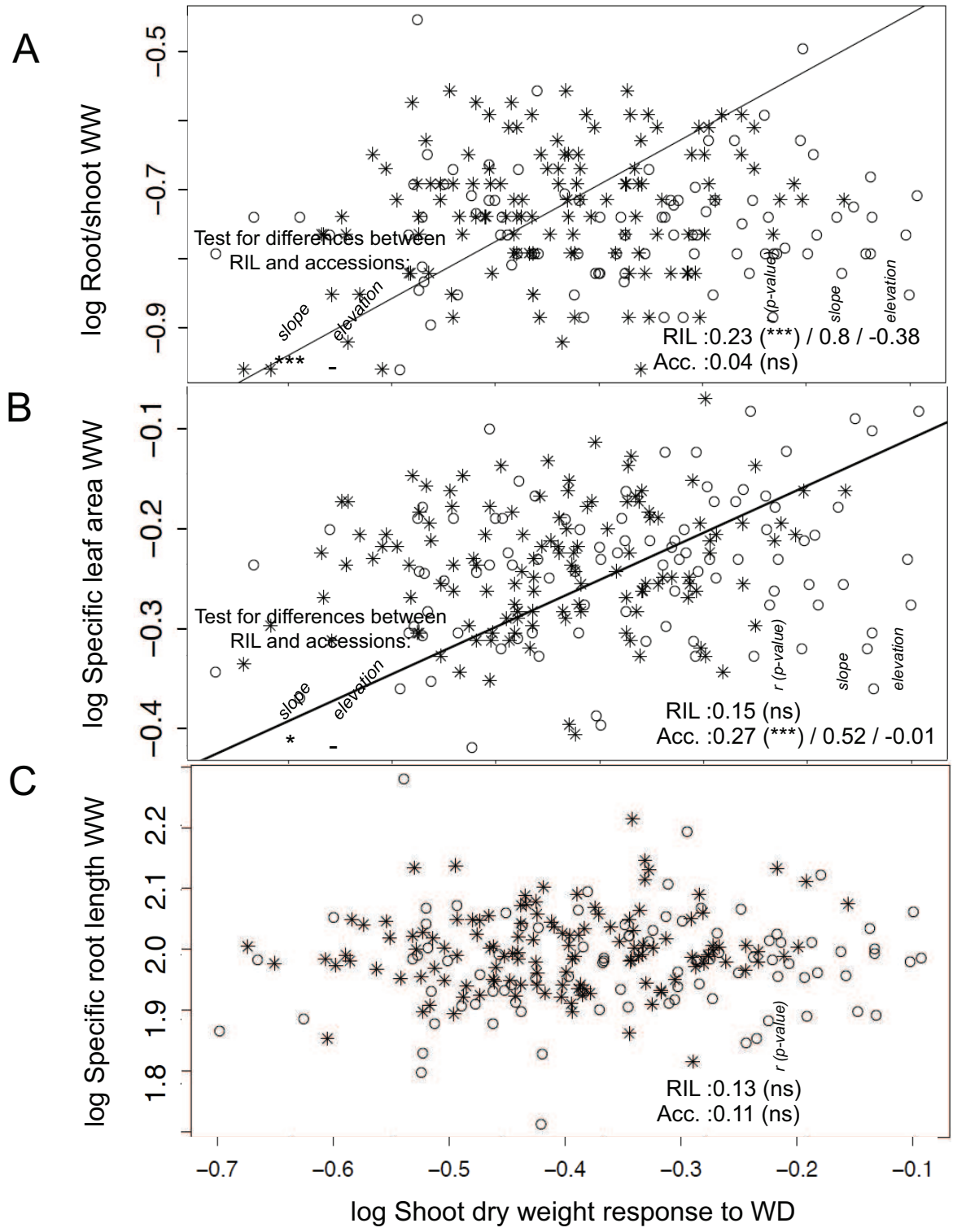


Fig. 6. Standardized major axis regressions (SMA) of the Shoot dry weight response to water deficit (*ie*, ratio of Shoot dry weight under water deficit conditions over Shoot dry weight under well-watered conditions) and the Root/shoot (**A**), the Specific leaf area (**B**) or the Specific root length **(C)** in "well-watered" (WW) conditions, in both sets of genotypes (asterix: RIL, circles: accessions). When correlations are significant, thin lines correspond to regression lines for the RIL population, and thick line to regression lines for the accessions. Values are log transformed. The bottom-right legend indicates for both sets of genotypes (RIL and accessions): (From the left to the right) the Pearson´s coefficient (r) , the p-value associated (*:p-value<0.05; ***:p-value<0.001; ns: non significant), the slope and elevation of the correlation. The bottom-left legend indicates the effect of the set of genotypes on slope and elevation of the correlation (When slopes are significantly different, differences for elevations are not tested (-)).

The maintenance of Shoot dry weight under water deficit was negatively correlated with the Shoot dry weight under well-watered conditions

A highly significant negative correlation was observed between the Shoot dry weight response to water deficit and the Shoot dry weight value in well-watered conditions (Fig. 4). As this ratio is log transformed, a value close to 0 indicates that the genotype maintains its Shoot dry weight under water deficit conditions. Correlation coefficients were high for the RIL population (-0.73) and lower but still significant for the accessions (-0.27) suggesting a size effect on the tolerance to soil water deficit. The genotypes with smallest biomass values under well-watered conditions were less affected by water deficit. The slopes and the elevations of this correlation were not significantly different between the two sets of genotypes.

In order to evaluate the link between rosette biomass and biomass partitioning variables, the relationships between the three biomass partitioning variables (Root/shoot ratio, Specific leaf area, Specific root length) under well-watered conditions and the Shoot dry weight under well-watered conditions were drawn (Fig. 5). The correlation between Root/shoot ratio and Shoot dry weight was not significant for both sets of genotypes (Fig. 5A). The correlations between Shoot dry weight and Specific root length was significant but very weak (r = -0.17) for RIL and for accessions (Fig. 5C). By contrast, an important (accounting for 10 to 35% of the variance) negative correlation between Specific leaf area and Shoot dry weight was detected (Fig. 5B). Slopes and elevations were not significantly different for both sets of genotypes. These results suggest that larger genotypes have a slight tendency to develop denser leaves and roots.

Root/shoot *and Specific leaf area under well-watered conditions were slightly associated to growth maintenance of the* Shoot dry *weight under water deficit conditions*

We aimed at finding potential variables whose value under well-watered conditions could be related with plant growth maintenance under water deficit. A negative correlation between plant size under well-watered conditions and growth maintenance in water deficit was previously observed (Fig. 4). We therefore examined the correlations between partitioning variables (Root/shoot ratio, Specific leaf area, Specific root length) and Shoot dry weight response to water deficit in both RIL and accessions (Fig. 6). Correlations were either non-significant

Tab.1. Multiple regression linear models using biomass partitioning variables (Root/shoot ratio, Specific leaf area, Specific Root Length) in well-watered conditions to explain the Shoot dry weight response to water deficit. Values were log transformed. For accessions and RIL, the coefficients in the model and the p-value associated is indicated when significantly associated to Shoot dry weight response to water deficit associated (*:p-value<0.05; **:p-value<0.01). The percentage of variance explained by the global model is indicated.

	log Root/shoot $_{ww}$	log Specific leaf area $_{ww}$	log Specific root length $_{ww}$	% variance explained by the model
Accessions		+0.48(**)		0.14
RIL	+0.35(**)		+0.39(*)	0.21

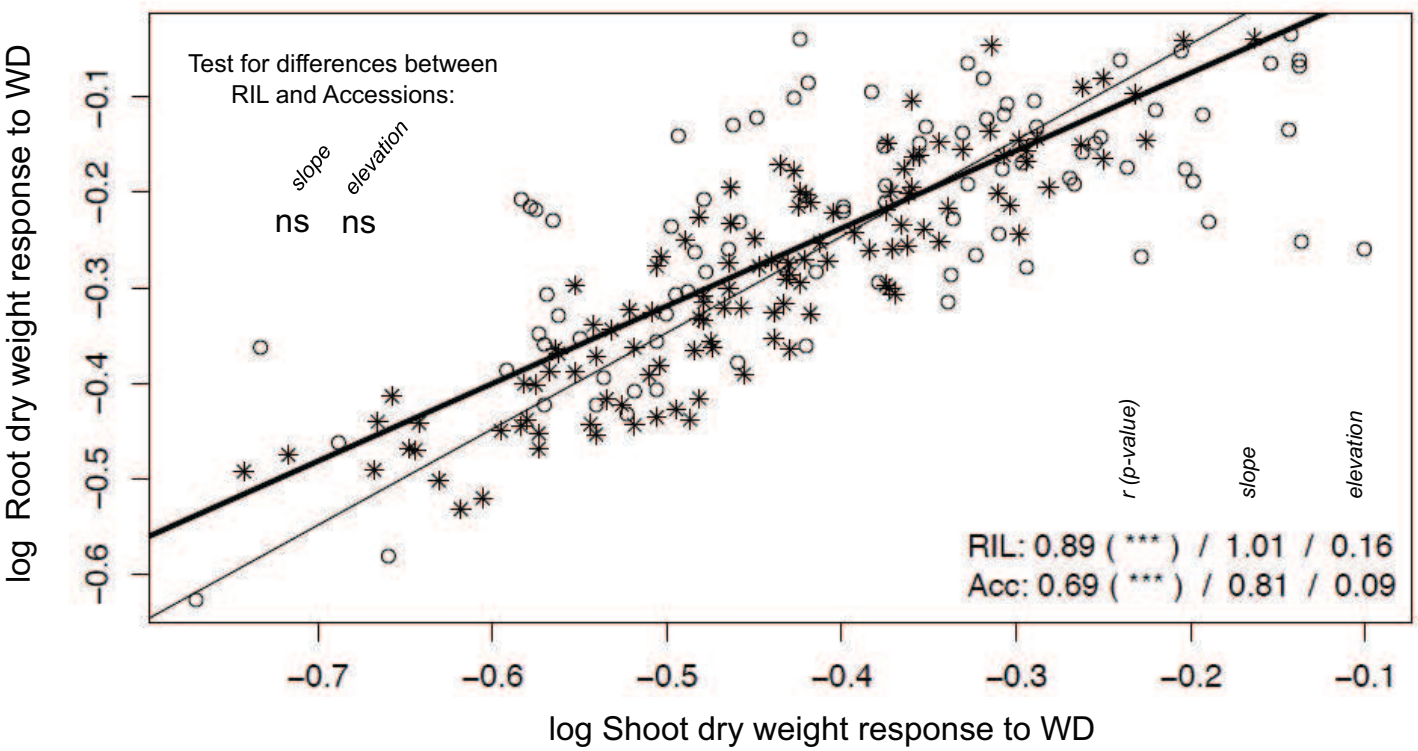


Fig. 7. Standardized major axis regressions (SMA) of the correlation between Shoot dry weight response to water deficit and Root dry weight response to water deficit (*ie*, ratio of the variable value under water deficit conditions over variable value under well-watered conditions) in both sets of genotypes (asterix for RIL, circles for accessions). The thin lines correspond to regression lines for the RIL population, and the thick line to regression lines for the collection of accessions. Values are log transformed. The bottom-right legend indicates for both sets of genotypes (RIL and accessions): (From the left to the right) the Pearson´s coefficient (r) , the p-value associated (***:p-value<0.001;ns:non significant), the slope and elevation of the correlation. The top-left legend indicates the effect of set of genotypes on slope and elevation of the correlation.

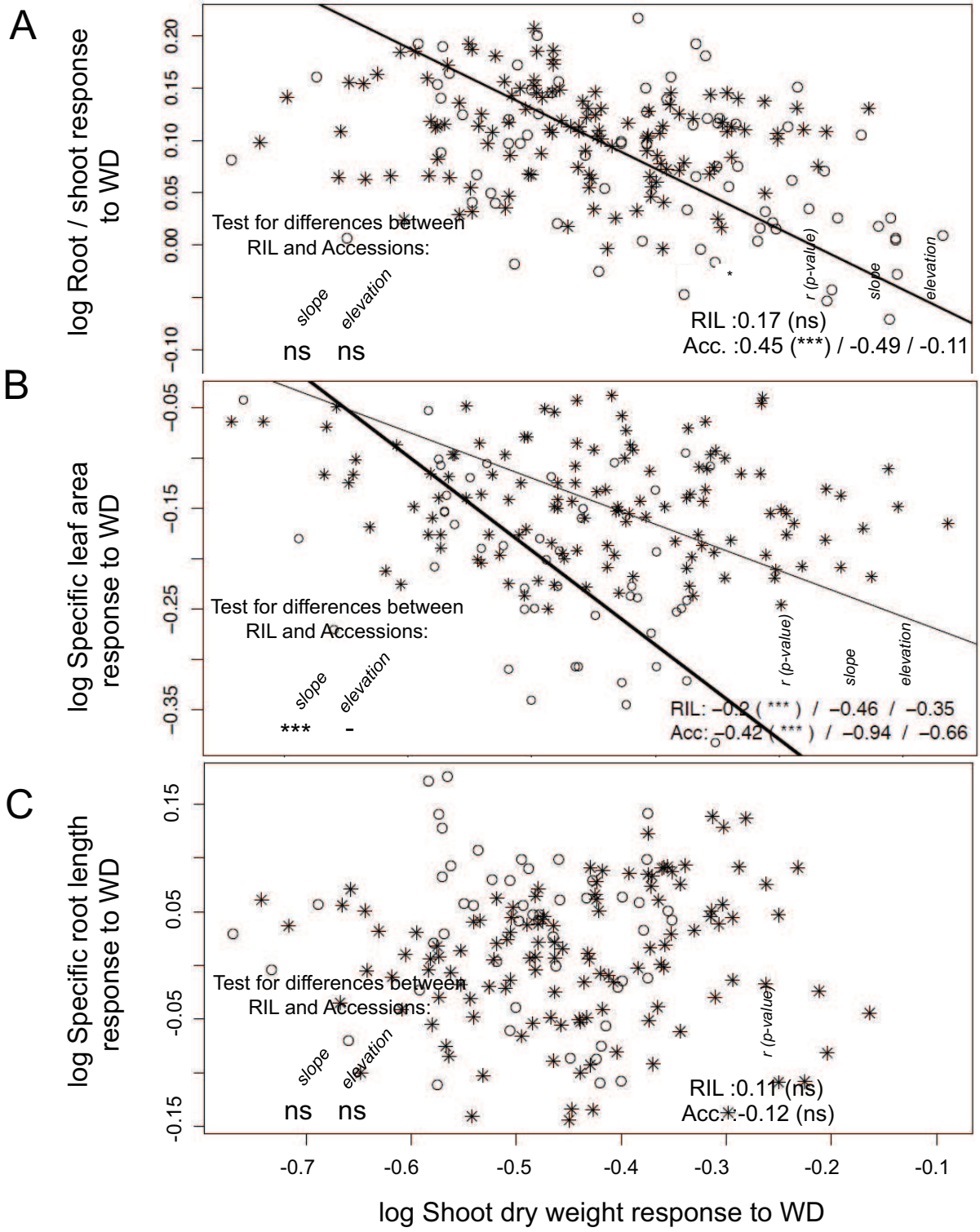


Fig. 8. Standardized major axis regressions (SMA) of correlations between the Shoot dry weight response and the response of Root/shoot (**A**), Specific leaf area (**B**), and Specific root length (**C**) to water deficit (*ie*, ratio of the variable value under water deficit conditions over variable value under well-watered conditions), in both sets of genotypes (asterix for RIL, circles for accessions). The thin lines correspond to regression lines for the RIL population, and the thick line to regression lines for the collection of accessions. Values were log transformed.
The bottom-right legend indicates for both sets of genotypes (RIL and accessions): (From the left to the right) the Pearson´s coefficient (r) , the p-value associated (*:p-value<0.05; **:p-value<0.01; ***:p-value<0.001; ns: Non significant), the slope and elevation of the correlations. The bottom-left legend indicates the effect of set of genotypes on slopes and elevations of the correlations.

or very moderate in all cases. The only significant correlation involved the Root/shoot ratio for the RIL (Fig. 6A) and the Specific leaf area for the accessions (Fig. 6B). In both cases, the correlation accounted for less than 10% of total variance. The Specific root length was not significantly correlated with the maintenance of the Shoot dry weight in water deficit conditions, whatever the set of genotypes (Fig. 6C).

In order to further explore the possibility that response of Shoot dry weight to water deficit would be explained by biomass partitioning variables and since they all were only weakly correlated to plant size, multiple regression models using those variables were tested (Tab. 1). This analysis confirmed that Specific leaf area was the only factor positively associated to Shoot dry weight response to water deficit in the collection of accessions, explaining 14% of the variance of Shoot dry weight response to water deficit. In the RIL population, the Root / shoot was positively involved in Shoot dry weight response, as well as the Specific root length. These two factors explained together 21% of the variance.

Growth maintenance under water deficit was positively correlated to the response of the other growth variables, and negatively correlated with the response of biomass partitioning variables

We then investigated the correlation between both the response of growth and biomass partitioning variables and the Shoot dry weight response. The correlation coefficient between the Root dry weight response and the Shoot dry weight response to water deficit was very high, 0.89 and 0.69 for the RIL and the accessions respectively (Fig. 7). The slopes and elevations of the correlations were not significantly different. The same trend was observed for the correlations between the response of other growth variables (Rosette area, Total root length, Primary root length) and the Shoot dry weight response (not shown).

Significant correlations were also detected between Shoot dry weight response and response of biomass partitioning variables (Fig. 8). In particular, strong significant correlations were detected for both RIL and accessions with the response of the Specific leaf area though with a lower slope for the accessions than for the RIL (Fig. 8B). Root / shoot response was only correlated with Shoot dry weight response in the accessions (Fig. 8A). By contrast, no significant correlation was detected with Specific root length response (Fig. 8C).

Tab.2. Multiple regression linear models using the response of biomass partitioning variables (Root/shoot ratio, Specific leaf area, Specific root length) to water deficit to explain the Shoot dry weight response to water deficit. Values were log transformed. For accessions and RIL, the coefficients in the model and the p-value associated is indicated when significantly associated to Shoot dry weight response to water deficit associated (*:p-value<0.05; **:p-value<0.01; ***:p-value<0.001). The percentage of variance explained by the global model is indicated.

	log Root/shoot response to WD	log Specific leaf area response to WD	log Specific root length response to WD	% variance explained by the model
Accessions	-1.04(**)	-0.82(***)		0.22
RIL	-0.71(**)	-0.44(**)		0.28

An attempt was made to correlate the Shoot dry weight response to a combination of responses of the biomass partitioning variables (Tab. 2). Both the Specific leaf area and the Root/shoot ratio showed highly significant parameters in a linear model accounting for shoot biomass response to water deficit. The coefficients of the regression had higher values in the case of the accessions but the percentage of variance explained was slightly higher in the case of the RIL. By contrast, Specific root length response was not involved in any of the models.

Discussion

In this study, plant response to a severe soil water deficit was investigated in two different sets of genotypes, a RIL population derived from two parents and thus having only limited genetic diversity, and a collection of accessions that represented much of the world-wide genetic diversity present in the species. The purpose of this combined analysis was to identify common responses of the two collections with regard to response to soil water deficit, focusing on organ biomass and dimension. We also aimed at identifying possible differences between a set of naturally selected genotypes originating from very contrasted areas of the northern hemisphere and a set of recombinant inbred lines that by construction represent artificial combinations of alleles that may not be present in natural conditions because some specific combinations might be maladaptive in the wild. This analysis was conducted by evaluating coordination between variables through correlations analysis. Another purpose was to evaluate to what extent the biomass partitioning variables or their plasticity could be related to plant tolerance to soil water deficit.

Water deficit differentially affects biomass allocation to the organs

The first objective was to characterize the effect of a severe soil water deficit on plant growth. As expected, the prolonged (10 days) exposure to soil water deficit led to a strong decrease of growth. Except the Primary root length, which was on average unaltered by water deficit, all the shoot and root growth variables were affected. In particular, the rosette area was strongly decreased by water deficit. This response related to the sensitivity of leaf expansion to drought is widely reported in the literature (reviewed by Granier and Tardieu 2010) and is thought to contribute to water saving by reducing transpiring surfaces. Root variables were less affected and this

contributed to a strong increase of the Root/shoot ratio. While several studies have reported an increase of the Root/shoot ratio in response to soil drying (Kalapos *et al.,* 1996 on wheat; Mingo *et al.,* 2004 on tomato, Padilla *et al.,* 2009 on different shrubs species), a significant number of other studies have reported a lack of effect or even a decrease (Rice *et al.,* 1979 on sorghum; Asch *et al.,* 2004 on rice). These differences could be due to intrinsic factors such as species, ecological origin (drought-prone environments, or more wet climates), but could also be explained by the design of the experiment itself. For example, Erice and coworkers (Erice *et al.,* 2010) reported that the strength of the water deficit influenced the effect on the Root/shoot. In experiments similar to ours (Hummel *et al.,* 2010), an increase of the Root/shoot ratio was already visible seven days after exposure to a moderate water deficit suggesting this effect is robust across temporal and intensity scales. By contrast, a significant proportion of the accessions evaluated (16/100) did not show any increase of this ratio suggesting the occurrence of a large genetic variation for this trait.

As severe water deficit had a stronger effect on Rosette area than on Shoot dry weight, a decrease of Specific leaf area was observed. This response has been reported in several studies, on various species (Cabuslay *et al.,* 2002 on rice; Liu and Stützel 2004 on amaranth; Chenu *et al.,* 2008 on maize). In our study, all the accessions and the RILs showed decreased Specific leaf area in response to soil water deficit and this response was also observed for shorter or milder stresses (see Hummel *et al.,* 2011). Variations of Specific leaf area could correspond to a mean to maintain a tight balance between transpiration and biomass production. Indeed, Specific leaf area is negatively correlated with water-use efficiency (Wright *et al.,* 1994, Craufurd *et al.,* 1999). Then, a reduction of Specific leaf area could then be associated with water-use efficiency improvement. This point would deserve further consideration by actually measuring transpiration in these lines.

Finally, the Specific root length (Total root length/ Root dry weight) was on average slightly increased under water deficit conditions but the SMA analysis showed that this trend was due to ontogenic effects rather than direct effect. There is no consensus in the literature on the modifications of the Specific root length in response to drought (decreased, Kage *et al.,* 2004 on cauliflower; or increased, Azhiri-Sigari *et al.,* 2000 on rice). In citrus, a higher Specific root length in some cultivars was correlated to a better soil water uptake, through higher root hydraulic conductivity and higher root proliferation (Eissenstat *et al.,* 1999). Our analysis thus could tend to rule out a rolle for Specific root length as a syndrome of different strategies of plants facing drought conditions.

Our analysis also suggests that modifications of Root/shoot and Specific leaf area significantly contribute to tolerance to water deficit when this is defined as the maintenance of Shoot dry weight under water deficit

conditions. More precisely, a higher tolerance was achieved through the combination of a poorly increased Root/shoot ratio, and a strongly decreased Specific leaf area (Fig. 8 and Tab. 2). Increasing the soil exploration by increasing the Root/shoot ratio may not be an advantage in our conditions in which pot size is limited. This could also be the case in the wild in the frequent situations of limited soil volumes. The strong involvement of the Specific leaf Area (high Specific leaf area in optimal condition and being strongly reduced under water deficit) in tolerance could illustrate that tolerant genotypes maintain a good trade-off between the necessity to avoid water loss by reducing leaf expansion (and thus stomatal density), and the maintenance of leaf area to achieve a sufficient level of photosynthesis. This result is in line with recent ones in other species but on much narrower genetic range, showing that Specific leaf area variation (Erice *et al.,* 2009; Tezara *et al.,* 2011) is positively related to drought tolerance. Interestingly, low Specific leaf area in optimal (thick, dense leaves) has in the past been rather associated with higher drought tolerance (Zhang *et al.,* 1997; Marron *et al.,* 2003). In our case, we observe the opposite on a much broader genetic panel. A possible interpretation of our results is that the accessions with a high Specific leaf area in optimal condition have a better ability to reduce Specific leaf area in water condition than the accession displaying low Specific leaf area in optimal condition.

Global allocation patterns are conserved in both sets of genotypes, but accessions displayed a larger plasticity than RIL

Strong correlations were found between the shoot and root growth variables, both under well-watered and water deficit conditions (Fig. 3). One of the main conclusions of this correlation analysis was the remarkable constancy of the allometric relationships between the variables in both sets of genotypes studied (RIL and accessions). The allometric coefficients (slopes) were not differentially affected by water deficit in both RIL and accessions. Contrary to that, the modification of elevations illustrated the effect of water deficit conditions on the biomass partitioning variables (Root/shoot, Specific leaf area) previously observed.

Water deficit induced different response range in both sets of genotypes. Especially, the modification of the relationship between Shoot dry weight and Rosette was lower for RILs than for accessions whereas the scatterplots of these two sets of genotypes were almost superimposed under well-watered conditions (also shown in the boxplots), the RIL seemed to less reduce their Rosette area for the same Shoot dry weight (*ie* their Specific leaf area) in water deficit conditions than the accessions. Moreover, the range of variability in the response of Specific leaf area to water deficit was larger in the collection of accessions. Contrary to RIL, in which

the response is very similar between genotypes, the different accessions displayed a large range of responses to water deficit conditions, with accessions that were able to maintain a high shoot growth under drought, and other accessions that were considerably affected. This difference could be explained by the genetic architecture of these two sets of genotypes. RIL correspond at the genetic level to a mosaic of randomly associated alleles, inherited from two parental lines, whereas in the collection of accessions, genome architecture is the result of evolutionary processes that allow the accession to adapt to its local environment (Orr *et al.,* 2005), with a large diversity of alleles that are not randomly distributed. This adaptation could have favoured very specific plant behaviour, enabling it to face different types of drought into the wild (see chapter 3; Yin *et al.,* 2005). Against the diversity of drought scenarii, a one-size-fits-all strategy is unlikely to be appropriated, and evolution could have favoured differentiation of phenotypic plasticity (Cornwell and Ackerly, 2009; Erice *et al.,* 2010) that could not be observed in RIL because of the fragmentation of the genomic regions involved in this plasticity.

How does plant tolerance to drought relates to its characteristics under optimal conditions and plasticity of biomass partitioning variables?

A negative correlation between tolerance to water deficit and plant size under well-watered conditions was observed, especially in the RILs (Fig. 4). This "plant size effect" was also observed in several other datasets, including RIL population (Vile *et al.,* unpublished; Tisné *et al.,* 2010), a collection of mutants (original data from Skirycz *et al.,* 2011), another collection of accessions (original data from Bouchabke *et al.,* 2008), as well as in other species (Monclus *et al.,* 2005, in poplar; He *et al.,* 2010, in *Centaurea stoebe*). By contrast, plant size was found to have no effect on plant response to drought stress in other studies (Boogaard *et al.,* 1996, on wheat; Yue *et al.,* 2006 on rice). Boogaard *et al.,* 1996 reported that the differences in allocation patterns or size found under well-watered conditions on wheat cultivars persisted under dry conditions. One hypothesis could be that our result is due to a bias of the experimental design. Indeed, large plants display a larger transpiration per day and thus may experience a lower soil content between 2 irrigations. However, in our experiments, plants were watered twice daily and the amount of evapotranspiration was rarely higher than 3 g / pot, which corresponds to 2% relative soil water content and variation between small and large accession was never higher than 1.5 g / pot which corresponds to 1% difference in relative soil water content. Such a small variation is unlikely to induce massive differences in water potential. Moreover, in our experiments, this negative relationship was retrieved when plants were watered once or twice daily (not shown).

An explanation may reside in some characteristics associated with small plants. There was a clear trend between plant size (Shoot dry weight) and Specific leaf area (Fig. 5), whereas no or looser relationships were detected with Root/shoot. Moreover, it has been shown that tolerance is related to the capacity to decrease Specific leaf area and this feature is associated with a large Specific leaf area and a small size. The importance of the Root/shoot and the Specific leaf area was different in RIL and accessions, with a stronger effect of the Root/shoot in the RIL population, and of Specific leaf area in the collection of accessions. These two inherent characteristics of genotype are both probably important under drought. A high Root/shoot is likely to favour water uptake, while the Specific leaf area reflects the trade off between leaf expansion and biomass accumulation that can be later used if conditions become better. The differences between Root/shoot and Specific leaf area importance in RIL and accessions could be explained by a stronger selective pressure on Specific leaf area, as compared to Root/shoot. Specific leaf area is a trait whose variation could have followed the worldwide spread of the different accessions used in this study and their adaptation to local environments (Ramirez-Valiente *et al.,* 2011).

Chapter 3 / Climates at sites of origin are reflected in drought tolerance of Arabidopsis thaliana accessions

Marie Bouteillé[1], Yasushi Kobayashi[2], Denis Vile[1], Detlef Weigel[2], and Bertrand Muller[1]*

[1] Institut de Biologie Intégrative des Plantes, Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, UMR759, INRA, Montpellier, France

[2] Max Planck Institute for Developmental Biology, Tübingen, Germany

Abstract

Drought is one of the major constraints for plant growth in natural and cultivated areas and is thought to have shaped the genetic make-up of wild species. Drought tolerance encompasses various responses including dehydration avoidance (as in resurrection plants), drought escape (early flowering), dehydration avoidance (stomatal control and improved water uptake by root growth) or stress tolerance (growth maintenance). The later strategy is targeted in crop plants which are bred for production maintenance under drought but is also found in wild species since biomass production can be an important component of fitness. Understanding how climates in the wild have shape stress tolerance is therefore important to decipher the basis of adaptation.

In this study, eight populations of *Arabidopsis thaliana* collected in contrasted environments of Eurasia, from Iberian peninsula-North africa to Central Asia were grown in controlled conditions, with or without severe soil water deficit. Stress tolerance was characterized as the capacity to maintain shoot biomass under stressful conditions. Two geographic regions, Iberian peninsula-North africa and Central Asia, displayed higher levels of tolerance to drought, but also larger variability of climates at collection sites. High levels of tolerance were associated with warmer temperatures within Spanish accessions, whereas tolerance of Asian accessions was mainly associated with differences of hygrometry (air relative humidity, number of rainy days). For both regions and to a lesser extend for Caucasus, the climatic water balance (difference between the precipitations and the potential evapotranspiration) was negatively associated to plant tolerance, but the strength of this correlation varied according to seasons. The importance of seasonal climates was also illustrated by the flowering strategies of these accessions. We investigate the response to prolonged cold treatment (vernalization) and found differences between vernalization requirements of the accessions. Non vernalization-requiring accessions were generally more tolerant to drought conditions, maybe because of dryer environmental conditions that they encountered in nature at the time of year these plants complete their life cycle.

Introduction

Natural variation in ecologically important traits is considered to be strong evidence for adaptation to the environment to geographically varying factors (Mayr, 1956; Endler, 1977; Stinchcombe *et al.,* 2004; Stillwell *et al.,* 2007). Sessile organisms, such as plants, can experience considerable variation in natural selection across their range, and local adaptation to such selection can result in geographic differentiation of populations (Joshi *et al.,* 2001; Streisfeld & Kohn, 2005; Springer, 2007).

Arabidopsis thaliana grows in a wide variety of climates across its native range (Hoffmann, 2002). The analyses of the amount and patterns of genetic variation on a worlwide scale have found significant population structure in the native Eurasian rang in the different world regions (Sharbel et al., 2000; Nordborg et al., 2005; Ostrowski et *al.,* 2006; Schmid et *al.,* 2006; Beck et *al.,* 2008, Platt *et al*., 2010). In addition, several laboratories have recently initiated the development of new Arabidopsis thaliana collections to study population structure on a regional scale in regions of the native distribution area such as northern Europe (Stenøien et *al.,* 2005; Bakker et *al.,* 2006), France (Le Corre 2005), central Asia (Schmid et *al.,* 2006), and China (He et *al.,* 2007). Phenotypic variation, suggestive of adaptive differentiation in these different populations, has recently been observed in several ecologically important traits such as flowering time (Stinchcombe *et al.,* 2004; Wilczek *et al.,* 2009), response to vernalization (Hopkins *et al.,* 2008), freezing tolerance (Hannah *et al.,* 2006; Zhen & Ungerer, 2008), and size and growth rate (Li *et al.,* 1998), among others. However, the study of the natural variation in ecologically important traits in the field and its ecology in natural populations (Montesinos *et al.,* 2009; Bomblies *et al.,* 2010) is just beginning. An understanding of the process of adaptive evolution in Arabidopsis thaliana in nature requires the identification of adaptive traits and the linkage of these traits to the key environmental features that are known to exert selective pressures (Metcalf & Mitchell-Olds, 2009).

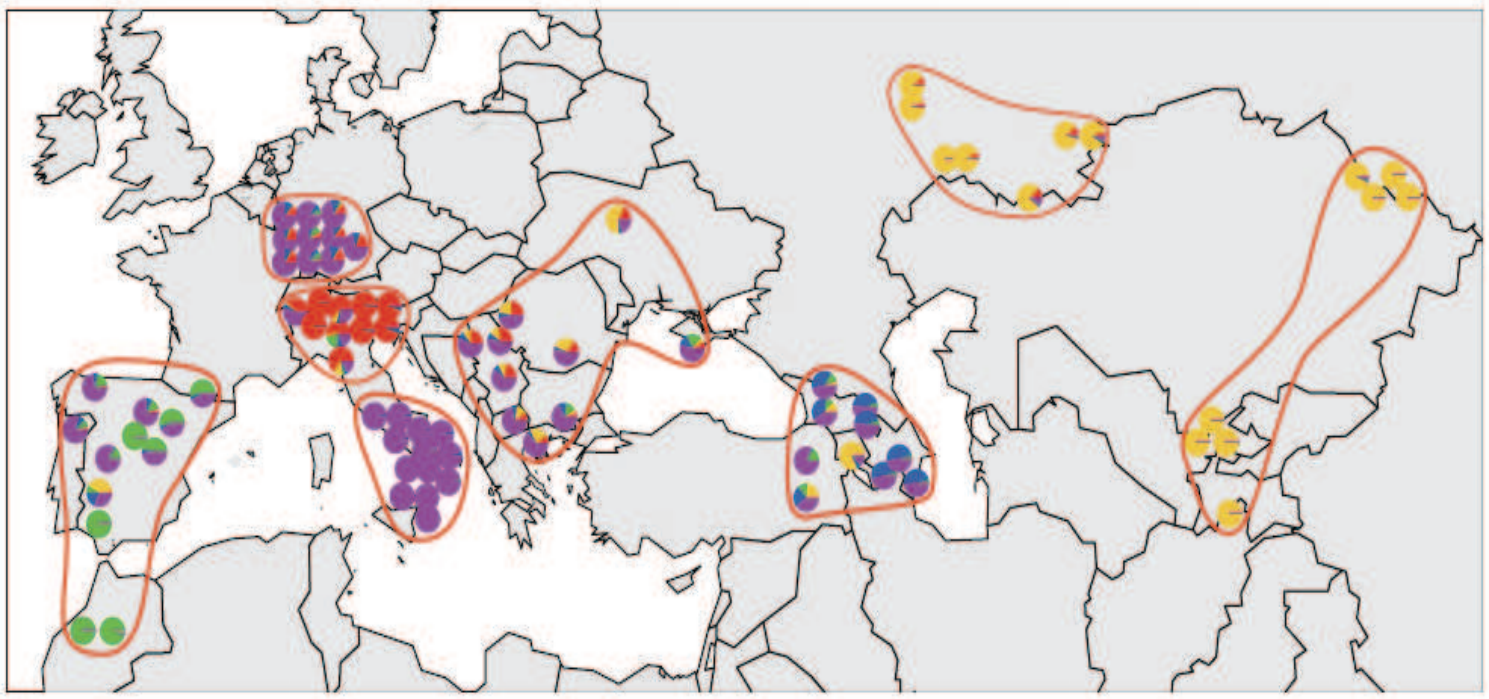
Drought is well known to affect distribution of plant species worldwide (Schimper, 1903, Cornwell and Grubb, 2003, Engelbrecht *et al.,* 2007). With climate change scenarios predicting increased aridity in many regions on the globe (Hulme *et al.,* 2005), efforts to understand the basis of plant adaptation to water deficit are essential, to give insights into potential impacts of climate change on natural variation, and to define targets to improve plant tolerance to such changes (Araus *et al.,* 2002).

Drought is the consequence of a difference between water supplies (precipitations, *plus* irrigation in agricultural

areas) and demand. The demand for water depends on evaporative demand, driven by climatic factors and stomatal control. Evaporative demand is driven by air humidity, leaf and air temperature, wind-speed and solar radiation (Monteith, 1973). At the plant level, a variety of strategies have been selected essentially falling in one of the following categories: dessication tolerance (involving survival at low water, such as in resurrection plants or in seeds), dehydration avoidance (maintenance of cell water status by stomatal control and increased soil water uptake), drought escape (= early flowering, Lempe *et al.,* 2005; Sherrard and Maherali, 2006; Franks *et al.,* 2011) and stress tolerance (= growth and production maintenance). These strategies can be favoured by a variety of mechanisms at different levels (molecule, cell, organ, plant, canopy) (Geber and Dawson 1990; Ingram and Bartels 1996; Ackerly *et al.,* 2000; Araus *et al.,* 2002; Maggio *et al.,* 2006, see Chapter 2). Dehydration tolerance is not related to other types of tolerance. Indeed, cells enter dehydration and peculiar metabolisms are engaged. Accordingly, it was recently found that mutant impaired in genes showing up in extreme dehydration are not stress tolerant (Skirycz *et al.,* 2011).

Adaptation of phenology is a typical drought escape strategy that enables plant to complete its life cycle before the onset of summer drought and display large genetic variation along environmental gradients (Amasino *et al.,* 2010, Koornneef *et al.,* 2004). Moreover, drought escape and stress avoidance (WUE) are negatively related (McKay *et al.,* 2003) suggesting the occurrence of a trade-off between both strategies. Whereas growth maintenance has been often focused on in crop plants as a major determinant of production maintenance, it has been less often studied in wild species and results suggesting the physiological basis of growth maintenance are rare (Cody and Monney, 1978; Lei *et al.,* 2006; Liu *et al.,* 2010). Recently a major QTL accounting for growth maintenance in Arabidopsis was detected from the Ler x An1 population (Tisné *et al.,* 2010).

The purpose of the present study was to explain differences in soil water deficit tolerance in natural populations of *Arabidopsis thaliana*, using climatic characteristics of their habitat of origin. Eight populations of Arabidopsis thaliana (88 accessions in total) were chosen for this study because they are widespread throughout Europa and Asia, therefore reflecting adaptations to a large range of environments. These populations are also supposed to reflect the evolutionary history of the species colonization. They were grown in a growth chamber under controlled conditions, with or without soil water deficit, and their response to water deficit was characterized. In parallel, climatic features of the natural habitats in which these accessions were collected were obtained from databases.



(Cao et al., 2011, submitted to Nature genetics)

Fig. 1. Location of the 80 accessions, in the eight geographic regions sampled (Spain, South Italy, Caucasia, South Tyrol, East. Europa, Central Asia, Tübingen, Russia). The Tübingen region is not indicated to scale. Pie charts indicate STRUCTURE results for 5 genetic clusters.

Materials and methods

Plant material and experiments

A collection of 88 accessions was used in this study. They were collected in specific regions in Eurasia that are known to have been refugees during the last glaciation (Sharbel *et al.,* 2000; Schmid *et al.,* 2006). Those accessions were also chosen as a starting point of an ambitious sequencing program of hundreds Arabidopsis accessions (www/1001genomes.org, Weigel and Mott, 2009). The genetic structure of 80 of these accessions has been analysed using the STRUCTURE program (Pritchard *et al.,* 2000), choosing 5 genetic clusters (k=5) (see Fig.1). Seeds of these collections were obtained from the Max Planck Institute for Developmental Biology (Tübingen, Germany).

Experiments and plant growth conditions

Plants were grown in four independent experiments. Experiments 1 and 2 were performed in the PHENOPSIS automated phenotyping platform (Granier *et al.,* 2006) in Montpellier, whereas experiments 3 and 4 were performed in the growth chambers, in Tübingen.

Growth experiments

During the experiments 1 and 2, the 88 accessions were grown under well-watered and water deficit conditions, respectively. All micro-meteorological conditions were kept constant during the whole growing period. Day-length was maintained at 10 h, and light was provided by HQI lamps with additional cool white fluorescent tubes. Photosynthetic photon flux density (PPFD) was measured continuously at the plant level, using a photosynthetic sensor (LI-190SB, Li-Cor, Lincoln, NE, USA) and set to 180 µmol m^{-2} s^{-1} in all cases. Air temperature and relative humidity were measured every 20 s (HMP35A Vaisala Oy, Helsinki, Finland) and set to 21-20°C (day and night) and 75% respectively. All measurements of temperature, PPFD and relative humidity were averaged and stored every 600 s in a data logger (Campbell Scientific, LTD-CR10Wiring Panel, Shepshed, Leicestershire, UK) and automatically sent to a database (http://bioweb.supagro.inra.fr/phenopsis/).

Seeds were sown in 200 mL conical pots (9 cm height and 4.5 cm diameter) filled with a mixture (1:1, v/v) of a loamy soil and organic compost. Soil water content was determined before sowing and set to 0.35 g(H_20) g(dry soil)$^{-1}$. Subsequent changes in pot weight were attributed to a change in soil water content. Soil water content was adjusted daily automatically with the automaton in the PHENOPSIS platform to two different values, 0.35 g H_2O g^{-1} dry soil corresponding to well-watered (WW), and 0.18 g H_2O g^{-1} dry soil corresponding to water deficit (WD) conditions (Granier *et al.,* 2002). These values correspond to a predawn leaf water potential of -0.3 MPa, and -1.1 MPa respectively (Granier *et al.,* 2006; Hummel *et al.,* 2010).

In all cases, germination occurred within 3-6 days after sowing. Pots were maintained at a soil water status of 0.35 g H_2O g^{-1} dry soil corresponding to well-watered conditions during the first 15 days after germination, and the soil water status was either maintained at this value in experiment 1, or reduced down to 0.18 in experiment 2. This value was reached within 3-4 days. Then soil water content was kept at this value by automatic irrigation twice a day. Each accession was grown in 3 and 5 pots respectively, randomly located in the growth chamber. The lack of block effect was later tested. After 2 weeks, 1 to 3 homogeneous plants were kept per pot, depending on plant size to avoid overlapping. Plant were harvested 25 days after germination, and six to ten plants per accession were collected and individually measured.

Flowering time experiments

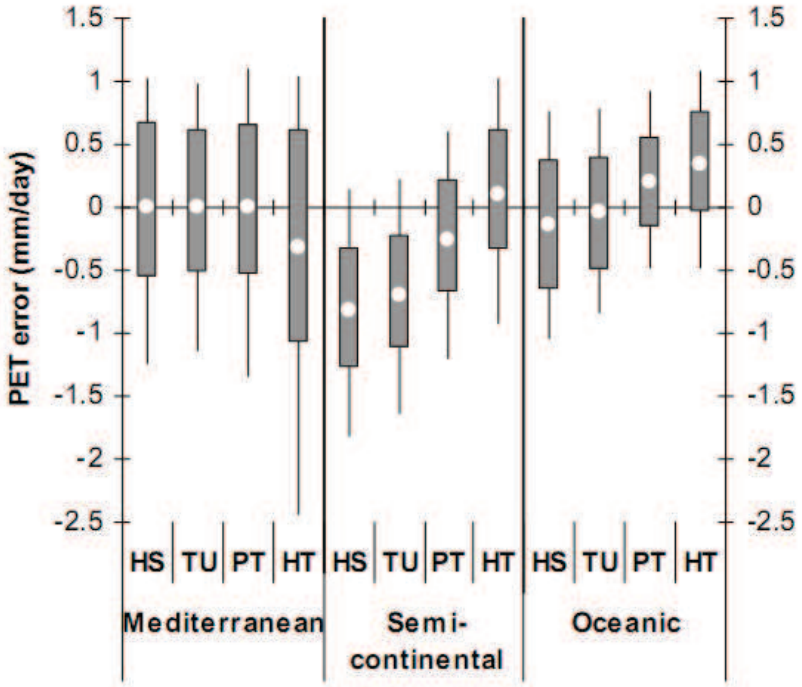
Experiments 3 and 4 were dedicated to flowering time measurements. Plants were grown in controlled growth rooms with 16 hours of light, a temperature of 23°C (± 0.1°C) under a 1:1 mixture of Cool White and Gro-Lux Wide Spectrum fluorescent lights, providing a PPFD of 150 μmol m^{-2} s^{-1}. All light bulbs were of the same age. Maximal humidity was 65%. Light, temperature, and humidity were continuously monitored online and logged data were stored in a Structured Query Language (SQL) database. Seeds were stratified at 4°C for 7 days (to minimize variation due to differences in stratification requirements) in 0.1% agarose, and then sown at the surface of a compost-like substrate. For the "long-days" experiment (expt. 3), plants were maintained in the growth room until the flowering. For the "Long days + vernalization" experiment (expt. 4), seed germination was induced at 23°C for 24 h, before the plants were transferred for 6 weeks at 4°C in a vernalization room with 8 h light of about 50 μmol m-2 s-1. After this "cold" treatment, they were transferred again in the 23°C long days room.

Plant growth measurements and statistical analysis

Calculation of the potential evapotranspiration

Details of the different formulae available in the literature to calculate the potential evapotranspiration

Name	Acronym	Input data	Formulae	Equation term
Hargreaves Temperature	***HT***	Daily minimum and maximum temperature	$HT=0.0023R_a(T+17.8)(T_\Delta)^{0.5}$	R_a=daily extra-terrestrial radiation (MJ.m^{-2}) T=daily mean temperature (°C) T_Δ=daily maximum-minimum temperature difference (°C)
Priestley-Taylor[1]	***PT***	Daily minimum and maximum temperature Solar radiation	$PT=\alpha\frac{\Delta}{\Delta+\gamma}R_n$	α=empirical coefficient[1] (=1.56) Δ=slope of saturation vapour pressure curve (kPa.°C) γ=psychometric constant (kPa.°C) R_n=net daily radiation (mm.day^{-1})
Turc Radiation[2]	***TU***	Daily mean temperature Solar radiation	$TU=\beta\frac{T}{T+15}(Rs+50)$	β= empirical coefficient[2] (=0.0142) T=daily mean temperature R_S=daily solar radiation (MJ.m^{-2})
Hargreaves Radiation	***HS***	Daily mean temperature Solar radiation	$HS=0.0135(T+17.8)R_s$	T=daily mean temperature R_S=daily solar radiation (MJ.m^{-2})



Box plots of daily deviations between empirical and Penman-Monteith models, from April to September. Lower and upper limits show the 1st and 9th deciles. Lower and upper sides of the boxes show the 1st and 4th quartiles. Central white point is the mean error (bias).

In experiments 1-2, 25 days after germination, six to ten plants of each accession were harvested. Different growth and biomass partitioning variables were measured (Rosette area, Shoot dry weight, Total and Primary root length, and Root dry weight) or calculated (Root/shoot, Specific leaf area (ratio of the Rosette area over the Shoot dry weight) and Specific root length (ratio of the Total root length over the root dry weight) following the methods fully described in the chapter 2. For all these variables, the response to water deficit was expressed as the ratio between the value in water deficit condition and the value in optimal well-watered conditions. In experiments 3-4 flowering time was scored as the number of days between the transfer into the 23°C long days room, and the opening of the first flower. All the statistical analyses (one-way ANOVA, clustering, Principle Component Analysis) were done with R (R Development Core Team, 2009). The clustering analysis was performed with the *hclust* function in R, using the Ward method of distance calculation.

Climatic variables acquisition

All the climatic variables used in this study were obtained from a database of the Climatic Research Unit (http://www.cru.uea.ac.uk/cru/data/hrg.htm). This database stores the mean monthly climatology of a 10' latitude/longitude grid of surface climate over global land areas, excluding Antarctica (for details, see New *et al.,* 2002). The climatology comprises a suite of variables collected at a daily timestep: **M**ean temperature, daily temperature range, air relative humidity, sunshine duration, ground-frost frequency, rainy-day frequency, wind speed and precipitations. The place where each accession was collected into the wild was identified by its latitude/longitude coordinates, which allowed extracting precisely climatic data from this database. The monthly data were extracted and averaged over the year or on a 3 months basis corresponding to seasons (autumn, spring, summer, winter for sept-nov, dec-feb, march-may and june-august respectively). Potential Evapo-Transpiration (PET) and Climatic water balance (Difference between precipitations and PET) was estimated using available methods used to estimate the Penman-Monteith PET when some of the variables needed are lacking (Bois *et al.,* 2005). We choose a method based on the average temperature, the daily temperature variation and total irradiance intercepted by the earth surface at each location (Hargreaves-Samani 1982, HT method in Table below). This method showed minimum error and lack of bias when tested on Mediterranean, semi-continental and oceanic climate (Figure below from Bois *et al.,* 2005). Extra-terrestrial radiation was evaluated from databases using latitude and longitudes.

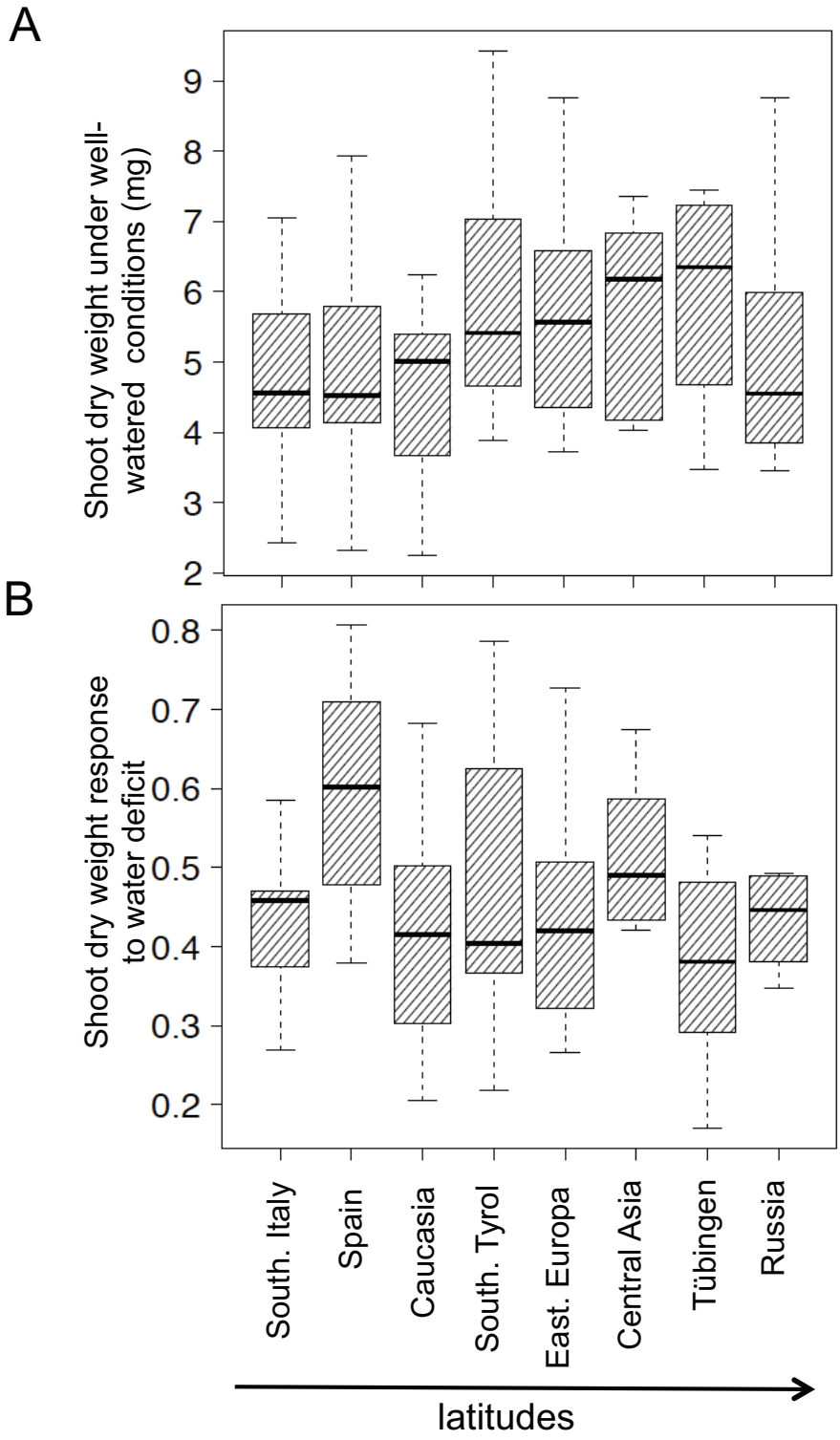


Fig. 2. Boxplots of the Shoot dry weight in well-watered conditions (**A**), and of the Shoot dry weight response to soil water deficit (*ie*, ratio of Shoot dry weight under water deficit conditions over Shoot dry weight under well-watered conditions) in the eight geographic regions. Data correspond to the mean values of all the accessions in each region. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

Sequence analysis of FRI and FLC alleles

The sequence patterns of FRIGIDA (FRI) and FLOWERING LOCUS C (FLC) alleles of all the accessions studied were analysed to determine whether they were functional or not. These multiple sequence alignments were carried out by Clustal-X version 2.1 (Thompson *et al.,* 1997). Naturally occurring loss-of-function of FRI gene can be largely explained by two major haplotypes, a Col-type 16bp deletion in the coding region causing frame-shift stop codon or Ler-type 345bp deletion in the 5' promoter region (Johansson et al., 2000). These types of sequence variations were investigated, and then any deleterious sequence variations, such as a large deletion in the coding region or stop codon caused by nonsense change or insertion/deletion based frame-shift change were evaluated. Several independent large insertions, disrupting FLC function in the FLC 1st intron that have been reported in the literature (Liu et al., 2004; Lempe et al., 2005) were not detected in this analysis.

Results

Variability of plant size and responses to soil water deficit

Variability of Shoot dry weight in well-watered conditions was very high among the accessions (Fig. 2A), ranging from 2.2 to 10.9 mg of Shoot dry weight for an accession (DOG-4) of Caucasia and an accession (ICE-79) originating from south Tyrol respectively. Within each geographic region, accessions showed similar variation in Shoot dry weight by 5 mg on average from the smallest to the largest accession. There was a slight general but non-significant trend towards an increased median shoot dry weight with higher latitude, except for the Russian accessions. The accessions originating from South Italy and Iberian peninsula-North africa, at low latitudes, had on average a lower weight (4.5 mg) than the accessions originating from Caucasia, South Tyrol, Eastern Europa, or Central Asia (5 to 6.5 mg). Plant tolerance to soil water deficit was estimated from the ratio of shoot dry weight under water deficit *vs.* control conditions (Fig. 2B). Individual tolerance ranged from 0.2 (*ie* a 80% decrease in Shoot dry weight) for some Tübingen accessions to 0.8 (*ie* a 20% decrease in Shoot dry weight) for some Spanish accessions. On average, Spanish and Asian accessions displayed the highest level of tolerance,

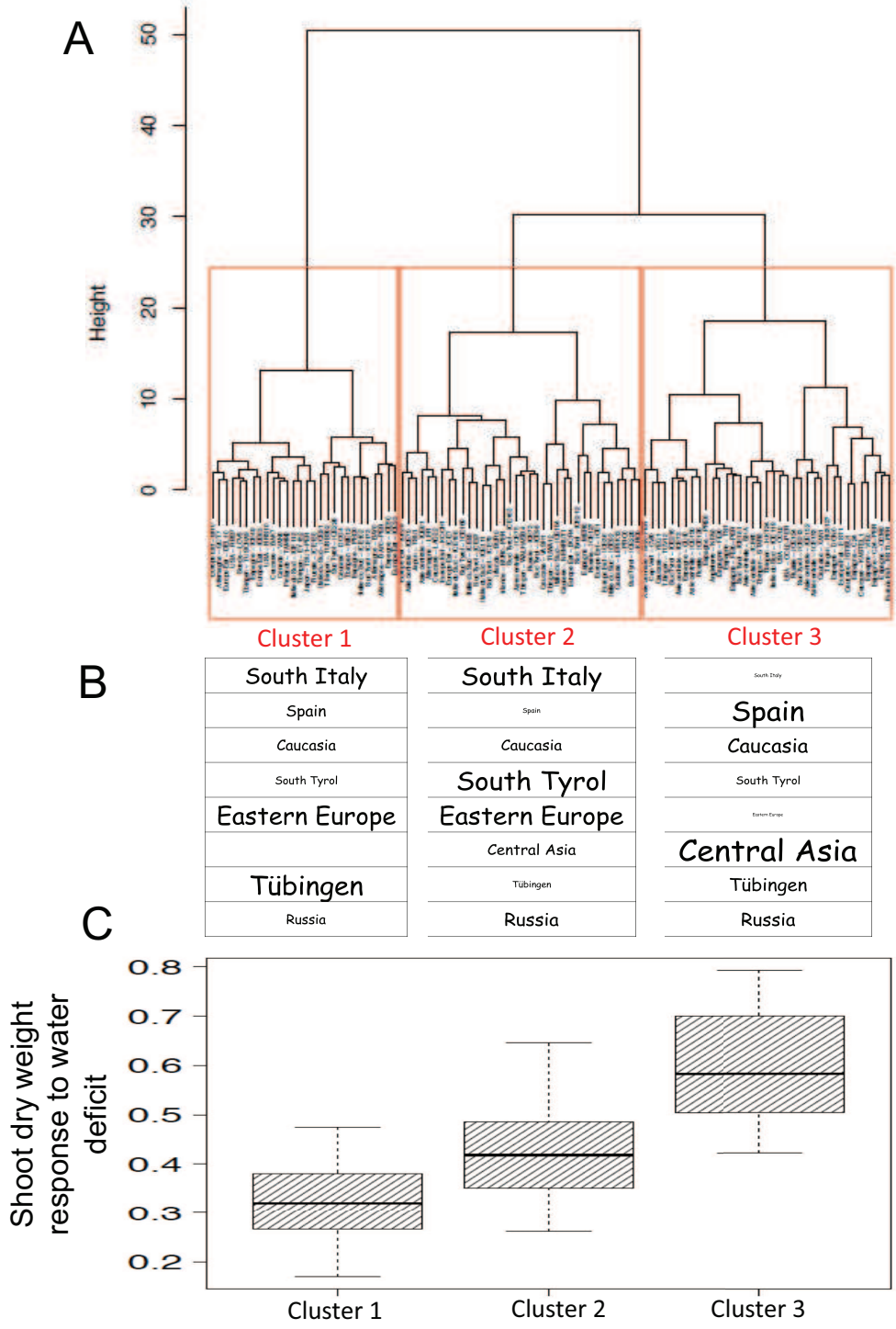


Fig. 3. Clustering of the accessions based on the different growth variables responses to water deficit (Shoot dry weight, Rosette area, Root dry weight, Total root length, Primary root length, Root/Shoot ratio, Specific leaf area, Specific root length). The response to water deficit is defined for each variable as the ratio of the value under water deficit conditions over the value under well-watered conditions. **A.** Clustering diagram. **B**. Schematic representation of the importance of each geographic region in each clusters. **C.** Shoot dry weight response of the accessions of the three different clusters.

with a shoot dry weight response of 0.62 and 0.50 respectively. In the other six regions, the response ranged from 0.39 (Tübingen) to 0.46 (South Italy).

Region-specific differences in tolerance to water deficit

In order to confirm the unequal proportion of geographical origins within tolerant and sensitive lines, we performed a clustering analysis based on the responses to water deficit (ratio of the value under water deficit conditions over the value under well-watered conditions) of growth variables (Shoot dry weight, Rosette area, Root dry weight, Total root length, Primary root length, Root/shoot ratio, Specific leaf area, Specific root length) (Fig. 3). Three main clusters could be defined (Fig. 3A), and the accessions of the different geographic regions were not equally partitioned in these three clusters (Fig. 3B). Whereas accessions from some regions such as Russia or Caucasia were equally partitioned in the three clusters, accessions from other regions were mainly represented in only one or two clusters. This is observed for the accessions from South Italy, Eastern Europa (mainly in clusters 1 and 2), or Tübingen (mainly in clusters 1 and 3). To a higher extent, accessions originating from Iberian peninsula-North africa and central Asia were almost only represented in the cluster 3. As expected, the accessions belonging to three clusters displayed very different values of Shoot dry weight response to water deficit (Fig. 3C), ranging from 0.32 for the accessions of cluster 1, 0.44 for cluster 2, and 0.58 for cluster 3.

Absence of clear correlation between local climate and tolerance to water deficit

As differences between tolerances of the accessions originating from different geographic regions were observed, we hypothesized that these differences could be partially related to climatic features of the different regions. We first characterized the climate encountered by the accessions in their region of origin with different annual climatic variables (see Material and methods for a complete description of these variables). In order to provide a broad view on the climate of the geographic regions synthetically, we performed a PCA on their annual climate (Fig. 4A). The first two axis of the PCA captured most of the inertia of the data, with 34 and 30% of variance explained for principle component 1 and principle component 2 respectively. The first two principal components successfully separated hygrometric variables (number of rainy days and air relative humidity), day-

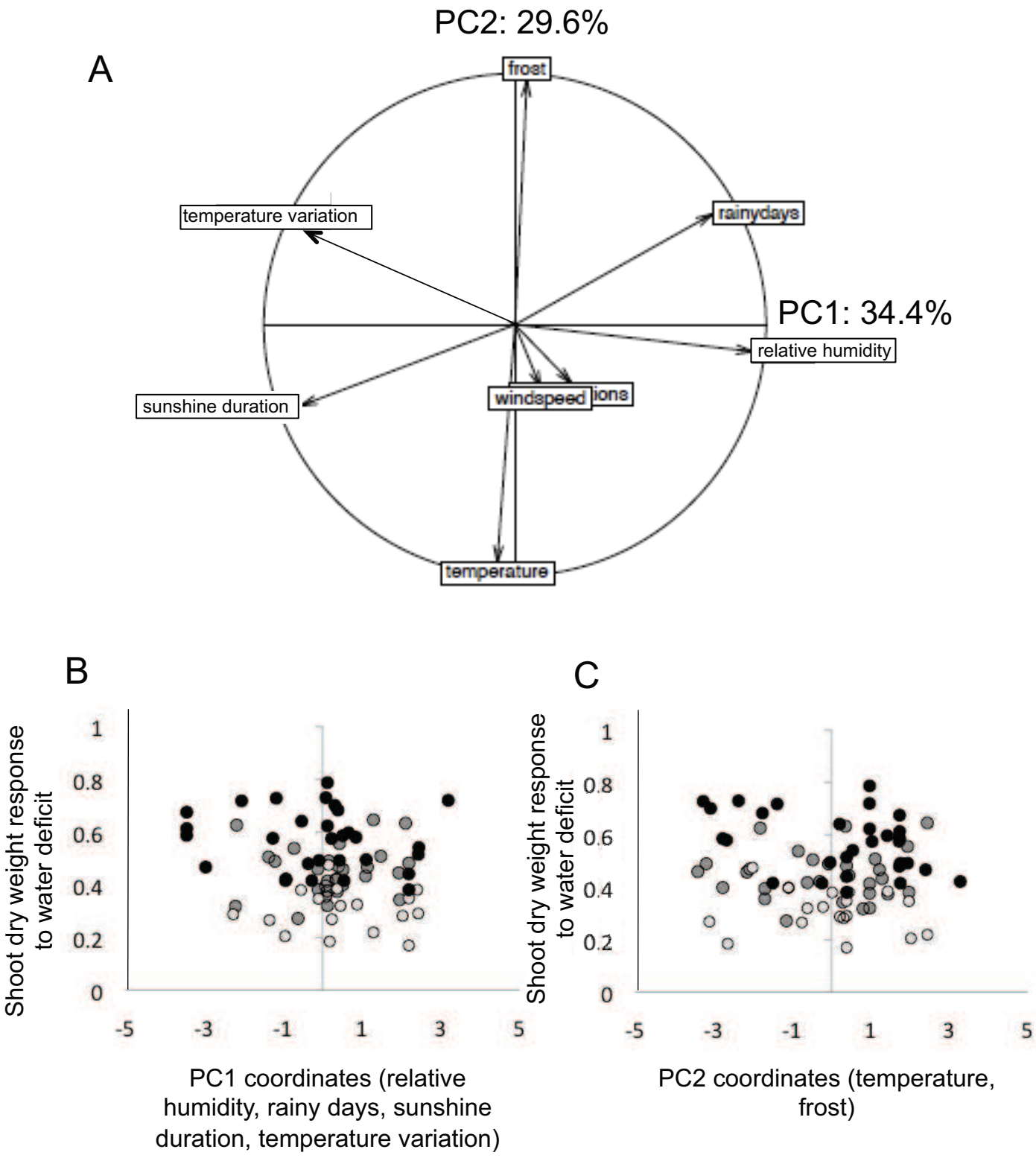


Fig. 4. A. Principal components **1** and **2** of a Principal component analysis performed with nine annual climatic variables describing the original habitat of the 88 accessions: **Frost** (number of days with negative temperature per month averaged on the year), **Precipitations** (mm.month^{-1}), air **Relative humidity**, **Rainy days** (number of days with precipitations>0.5mm per month, averaged on the year), **Sunshine duration** (% of day-length with full light), daily mean air **temperature** (°C), **temperature variation** (difference between daily minimal and maximal temperature, °C), **windspeed** (m.s^{-1}). **B,C.** Correlations between the Shoot dry weight response to water deficit (*ie*, ratio of Shoot dry weight under water deficit conditions over Shoot dry weight under well-watered conditions) for the accessions in the different clusters (white for cluster 1, grey for cluster 2, and black symbols for cluster 3), and the coordinates of the climatic values of the habitat of these accessions on principal components 1 (**B**) and 2 (**C**).

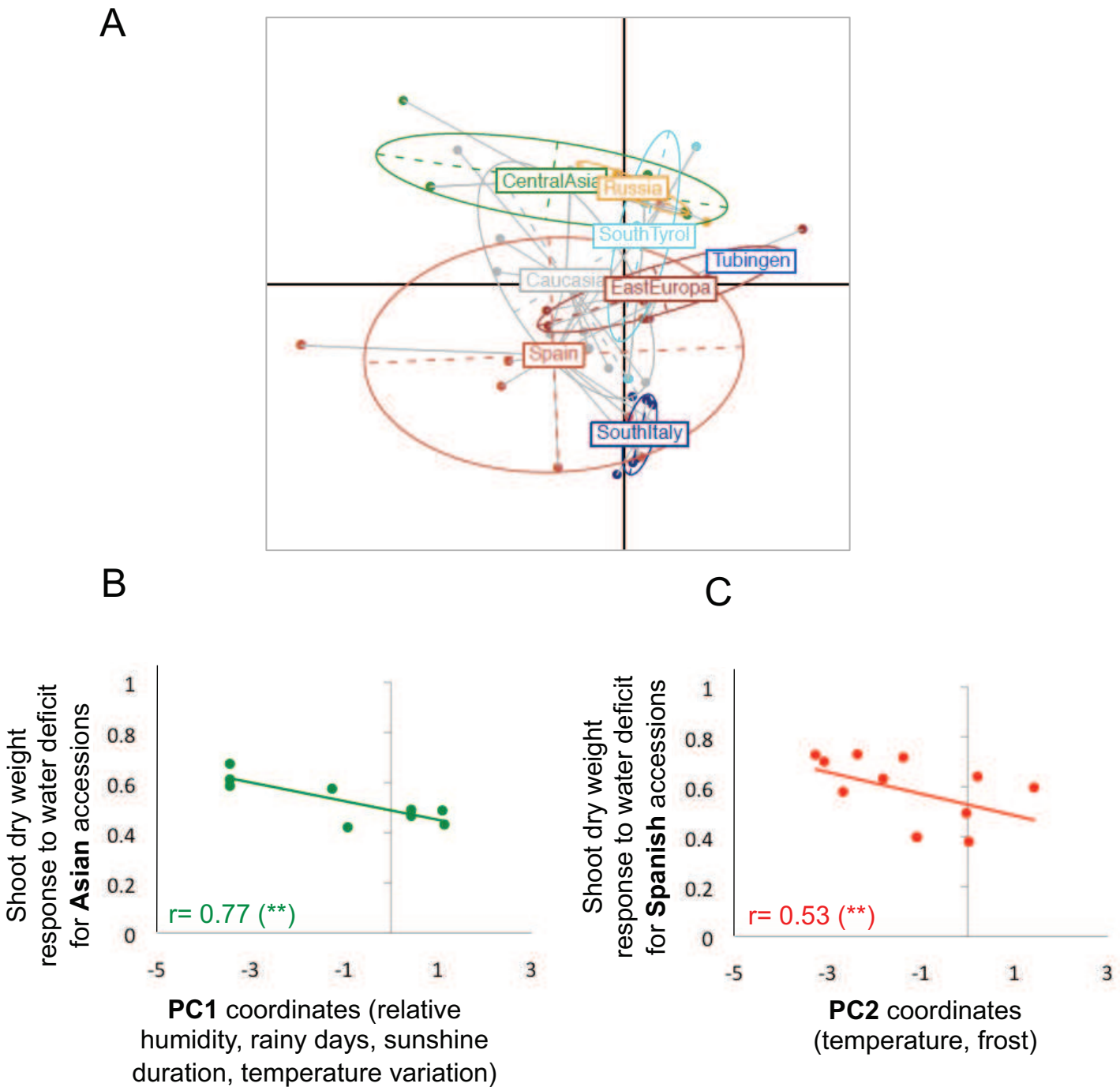


Fig. 5. Projection on principal components 1 and 2 of climatic characteristics of the habitats for the accessions. **A**. Projections are gathered according to the eight geographic regions (South Italy, Spain, Caucasia, South Tyrol, East Europa, Central Asia, Tübingen and Russia). Correlations between the Shoot dry weight response to water deficit (*ie*, ratio of Shoot dry weight under water deficit conditions over Shoot dry weight under well-watered conditions) for the accessions coming from central Asia and coordinates of climatic values of their habitat on principle component 1 (**B**), and between shoot dry weight response of accessions coming from Spain and coordinates of climatic values of their habitat on principle component 2 (**C**). Pearson´s coefficients value (r), and the p-value associated (**:p-value<0.01) are indicated.

night temperature variation and sunshine duration on principle component 1, and mean temperature and days under frost on principle component 2. Annual precipitations were mainly represented on the third principle component that accounted for 14% of the total variance of the data (Suppl. Fig. S1). There was no clear correlation between the coordinates of the climatic characteristics of the geographic region in which accessions were collected and the tolerance of these accessions to water deficit, neither for the whole accession set, nor considering the three clusters previously defined (Fig. 4B).

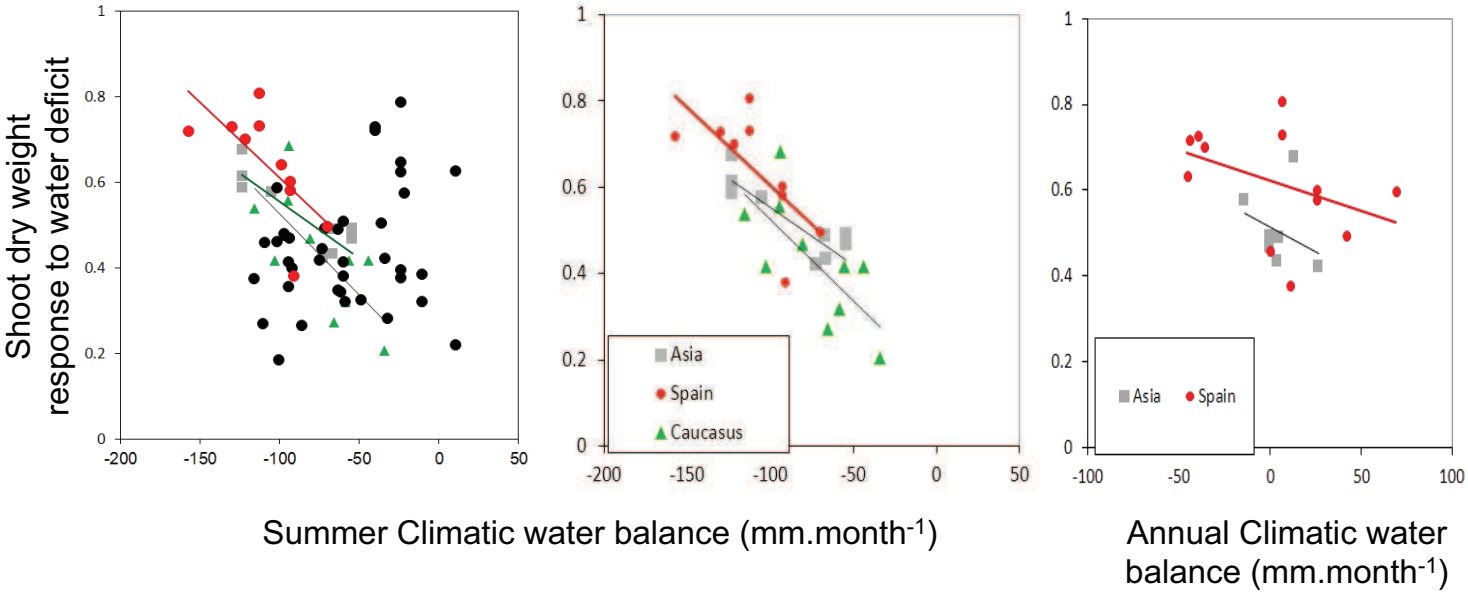
Variation in tolerance within different regions explained by climate variables

Gathering projections of the climate characteristics of the habitat of the accessions on the principal components 1 and 2 of the previously described PCA revealed that distinct geographic regions have distinct climate (Fig. 5A). The inter-regions variability of climate was mainly described by the second principal component, which represented a temperature gradient. South Italy climate was the warmest and Russia and central Asia climate the coldest. The principal component 1, related to hygrometric variables (see Fig. 4) did not really discriminate the different regions, apart from Tübingen, where climate was more humid compared to the mean climate of Caucasia, central Asia and Iberian peninsula-North africa regions.

Climatic variability was also observed within regions and to a lesser extent for Tübingen, Russia and South Italy. In these three regions, climatic variability was low and then could not be discriminated by any of the three climatic components. The locations in which accessions were collected in Iberian peninsula-North africa displayed the greater variability. This variability was described by both principal components 1 and 2. Central Asia and East Europa climates were mainly discriminated by principle component 1, whereas Caucasia and South Tyrol climates were mainly described by principle component 2. A large part of this climatic variability was related to the size of the region in which accessions were collected (see Fig. 1).

Noteworthy, significant correlations between tolerance to water deficit and climatic characteristics were observed in the two regions displaying the highest levels of climatic variability, central Asia and Iberian peninsula-North africa (Fig. 5B and Fig. 5C). For central Asia accessions, Shoot dry weight response to water deficit was strongly negatively correlated with the first principal component coordinates of the climatic characteristics of the locations in which accessions were collected in this region (r=0.77) (Fig. 5B). This suggests that tolerance in central Asia

A



B

	annual Climatic water balance	**spring** Climatic water balance	**summer** Climatic water balance	**autumn** Climatic water balance	**winter** Climatic water balance
Caucasia	ns	-0.31(*)	-0.72(***)	+0.65(*)	ns
Central Asia	-0.47(*)	ns	-0.87(***)	ns	ns
East. Europa	ns	ns	ns	ns	ns
Russia	ns	ns	-0.52 (*)	ns	ns
South. Italy	ns	ns	ns	ns	ns
South.Tyrol	ns	ns	ns	ns	ns
Spain	-0.51(**)	-0.48(**)	-0.68(***)	-0.33(*)	ns
Tubingen	ns	ns	ns	ns	ns
all the accessions	ns	ns	ns	ns	ns

Fig.6. A. Correlations between **summer or annual** climatic water balance (difference between precipitations and potential evapotranspiration) and Shoot dry weight response to water deficit for the whole set of accessions (black symbols), or for accessions for which the correlation is significant (represented by colored symbols: Accessions coming from central Asia (grey symbols), from Spain (red symbols), or from Caucasia (green symbols). **B**. Pearson´s coefficients of the correlations between climatic water balance and Shoot dry weight response to water deficit, averaged on year, or by seasons (spring, summer, autumn, and winter), in the different geographic regions. A one-way ANOVA was performed to test for differences between mean values. ***, pvalue<0.001; **, p-value<0.01; *, p-value<0.05; ns:non significant.

is higher under dry and sunny locations with high temperature fluctuations (see Fig. 4A). The low variability of Asian climates as expressed in the second principal component could explain the absence of correlation between this component and tolerance to water deficit. For Iberian peninsula-North africa, a negative correlation was observed between shoot dry weight response to water deficit and the second principal component (r=0.53) (Fig. 5C). This suggests that tolerance in Iberian peninsula-North africa is higher under warmer regions.

Negative correlation between climatic water balance and tolerance to water deficit in specific regions

Temperature gradients, sunshine duration and relative humidity that are well represented on the two first principal components, are the main determinants of potential evapotranspiration (Allen *et al.,* 1998) (Suppl. Fig. S2). We then calculated the daily mean value of this variable during the year (see material and methods) for the locations in which the 88 accessions were collected. Potential evapotranspiration was correlated to Shoot dry weight response to water deficit, both for accessions originating from central Asia (r=0.84, p-value<0.01) and from Iberian peninsula-North africa (r=0.61, p-value<0.05) (not shown). Nevertheless, potential evapotranspiration only refers to the evaporative demand and does not completely describe plant water balance, as water supplies are not taken into account. We therefore calculated the climatic water balance as the difference between precipitations and potential evapotranspiration. A low value of this variable refers to drought conditions. Considering all the accessions together, no correlation was observed between annual climatic water balance and the Shoot dry weight response to water deficit (Fig. 6A). However, a strong and unique negative correlations between annual climatic water balance and Shoot dry weight response to water deficit was observed for accessions originating from central Asia (r=-0.47), and Iberian peninsula-North africa (r=-0.51). In these two regions, the most tolerant accessions are found in locations in which they face water deficit conditions on a yearly average. Interestingly, the strength of this correlation varied when considering the climatic water balance at a seasonal scale (Fig. 6B). For Asian accessions, the coefficient of this correlation was stronger in summer (r=-0.87), and not significant for the other seasons. For spanish accessions, there was less variability of this relationships, and a negative correlation between tolerance to water deficit and climatic water balance was observed all along the year (except during winter). Noteworthy, the tolerance of accessions originating from Caucasia and to a lesser extent from Russia was also negatively related to seasonal water balance while the correlation did not appear at an annual scale. For Caucasian accessions, the shoot dry weight response to water deficit was negatively correlated to water balance in spring and summer, but positively in autumn.

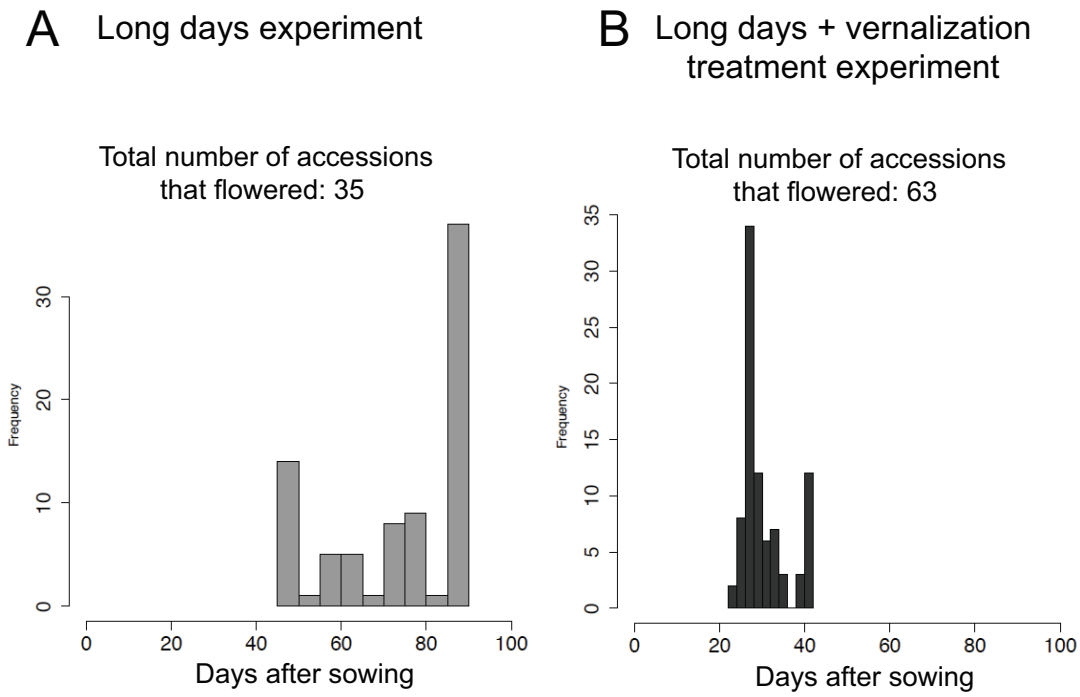


Fig. 7. Histograms of the flowering time observed in the 88 accessions, in long days conditions (**A.**16h daylength, 23°C), and in long days conditions (**B.**16h daylength, 23°C) preceded by a period of 6 weeks at 4°C that mimics the vernalization process that occurs into the wild. The flowering time corresponds to the number of days until the opening of the first flower, from the sowing for the “Long days” experiment, and without considering the 6 weeks at 4°C for the “Long days + vernalization” experiment.

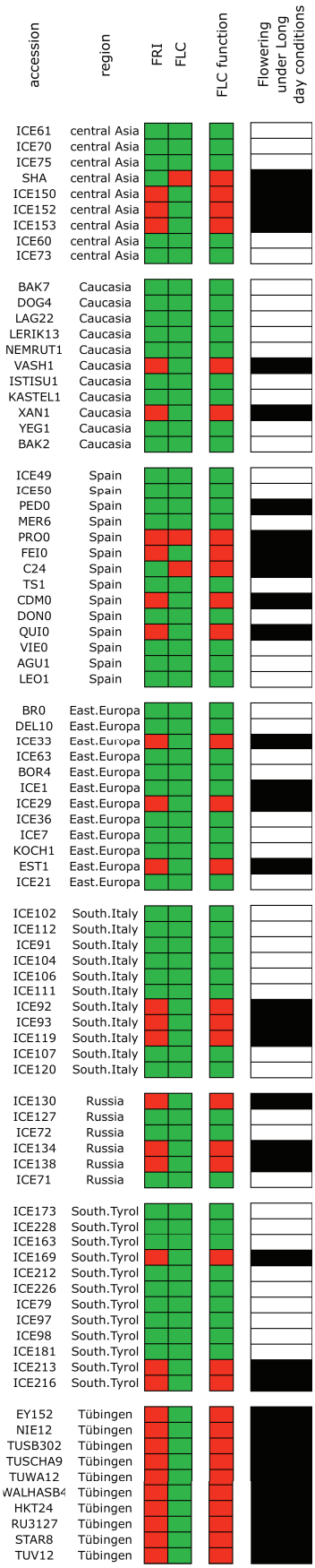


Fig.8. Correspondence between the functionality of *FRIGIDA* and *Flowering Locus C (FLC)* alleles in the 88 accessions (red cases when the allele is non-functional, green cases when the allele is functional), and the expected FLC function (red cases when the FLC function is expected to be weak, green when expected to be strong). The experimental validation of this vernalization requirement is indicated, with black cases when accessions flowered in long day conditions, without vernalization, and white cases when vernalization treatment was required for flowering to occur.

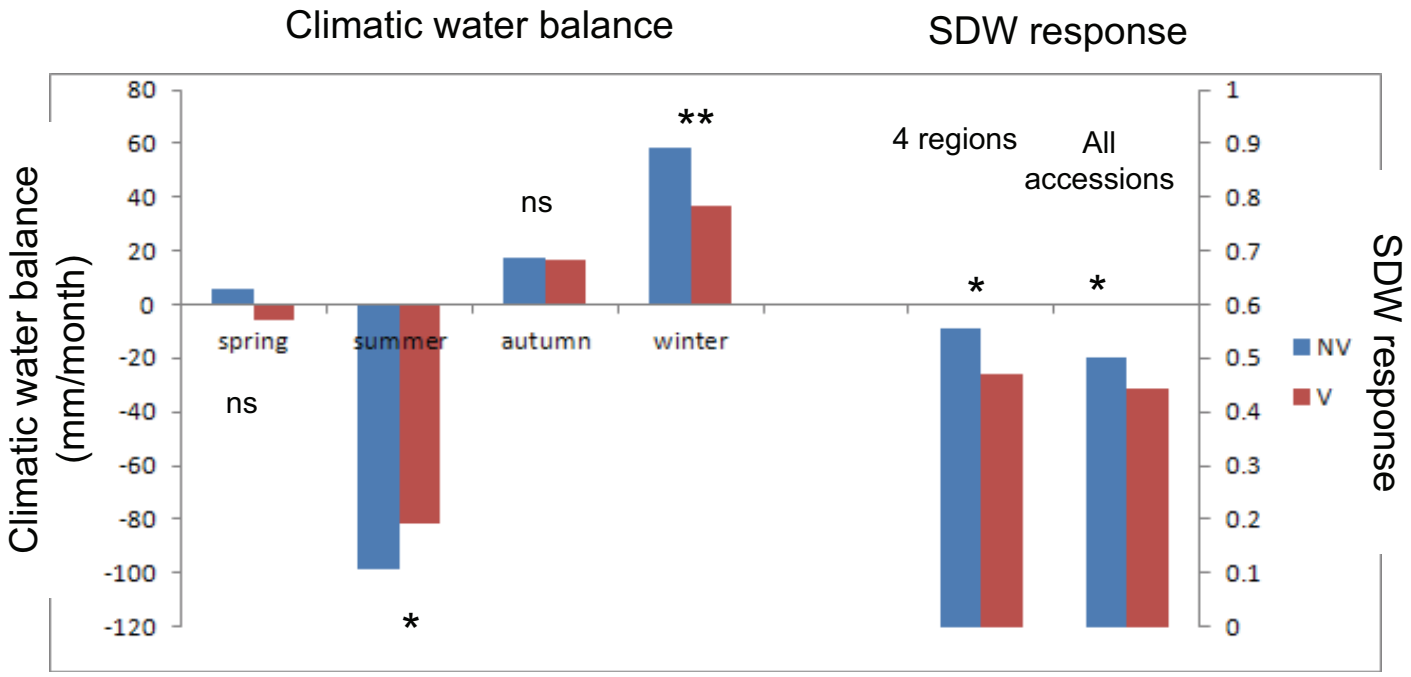


Fig. 9: Interaction between vernalization and tolerance to soil water deficit. On the right, the shoot dry weight response to water deficit is shown for accessions that need (V) or not (NV) vernalization to flower. Results correspond to the 4 regions that showed a relation between the Shoot dry weight response to water deficit and the climatic water balance, or for the whole dataset. On the left is shown the seasonal climatic water balance in those regions where the accessions of the 4 regions were collected. A t-test was performed to test for differences between mean values. ***, pvalue<0.001; **, p-value<0.01; *, p-value<0.05; ns: non significant.

Importance of the growing period for drought adaptation: response to vernalization

The correlation observed between water availability and tolerance to water deficit was variable according to seasons (Fig. 6B). We therefore hypothesized that this correlation could depend on the growing period of the accessions in the different geographic regions, that can occur at two different periods in Arabidopsis, either during Spring-Summer, or from Autumn to Spring, with a period of slow growth during winter (vernalization). In order to evaluate to what extent this vernalization requirement could interfere with tolerance, the flowering time was evaluated in dedicated experiments, performed with or without a period of cold. This analysis showed that 60% of the accessions used in this study flowered only after being exposed to this prolonged cold period (Fig. 7) (these accessions are called vernalization-requiring accessions because they need vernalization to flower, whereas non vernalization-requiring accessions do not need it). Moreover, a quantitative effect of this vernalization treatment was observed, as the flowering time was shortened (80 days after sowing in Long day experiment, 30 days after sowing for long day with vernalization experiment), even for non-vernalization requiring accessions. Whether accession need or not vernalization to flower is mainly determined by the activity of two genes, FRIGIDA (FRI) and Flowering Locus C (FLC). When the alleles of these two genes are both functional, the FLC protein strongly repress flowering, which is overcome after plants being exposed to a prolonged period of cold. We therefore determined the functionality of the FRI and FLC alleles of the 88 accessions (Fig. 8). Except for two accessions (Ice 1 and Ped-0), there was a complete agreement between the vernalization requirement observed during the experiments and the status of the FLC protein. Interestingly, the proportion of "non-vernalization requiring" and "vernalization requiring" accessions was different in the different geographic regions. All the Tübingen accessions do not need vernalization to flower, and in Iberian peninsula-North africa, the proportion of "non-vernalization requiring" accessions is greater than in South Tyrol, South Italy, or Caucasia.

Non-vernalization-requiring accessions tend to be more tolerant to water deficit

The shoot dry weight response to water deficit of "vernalization requiring" and "non-vernalization requiring" accessions was compared (Fig.9). When all the accessions were considered, the "non-vernalization requiring"

accessions were significantly more tolerant than the "vernalization requiring" ones (0.50 vs 0.44, p=0.04). The same trend was observed within the subset of regions in which a relationship was found between tolerance and climatic water balance (Iberian peninsula-North africa, Central Asia, Caucasus, Russia; 0.56 *vs.* 0.47, p=0.02). The greater tolerance to water deficit of "non-vernalization requiring" accessions was also associated to climatic characteristics encountered by these accessions in their natural habitats. Indeed, "non-vernalization requiring" accessions habitats were wetter in winter and dryer in summer.

Discussion

Evidence for variation in drought tolerance and drought escape

Drought escape is certainly the best understood strategy for wild species (McKay *et al.,* 2003; Li *et al.,* 2010). It relates to early flowering time in order to avoid drought conditions, in particular in summer. Adaptation is suspected to occur very rapidly over just few years as recently demonstrated using brassica populations harvested before or after a series of droughted years (1999-2003, Franks, 2011). Drought escape is also recorded after breeding schemes in crop plants such as maize (Bolanos and Edmeades, 1993) or rice (Lafitte et al 2007). In crop species, stress tolerance, ie. growth maintenance under drought, has been extensively studied since it directly relates to yield maintenance and is therefore a positive trait to breed for. By contrast, demonstration of growth maintenance in wild species is rarer (but see Bouchabke *et al.,* 2008: Tisne *et al.,* 2010). In the present study, we show that such strategy exists in Arabidopsis and displays a large degree of variation, from 0.2 to 0.8 (expressed as a ratio of stressed / non stress shoot biomass, after 10 days of severe soil water deficit). It thus confirms earlier results showing that the species carries some important sources of tolerance, especially in particular accessions (such as An-1, Granier *et al.,* 2006, Aguirrezabal *et al.,* 2007, or Bl-1, Bouchabke *et al.,* 2008). Here we show that sources of tolerance exist in all regions sampled suggesting selection has operated independently in various regions.

High variability of drought tolerance was identified between defined geographic regions but no climatic trend was able to account for inter-regions variation.

Stress tolerance was on average variable between the various regions. This was highlighted by our box plot analysis but as well by our clustering analysis. Central Asia and Iberian peninsula-North africa were overrepresented in the cluster associated with tolerance whereas east Europa and Tübingen were more abundant in the sensitive cluster. The climatic analysis (PCA) separated climates humidity on PC1 (coastal vs continental, little vs high temperature variation), climate temperature on PC2 and precipitation on PC3. Neither individual climate variable, nor any of these 3 composite axis integrated over the whole year or over single season was able to account for dry weight accumulation maintenance under drought. The same conclusion was drawn either using the whole population or averaging values for individual regions. The variability of the climates of collection among sites within individual regions differed strongly between Asia (massive variation) and Tübingen (almost no variation). This prompted us to search for correlations within those regions showing climate variation.

A strong relation between drought tolerance and water balance.

A first analysis identified correlation between Principal components 1 and 2 and tolerance. However, these correlations were not unique as Asia and Iberian peninsula-North africa showed correlation with PC1 and 2 respectively suggesting tolerance was higher in accessions originating from warm sites in Iberian peninsula-North africa and dry sites in central Asia. In order to get closer to the drought regime of the climate of collection site, we evaluated the local potential evapotranspiration using commonly used models and PET was well correlated with PC1. This estimate was subtracted from precipitations to approach a climate water balance (P-PET). This balance was in summer strongly related to growth maintenance in Iberian peninsula-North africa, Asia and Caucassus with a unique relationship. Relationship during other seasons were on average weaker but remained significant on a yearly basis for Iberian peninsula-North africa and Central Asia. This result is one of the first demonstrating a strong link between local climate and stress tolerance despite several attempts. The unique nature of the correlation suggests that drought has driven adaptation in a similar way in independent regions. In the past years, a series of converging results were accumulating to support such hypothesis but it was remaining undemonstrated yet. Indeed, using a range of sensitive and tolerant tree species along the Panama canal, Engelbrecht *et al.,* (2007) demonstrated a relationship between species sensitivity and their presence along a climatic gradient from the wet Atlantic to the dry Pacific side along the canal. Joshi *et al.,* (2001) showed in forage species using reciprocal transplant experiments that local genotypes performed better in their native environment than any other. Li *et al.,* (2010) showed a correlation between latitude and presence of flowering time alleles. Weisshuhn *et al.,* (2011) were able to connect responsiveness to drought in root

allocation and climate at the site of connection but no relation was found with drought tolerance. In our case, the link between tolerance gradient and biomass partitioning remains to be done.

Although we did not have access to the irradiance and were not able to estimate the 'Penman-Monteith' Potential Evapotranspiration, we used a method that proved robustness and lack of bias in a variety of climates (see Material and Methods). Although errors are possible because of the low resolution of our climatic template (400 km²), this is not likely to contradict the results obtained.

Our results remain however puzzling since the strongest correlations were obtained with summer climate whereas Arabidopsis is thought to have completed its cycle in April-May in the wild. This could be due to the fact that summer water balance is a better descriptor of aridity than winter water balance. This point clearly needs further examination.

While we found correlations between climatic components and plant tolerance to drought in three regions, Iberian peninsula-North africa, Caucasus and central Asia, we failed to identify such correlations in the other regions (South Italy, Russia, Tübingen, South Tyrol, Caucasia, Eastern Europa). In these regions, the variability of climate was limited, mainly because the collection strategy was to get geographically close populations and study local adaptation. However, there was an appreciable variation of tolerance in these regions suggesting climate itself (at least as it was estimated here since the climate grid is 400 km²) is unable to account for tolerance. This is suggestive that factors other than climate operate and edaphic conditions are obvious candidates. For most of the accessions collected, we had information on the precise collection site but the description was too limited (rocky path, meadow…) and the number of individuals within each region was too low to allow conclusions to be driven. The drivers of local adaptation are the matter of studies in the ecology and evolution community (eg Joshi *et al.,* 2001). In this line, it was shown that populations of the rare endemic plant Arabis fecunda are physiologically adapted to the local microclimate despite the absence of divergence at almost all marker loci and very small effective population sizes (McKay *et al.,* 2001).

By contrast with the Tübingen accessions, the accessions originating from Southern Italy and from Russia, displayed a relatively low variability of responses to drought. Therefore, it was difficult to find causal relationships between climatic features of these environments and tolerance to water deficit. A large geographic area was sampled to collect these accessions, but the phenotypic variability may not be representative of the variability of drought tolerance in these two regions. Indeed, these populations were collected because they represented a

genetic gradient reflecting the evolutionary history of the species, and not in the specific goal to distinguish phenotypic responses to dry environments.

Vernalization requirement appears to be a consequence of adaptation to specific climates

Because Spanish and Asian accessions showed gradients of tolerance depending on average temperature and temperature variation, we hypothesized that these gradients could be linked to flowering strategies associated with winter-spring or spring-summer growth habits. In order to indirectly approach this strategy, we evaluated the vernalization requirement of these accessions (ie the need to experience long term exposure to cold temperature to induce flowering, Bastow *et al.,* 2004; Amasino, 2005; Trevaskis *et al.,* 2007). This was done both using molecular information and a dedicated experiment. In *Arabidopsis thaliana*, the flowering response to vernalization requires the interaction of two genes, *FLOWERING LOCUS C* (FLC) and *FRIGIDA* (Michaels and Amasino, 1999; Sheldon *et al.,* 1999; Johanson *et al.,* 2000). Having functional alleles at both FRI and FLC loci imply that the accession FT will be modified by vernalization. FLC is a MADS domain-containing transcription factor that acts as a floral repressor, and FRI is a plant-specific gene of unknown biochemical function that is required for high levels of FLC expression. From an ecological point of view, rapid cycling corresponds to a summer-annual habit, whereas functional alleles at both FRI and FLC loci confer a winter-annual habit (Shindo *et al.,* 2005). Natural allelic variation at the FLC locus has also been identified, suggesting that non-vernalization requiring accessions have been derived from late-flowering ancestral accessions through loss-of-function mutations of FRI and/or FLC (Gazzani *et al.,* 2003; Michaels *et al.,* 2003).

Experiments and molecular analysis gave similar results with very few exceptions since vernalization requiring accessions were those carrying the functional alleles at both FRI and FLC loci. Vernalization significantly shortened the vegetative phase duration and tended to homogenize flowering time for all the accessions. We can raise the hypothesis that vernalization requiring accessions grow through winter whereas non vernalization requiring ones are more likely to grow during spring - early summer. Indeed, differences in vernalization requirements seem to be an adaptive response to temperature and season length in a particular latitude (Boudry *et al.,* 2002)

Plant tolerance was higher in "non-vernalization requiring" accessions, compared to "vernalization requiring"

ones. If we assume that non vernalization requiring accessions grow later in the season, they should be likely to experience more negative water balance during their cycle than vernalization requiring ones. This result would then fit with the idea that drought experienced by the plants during their development contributes to selecting growth maintenance phenotype, maybe for guaranteeing biomass production and fitness.

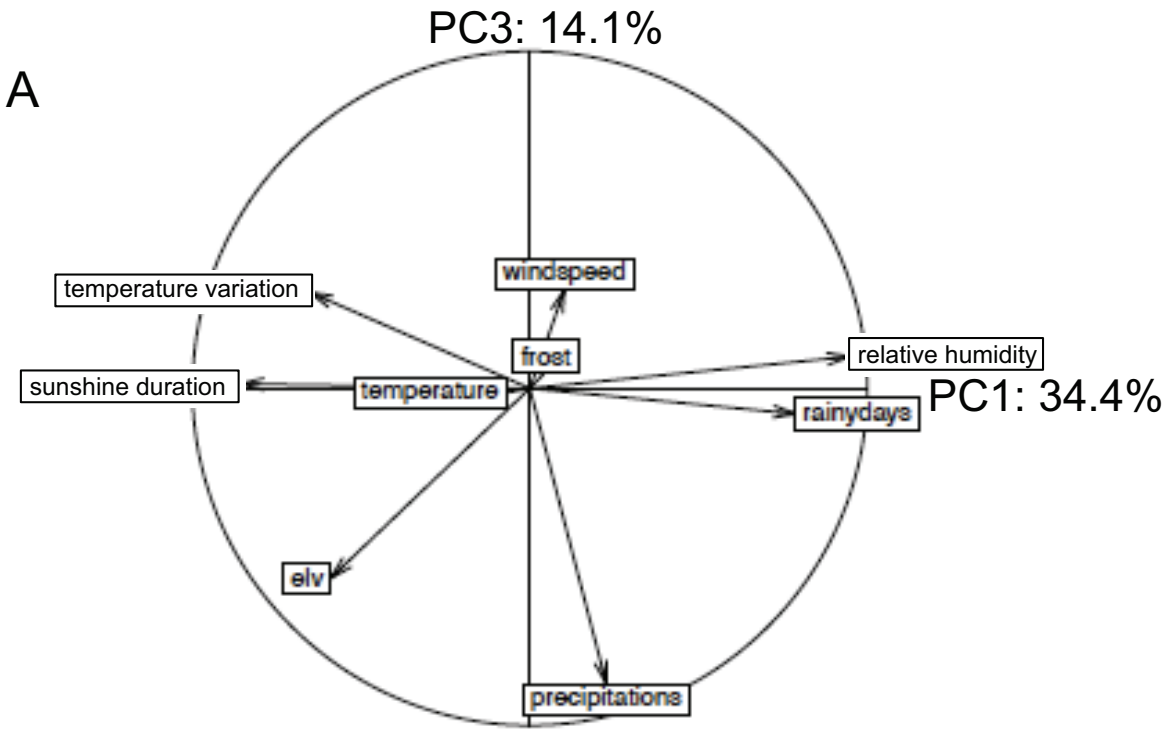


Fig. S1. Principal components 1 and 3 of a Principal component analysis performed with nine annual climatic variables describing the original habitat of the 88 accessions: **Frost** (number of days with negative temperature per month averaged on the year), **Precipitations** (mm.month^{-1}), air **Relative humidity**, **Rainy days** (number of days with precipitations>0.5mm per month, averaged on the year), **Sunshine duration** (% of day-length with full light), daily mean air **temperature** (°C), **temperature variation** (difference between daily minimal and maximal temperature, °C), **windspeed** (m.s^{-1}).

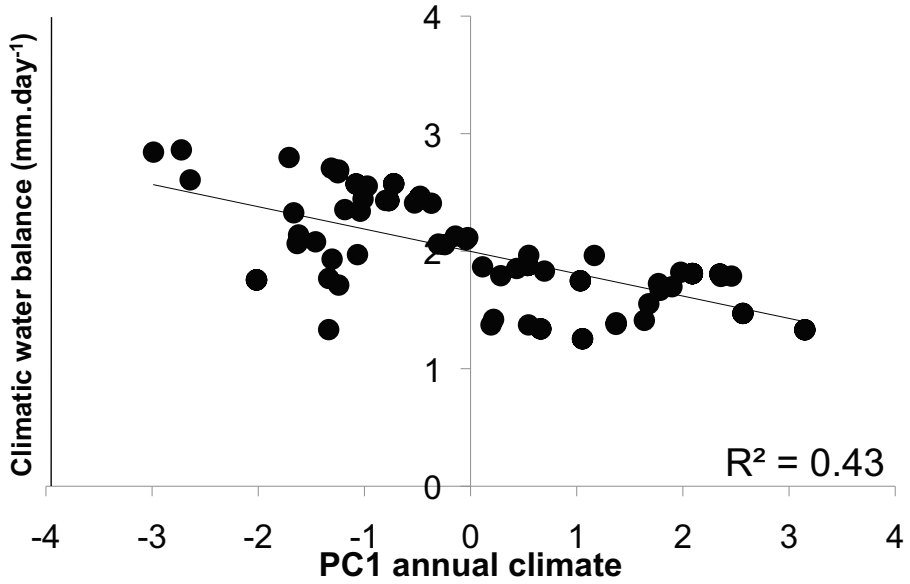


Fig S2. Correlation between the daily mean evapotranspiration calculated for the location of each accessions, and the coordinates of these locations on the principal component 1 of the PCA performed with nine annual climatic variables.

Tab. S1. Pearson´s coefficients of the correlation between the shoot dry weight response to water deficit of **asian** and **spanish** accessions, and (**A**) raw climatic variables (either averaged by year, or by seasons (autumn, winter, spring, summer)), or (**B**) principal components 1 and 2 of the PCA performed on climatic variables, either averaged by year, or by seasons.

A

	CENTRAL ASIA					SPAIN				
	Year	Autumn	Winter	Spring	Summer	Year	Autumn	Winter	Spring	Summer
temperature variation	0.8	0.76			0.83					
frost days						-0.41				
precipitations										
rainy days	-0.61									
relative humidity					-0.73					
sunshine duration	0.73		0.69		0.74					
temperature						0.43		0.44		
windspeed										

B

	CENTRAL ASIA					SPAIN				
	Year	Autumn	Winter	Spring	Summer	Year	Autumn	Winter	Spring	Summer
PC1	-0.71 (+rainy days, - sunshine duration)				-0.8 (+ rainy days, +precipitations , - sunshine duration)					
PC2						-0.59 (- temperature , + frost)	-0.58 (-temperature, + frost days, - precipitations)		-0.68 (- temperature, +frost days, + temperature variation)	-0.59 (+ temperature variation)

Chapter 4 /Genome wide survey of growth responses to drought conditions in Arabidopsis using combined association and linkage mapping

Marie Bouteillé[1,2], Oliver Stegle[2], Christoph Lippert[2], Gaelle Rolland[1], Karsten Borgwardt[2], Detlef Weigel[2] and Bertrand Muller[1]

[1] Institut de Biologie Intégrative des Plantes, Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, UMR759, INRA, Montpellier, France

[2] Max Planck Institute of Developmental Biology, Tübingen, Germany

Abstract

Drought tolerance partly relates on the phenotypic plasticity, ie the capacity for growth adjustment in the face of water deficit. While several QTL studies have been performed to identify genetic basis of shoot growth response to soil water deficit, analysis performed on root growth response are much rarer. Moreover, QTLs studies suffer from the limited allelic range while genome wide association mapping using large natural panels open the door to the identification of loci involved using a broader allelic source. In this study, we used growth and biomass partitioning data obtained from a population of Recombinant Inbred Lines originating from Bay-0 x Shahdara cross, and a collection of 88 accessions collected in very diverse habitats throughout Eurasia. These two sets of genotypes were grown in a soil based substrate under well watered and severe water deficit condition in the Phenotyping platform PHENOPSIS.

Results are globally very consistent across the two mapping populations with several regions in common, some of them controlling the same variable. To a much larger extend than in a previous study using hydroponics, specific regions were found for either root and shoot growth, in particular under water deficit conditions suggesting drought impairs shoot dependence of root growth. Regions specifically responsible for the response to soil water deficit were also detected. A series of candidate genes were identified and their expression profile stored in databases was examined. Among them, *ACD6* on the chromosome 4, and two other regions on chromosome 5 (At5g14920 and AGL101) already appear as valuable candidates controlling plant tolerance to water deficit.

Key words: Arabidopsis thaliana, shoot and root growth, plant tolerance to water deficit, Recombinant Inbred Lines, Quantitative trait Loci, genome wide association, expression profiles

Introduction

Plant growth is a complex trait that is controlled by many loci (Glazier *et al.,* 2002; Holland *et al.,* 2007; Mitchell-Olds, 2010; see Chapter 1), and which are exposed to important Genotype x environment interactions (Stanton *et al.,* 2000; van Eeuwijk *et al.,* 2010; Vlad *et al.,* 2010; Tardieu *et al.,* 2011; see Chapter 2). Identifying the loci involved in plant response to environmental constraints and especially to water deficit is required for deciphering mechanisms involved in this process as well as for helping breeding programs (Araus *et al.,* 2002; Chapman *et al.,* 2007; Cativelli *et al.,* 2008). Several studies reported the identification of loci responsible for variation of plant growth in response to water deficit (Price *et al.,* 2002; Welcker *et al.,* 2007; Mathews *et al.,* 2008; Hao *et al.,* 2009, Tisné *et al.,* 2008, 2010). Most of these analyses focused on either shoot or root traits. Shoot growth variation under drought conditions has been analysed at different scales, such as organ (Reymond *et al.,* 2003; Chenu *et al.,* 2009), or cell (Tisné *et al.,* 2008) or in different species (Welcker *et al.,* 2007 on maize; Mathews *et al.,* 2008 on wheat; Tisné *et al.,* 2008 on Arabidopsis). The genetic basis of the response to water deficit has also been characterized through changes of leaf physiology, such as modifications of transpiration and water-use efficiencies (McKay *et al.,* 2003; Haussman *et al.,* 2005; McKay *et al.,* 2008). Several studies have also been performed on genetic bases of root growth variation (de Dorlodot *et al.,* 2007), and especially under drought conditions on rice (Price *et al.,* 2002; Zheng *et al.,* 2003; Cui *et al.,* 2008), maize (Giuliani *et al.,* 2005), or Arabidopsis (Vartanian *et al.,* 1994; van der Weele *et al.,* 2000; Verslues and Bray, 2006; Xiong *et al.,* 2006). However, tolerance to drought implies a necessary optimization of both root and shoots growth, to maintain an efficient balance between water and minerals uptake on one hand and biomass production through photosynthesis on the other hand (see Chapter 2). The relative importance of root and shoot growth depends on biomass allocations patterns within the plant and even within the organs, and have been described through a series of dedicated variables : Root/shoot, Specific leaf area, and Specific root length (see Chapters 1&2). To our knowledge, only a very few studies have considered the genetic bases of these different growth and biomass partitioning variables (Cui *et al.,* 2008) and none has considered these variables under drought conditions.

The different studies evocated allowed the identification of Quantitative trait loci (QTL) in segregating populations (such as Recombinant Inbred Lines populations). But these populations are generally obtained from biparental crosses, and therefore only represent the allelic variation existing in parental lines. That leads to a limited genericity of the QTL detected (Bergelson and Roux, 2010), and could highlight polymorphisms that are specific to these crosses. For instance, QTL of leaf expansion detected under drought conditions in the Ler xAn-1 RIL population (Tisné *et al.,* 2008) were strongly linked to the *erecta* mutation, specific of the Landsberg erecta

(Ler) parent.

Association studies have recently emerged as an alternative to QTL studies to identify genomic regions responsible for the variation of quantitative traits (Rafalski *et al.,* 2002; Zhu *et al.,* 2008). These methods use the recombination events accumulated through the evolution of natural populations (Flint-Garcia *et al.,* 2003; Gupta *et al.,* 2005). Using natural populations allows increasing the allelic diversity compared to biparental populations (Bergelson and Roux, 2010). Nevertheless it also generates high levels of spurious associations due to confounding effects of population structure and relatedness between the accessions (Myles *et al.,* 2009; Platt *et al.,* 2010; Qin *et al.,* 2010; Mezmouk *et al.,* 2011). Association studies have been successfully performed as candidate genes approaches, to identify polymorphisms associated with resistance to pathogens (Nemri *et al.,* 2010), anthocyan synthesis (Fournier Level *et al.,* 2009), abcisssic acid content (Setter *et al.,* 2010) or flowering time (Zhao *et al.,* 2007) in various species, even without full sequence information (Saidou *et al.,* 2009). Recently, due to the increased number of genomic sequences available (Borevitz and Nordborg, 2003; Nordborg and Weigel, 2008; Weigel and Mott, 2009), association studies without *a priori* (genome wide) have been conducted on traits such as resistance to pathogens, leaf architecture (Tian *et al.,* 2011), flowering time (Atwell *et al.,* 2010), and adaptation along environmental gradients (Mariac *et al.,* 2011).

In this study, two types of analyses were performed to identify the genetic basis of shoot and root growth response to water deficit conditions in Arabidopsis thaliana. The first one consisted in a QTL analysis using the Bay x Sha RIL population that was chosen because Bay-0 and Shahdara accessions displayed contrasted root architecture in response to various abiotic stresses (Loudet *et al.,* 2002, 2005), possibly related to adaptation to diverse natural habitats (Bay-0 was collected in fertile plains of Gerseveral near Bayreuth, whereas Shahdara originates from high mountains of Tadjikistan, Loudet *et al.,* 2002). The second one consisted in a genome wide association study performed on a population of 88 accessions from throughout the Eurasian range of the species that has been shown to display a large variability of growth response to water deficit (see Chapter 3), reflecting the adaptation of these accessions to various drought conditions in their natural habitats. Genomic regions that control root and shoot growth under well-watered conditions and in response to water deficit conditions were identified combining the two approaches, and were compared to decipher polymorphisms related to plant drought tolerance using natural allelic variation.

Materials and methods

Plant material

wo sets of genotypes were used in this study: The first one corresponds to a population of 130 Recombinant Inbred Lines from the Bay-0 x Shahdara core-collection (Loudet *et al.,* 2002) genotyped with 69 microsatellites markers, selected to capture maximum recombination. This material was obtained from Versailles Biological Resource Centre (http://dbsgap.versailles.inra.fr/vnat/). Complete genetic and phenotypic information on this population are available at http://dbsgap.versailles.inra.fr/vnat/Documentation/33/DOC.html.

The second set of genotypes included two collections of 80 and 8 accessions (see Appendix 1 and Chapter 3). The parental lines of the RIL population, Bay-0 and Shahdara were also present in this collection of accessions. The 88 accessions were collected in specific regions in Europe and Asia that are know to have been the native range of the species (Sharbel *et al.,* 2000; Schmid *et al.,* 2006). Those accessions were also chosen as a starting point of an ambitious sequencing program of hundreds Arabidopsis accessions (www.1001genomes.org, Weigel and Mott, 2009, Clark *et al.,* 2007, Cao *et al.,* 2011).

Plant growth conditions

All the experiments were performed in the PHENOPSIS automated phenotyping platform (Granier *et al.,* 2006). In each experiment, all micro-meteorological conditions were kept constant during the whole growing period. Day-length was maintained at 10 h, and light was provided by HQI lamps with additional cool white fluorescent tubes. Photosynthetic photon flux density (PPFD) was measured continuously at the plant level, using a photosynthetic sensor (LI-190SB, Li-Cor, Lincoln, NE, USA) and set to 180 $\mu mol\ m^{-2}\ s^{-1}$ in all cases. Air temperature and relative humidity were measured every 20 s (HMP35A Vaisala Oy, Helsinki, Finland) and set to 20-21°C (day and night) and 75% respectively. All measurements of temperature, PPFD and relative humidity were averaged and stored every 600 s in a data logger (Campbell Scientific, LTD-CR10Wiring Panel, Shepshed, Leicestershire, UK) and automatically sent to a database (http://bioweb.supagro.inra.fr/phenopsis/).

Seeds were sown in 200 mL conical pots (9 cm height and 4.5 cm diameter) filled with a mixture (1:1, v/v) of a loamy soil and organic compost. Soil water content was determined before sowing and set to 0.35 $g(H_20)$. $g(dry\ soil)^{-1}$. Subsequent changes in pot weight were attributed to a change in soil water status. Soil water content was

adjusted daily automatically with the automaton in the PHENOPSIS platform to two different values, 0.35 g H_2O g^{-1} dry soil corresponding to well-watered (WW), and 0.18 g H_2O g^{-1} dry soil corresponding to water deficit (WD) conditions (Granier *et al.,* 2002). These values correspond to a predawn leaf water potential of -0.3 MPa, and -1.1 MPa respectively (Granier *et al.,* 2006; Hummel *et al.,* 2010).

Experiments and treatments

Plants were grown in four independent experiments. During the first two experiments, RIL were grown at optimal soil water content and under soil water deficit conditions respectively. In two other experiments, accessions were grown at optimal soil water content and under soil water deficit conditions respectively. In all cases, germination occurred within 3-6 days after sowing. Pots were maintained at a soil water status of 0.35 g H_2O g^{-1} dry soil corresponding to well-watered conditions during the first 15 days after germination, and the soil water status was either maintained at this value (well-watered experiments) or reduced down to 0.18 (water deficit experiments). This value was reached within 3-4 days. Then soil water content was kept at this value by automatic irrigation twice a day.

Each RIL and accession was grown in 3 and 5 pots respectively, randomly located in the growth chamber. The lack of block effect was later tested. After 2 weeks, each pot was thinned to let 1 to 3 homogeneous plants per pot, depending on plant size to avoid overlapping.

Variables measurements and calculation

Plants were then harvested 10 days after the onset of water deficit corresponding to 28-31 days after sowing. 6 to 9 individual plants per genotype were collected and individually measured. At this time, images were taken by the automaton. Photos were further used to estimate projected rosette area and rosette leaf number of each individual plant using Image-J software and customized macros. At harvest, all pots corresponding to one genotype were gathered, plants were gently removed from the pot, and rosettes were separated from the root system. Rosettes were then stored in paper bags for further measurements of shoot dry weight after the tissues had been dried down after 2 days at 80°C. In order to capture root biomass and dimension, the root system was

cleaned from every soil particles, and, spread at the surface of large (20 x 20 cm) Petri plates filled with water and a numerical image was taken at 600 dpi using a scanner in transmission mode. Total root length and primary root length were measured on those images, using Image-J software and customized macros. After image capture, root systems were individually stored into 96 well plates each containing pre-weighed aluminium cell-cup to facilitate weighing of dry material. The plates were then oven dried for 2 days at 80°C and the cups were weighed to measure root dry weight. All weights were measured using a 5 digits balance. Root/shoot ratio was calculated as the ratio between root dry weight and shoot dry weight, whereas Specific leaf area and Specific root length were calculated as the ratio between rosette area and shoot dry weight and between total root length and root dry weight, respectively. The response to water deficit was expressed as the ratio between the value in water deficit condition to the value in well-watered conditions.

QTL detection

All analyses were performed using the computer package SPSS 11.0.1 for Windows (SPSS) and the R software (R Development Core Team, 2008). Normality of the distributions of each variable among the lines was verified by evaluating skewedness. Heritability (broad sense) was estimated as the proportion of variance explained by between-line differences based on measurements of nine plants per line on average, at the harvest.

A first QTL detection using simple interval mapping (IM) was performed with the MapQTL5 software (MAPQTL®5, Kyazma BV, Wageningen, the Netherlands). Cofactors were then selected using the 'automatic cofactor selection' (ACS) chromosome per chromosome, and were used for Multiple QTL Mapping (MQM). The cofactors for which no QTL were detected (LOD score under a 95% LOD threshold (LOD < 2.4) estimated by permutation tests implemented in MapQTL5 using at least 1,000 permutations of the original dataset) were removed. The Epistat software was used to detect epistatic interactions between QTL (Chase *et al.,* 1997). Then, global QTL models combining main effects QTL and epistatic QTL were statistically tested using the GLM of the statistical package of SPSS 11.0.1 for Windows. The estimated additive effect, the percentage of variance explained by each individual QTL, and the total variance explained by the QTL model were obtained using the same package.

88 accessions genotypic information

For 80 of the accessions and the reference Col-0, we considered genotypic information that has been published in the context of the 1001 Arabidopsis project (www.1001genome.org; Cao *et al.,* 2011), and that has been projected on a SNP collection interrogated in a larger number of accessions with a custom Affymetrix single nucleotide polymorphism (SNP) chip containing 250k SNP (Kim *et al.,* 2007). For the 8 additional accessions (see Appendix 1), we used genotype information corresponding to the same 250k SNP chip and that has previously been published (Clark *et al.,* 2007).

All genotypes were binarized, distinguishing between the most frequent variant among the 88 accessions (major allele) and the second most frequent variant (minor allele). If other variants existed these were marked as missing values and ignored.

Association testing

Linear regression and linear mixed models (Kang *et al.,* 2008; Kang *et al.,* 2010; Zhang *et al.,* 2010) were used for association testing. For both models, p-values were computed from likelihood ratio tests, using a Chi-squared distribution with one degree of freedom.

Linear mixed models were applied in a two-step process. First, we estimated the genetic similarity matrix K between the strains by computing covariance based on all 216k standardized SNP, using the STRUCTURE program (Pritchard *et al.,* 2000). Covariance between two strains i and j was computed as Kij=1/S (Xi - E[Xi])T (Xj - E[Xj]), where S denotes the number of SNP and Xi is the vector of all SNP of individual i.

Then we used the similarity matrix to model random effects and the SNP weight plus a bias term as fixed effects in the mixed model. The mixed model likelihood with random effects integrated out is fully specified by N(y | Xw+b , e2 I+g2 K), where X, y and b denote the SNP vector across all individuals, the phenotype vector across all individuals and the bias term respectively. e2 denotes the environmental variance and g2 denotes the variance of the random effects. The model parameters w, b were estimated by maximum likelihood. To speed up computation, the ratio =e2/g2 of the variance components was estimated on the null model only, as has also been done in (Kang *et al.,* 2010; Zhang *et al.,* 2010).

Tab. 1. Heritabilities and percentage of variance explained by the QTL models for each variable in each condition.

	heritability	% variance explained by QTL models
SDW	65.2	54.9
AREA	71.2	57.4
RDW	66.5	61.5
TRL	58.9	42.5
PRL	59.2	47.8
RS	61.2	55.3
SLA	58.4	51.1
SRL	61.9	54.1
SDWstress	61.5	53.4
AREAstress	66.9	61.3
RDWstress	62.1	57.8
TRLstress	56.9	47.9
PRLstress	58.3	44.1
Rsstress	64.3	46.6
SLAstress	61.1	53.5
SRLstress	59.5	47.4
SDWresponse	66.4	58.9
AREAresponse	63.9	55
RDWresponse	62.2	57.2
TRLresponse	64.9	53.8
PRLresponse	61.2	58.9
Rsresponse	65.3	48.3
SLAresponse	65.8	62.9
SRLresponse	60.3	56.2

Gene expression patterns

The expression profiles of target genes underlying the detected associations were investigated with the meta profile analysis of Genevestigator (Hruz *et al.,* 2008), using the ATH1 22k array. This method, based on a survey of literature, allowed to investigate the differential expression levels of target genes in different plant tissues or in response to specific environmental condition (ABA treatment and different drought conditions in that case).

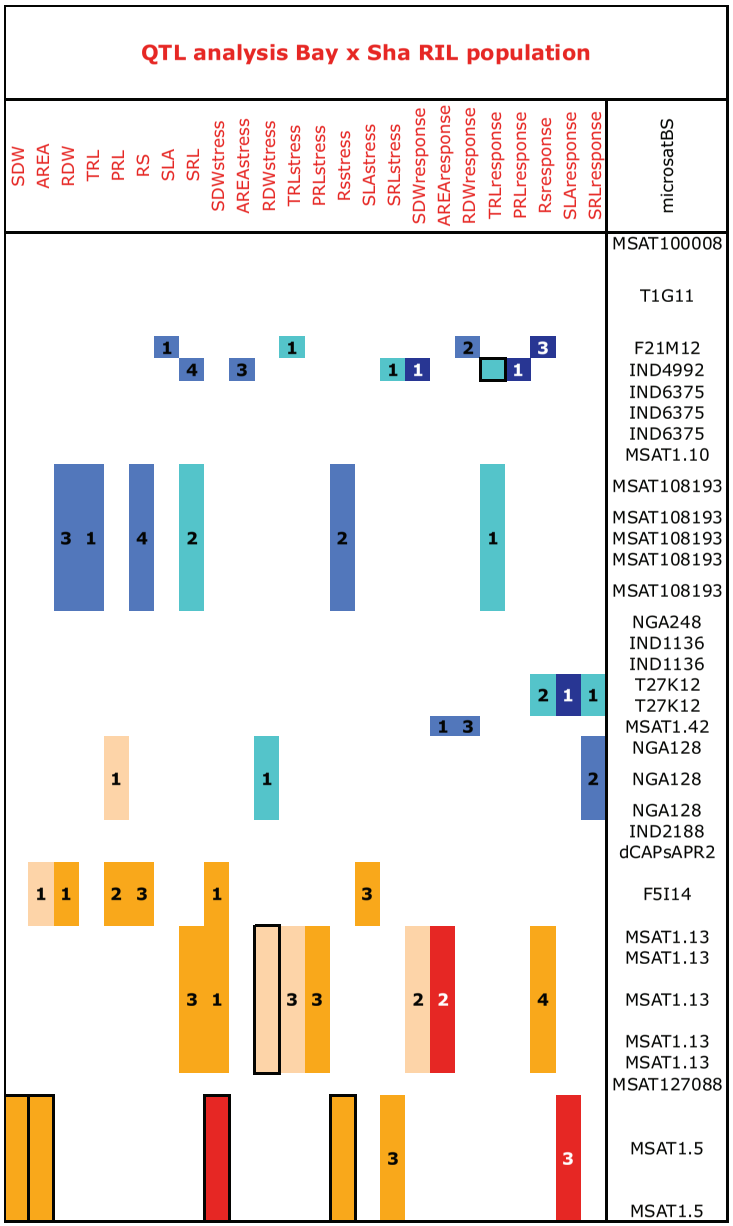
Results

Two analyses were performed to explore the genetic basis of growth traits related to drought tolerance. The first one consists in a QTL analysis using the Bay-0 x Shahdara RIL population that was genotyped with 69 microsatellites markers, and the second one corresponds to a genome wide association study on 88 accessions, with a 250K high-resolution SNP dataset.

QTL clusters affecting plant growth and biomass partitioning in the Bay-0 x Shahdara RIL population

Quantitative trait loci (QTL) were detected for eight variables related to growth or to biomass partitioning: Shoot dry weight, Rosette area, Root dry weight, Total root length, Primary root length, Root/shoot ratio, Specific leaf area, and Specific root length (Tab.1). QTL detection was performed for these variables under well watered and under water deficit conditions, but also on the variables corresponding to the response of each variable to water deficit conditions (*i.e.* ratio of the water deficit value over the well watered value). Heritability for these variables varied between 47 and 63 % (Tab 1). Both main effect and epistatic significant QTL (*i.e.* with LOD > 2.4) were detected for each variable, but the number of main effect QTL was lower than the number of epistatic ones (Figs. A). The QTL models including all the QTL detected for each variable explained a large part of the trait variance, ranging from 42.5% of the Total root length variance under well-watered conditions, and 62.9% for Specific leaf area response to water deficit conditions. Noteworthy, the percentage of variance explained by QTL

1A



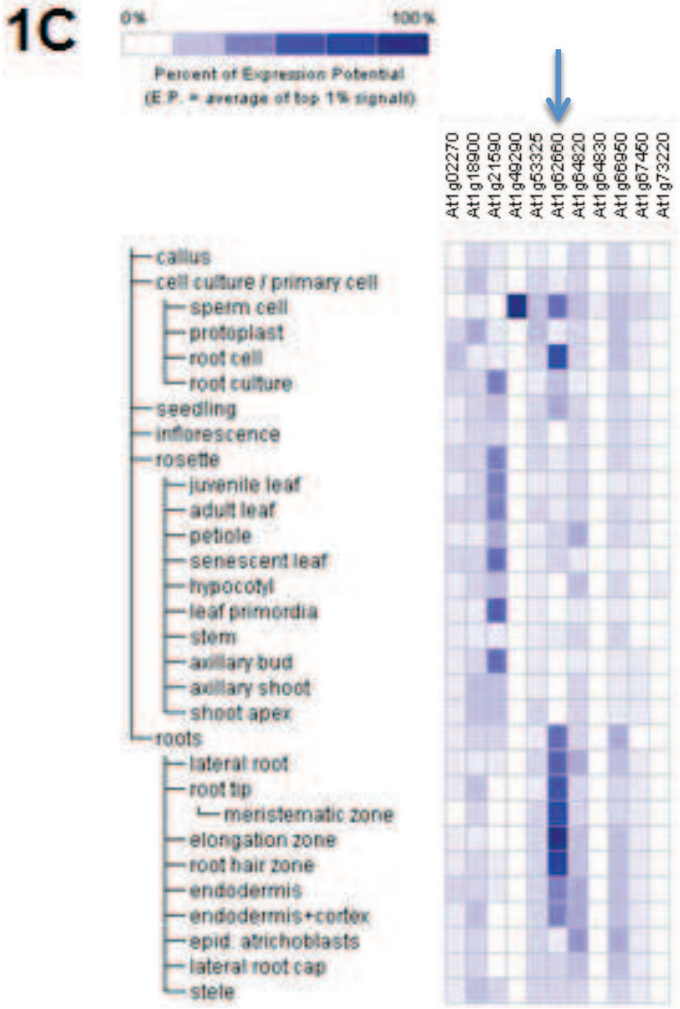
1B

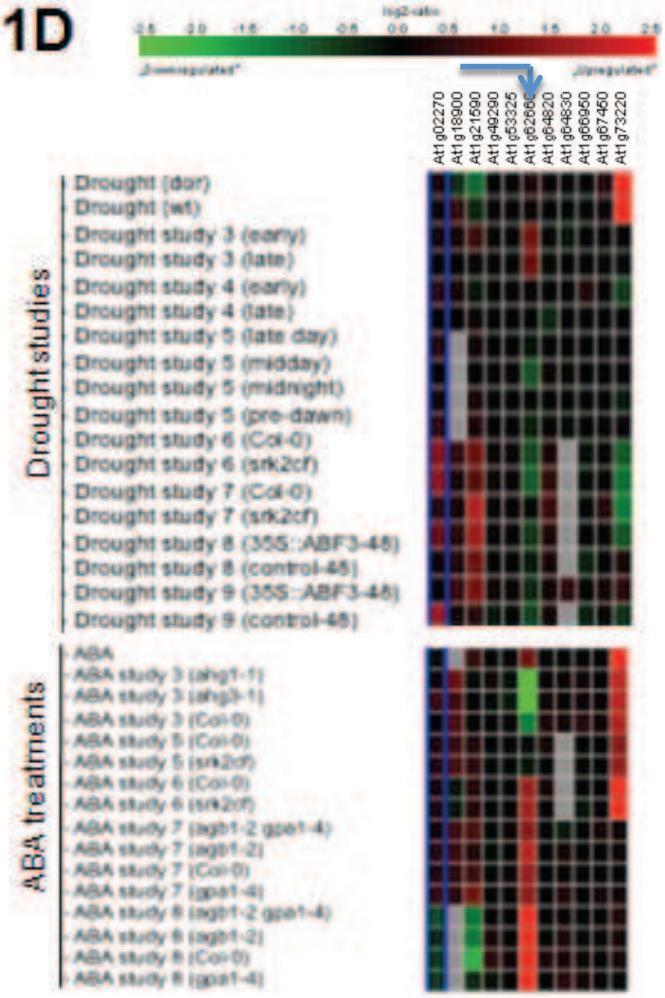
Chromosome 1

GWA analysis

variable	position	LOD	Qvalue	ExpVar	gene	gene Annotation	expression level in the different organs
SRLwell-watered	**1 _ 445336**	**16.9**	**0.000**	**4.8**	**AT1G02270**	**endonuclease/exonuclease/phosphatase family protein / calcium-binding EF hand family protein**	
respAREA	**1 _ 4366943**	**12.9**	**0.008**	**7.1**	**AT1G12805**	**nucleotide binding**	
respSDW	**1 _ 4433564**	**11.8**	**0.000**	**5.4**			
SLAwell-watered	**1 _ 4592326**	**10.4**	**0.000**	**3.7**	**AT1G13390**	**unknown protein**	
FT LDVwell-watered	1 _ 6531026	11.0	0.087	2.2	AT1G18900	pentatricopeptide (PPR) repeat-containing protein	
respAREA	**1 _ 6696977**	**16.1**	**0.001**	**6.9**			
LeafNbwater deficit	1 _ 6755774	12.5	0.020	6.8			
respTRL	1 _ 7061793	10.8	0.053	3.0			
SRLwell-watered	1 _ 7569451	11.4	0.036	5.2	AT1G21590	protein kinase family protein	rosette, young leaves
SRLwell-watered	1 _ 10023561	11.2	0.038	7.2			
AREAwater deficit	1 _ 10965274	10.9	0.047	6.8			
PRLwater deficit	**1 _ 13459634**	**14.6**	**0.000**	**5.3**			
RSwell-watered	1 _ 15925612	9.6	0.092	6.7			
RDWwater deficit	1 _ 16952996	10.1	0.081	6.1			
AREAwater deficit	1 _ 18232779	11.4	0.040	6.8			
respAREA	**1 _ 18232779**	**12.5**	**0.009**	**7.3**	**AT1G49290**	**unknown protein**	sperm cells, pollen
RSwater deficit	**1 _ 19401611**	**12.5**	**0.000**	**7.4**	**AT1G52130**	**jacalin lectin family protein**	
RSwell-watered	1 _ 19895281	9.5	0.092	5.5	AT1G53325	F-box family protein-related	
respTRL	1 _ 22945115	10.1	0.053	3.4			
FT LDVwell-watered	1 _ 23202855	10.6	0.087	6.9	AT1G62660	beta-fructosidase (BFRUCT3) / beta-fructofuranosidase / invertase	**root** cortex cells, radicle, elongation zone
FT LDVwell-watered	**1 _ 24090013**	**13.8**	**0.000**	**7.5**	**AT1G64820**	**MATE efflux family protein**	
FT LDVwell-watered	1 _ 24091392	10.5	0.087	6.1	AT1G64830	aspartyl protease family protein	
TRLwater deficit	**1 _ 24978234**	**11.2**	**0.000**	**5.5**	**AT1G66950**	**ATPDR11/PDR11 (PLEIOTROPIC DRUG RESISTANCE 11); ATPase**	ovule
RDWwater deficit	1 _ 25267585	12.3	0.024	6.8	AT1G67450	F-box family protein	
respTRL	1 _ 25653333	12.3	0.053	9.2			
SRLwell-watered	1 _ 27541562	11.1	0.038	5.9	AT1G73220	ATOCT1 (ARABIDOPSIS THALIANA ORGANIC CATION/CARNITINE TRANSPORTER1); carbohydrate transmembrane transporter/ carnitine transporter/ transporter	flower
RSwell-watered	1 _ 29910101	10.6	0.089	7.1			

See legend on Chromosome 5

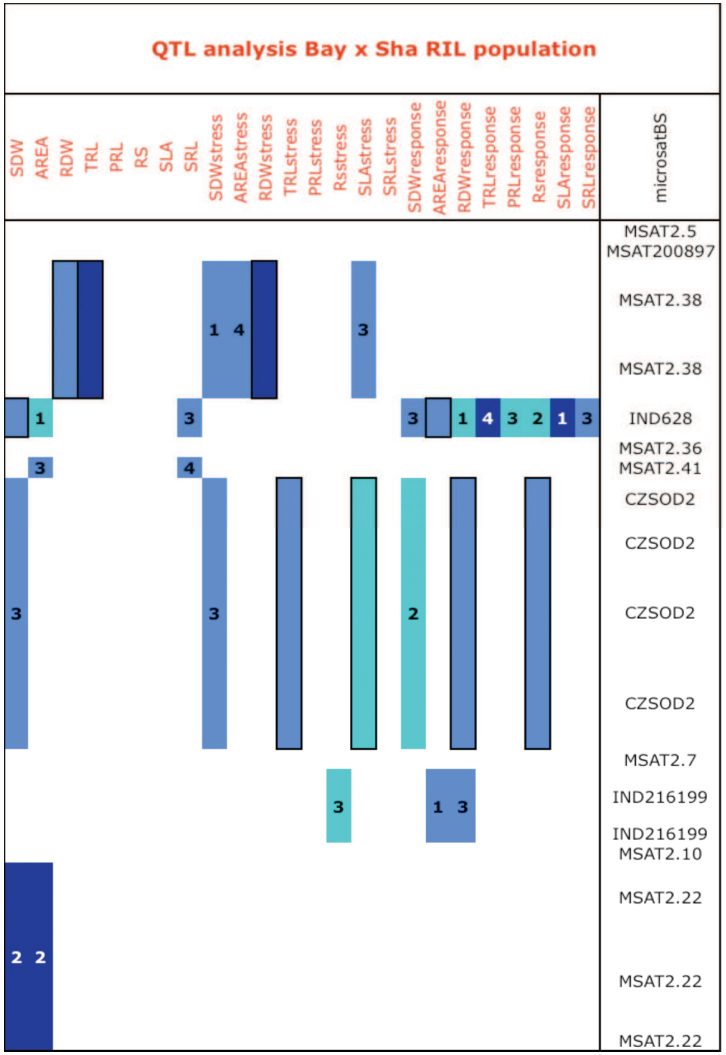

1C
0%
100%
Percent of Expression Potential
(E.P. = average of top 1% signal)
At1g02270
At1g18900
At1g21590
At1g49290
At1g53325
At1g62660
At1g64820
At1g64830
At1g66950
At1g67460
At1g73220
callus
cell culture / primary cell
sperm cell
protoplast
root cell
root culture
seedling
inflorescence
rosette
juvenile leaf
adult leaf
petiole
senescent leaf
hypocotyl
leaf primordia
stem
axillary bud
axillary shoot
shoot apex
roots
lateral root
root tip
meristematic zone
elongation zone
root hair zone
endodermis
endodermis+cortex
epid. atrichoblasts
lateral root cap
stele


1D
log2-ratio
2.5 2.0 1.5 1.0 0.5 0.0 0.5 1.0 1.5 2.0 2.5
"downregulated"
"upregulated"
At1g02270
At1g18900
At1g21590
At1g49290
At1g53325
At1g62660
At1g64820
At1g64830
At1g66950
At1g67460
At1g73220
Drought studies
Drought (dor)
Drought (wt)
Drought study 3 (early)
Drought study 3 (late)
Drought study 4 (early)
Drought study 4 (late)
Drought study 5 (late day)
Drought study 5 (midday)
Drought study 5 (midnight)
Drought study 5 (pre-dawn)
Drought study 6 (Col-0)
Drought study 6 (srk2cf)
Drought study 7 (Col-0)
Drought study 7 (srk2cf)
Drought study 8 (35S::ABF3-48)
Drought study 8 (control-48)
Drought study 9 (35S::ABF3-48)
Drought study 9 (control-48)
ABA treatments
ABA
ABA study 3 (ahg1-1)
ABA study 3 (ahg3-1)
ABA study 3 (Col-0)
ABA study 5 (Col-0)
ABA study 5 (srk2cf)
ABA study 6 (Col-0)
ABA study 6 (srk2cf)
ABA study 7 (agb1-2 gpa1-4)
ABA study 7 (agb1-2)
ABA study 7 (Col-0)
ABA study 7 (gpa1-4)
ABA study 8 (agb1-2 gpa1-4)
ABA study 8 (agb1-2)
ABA study 8 (Col-0)
ABA study 8 (gpa1-4)

2A

2B

Chromosome 2

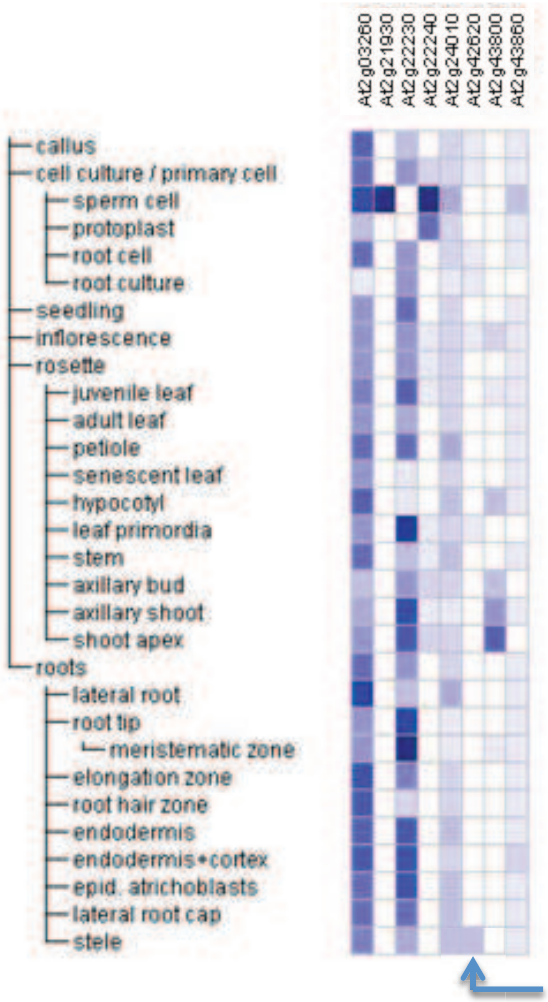


GWA analysis

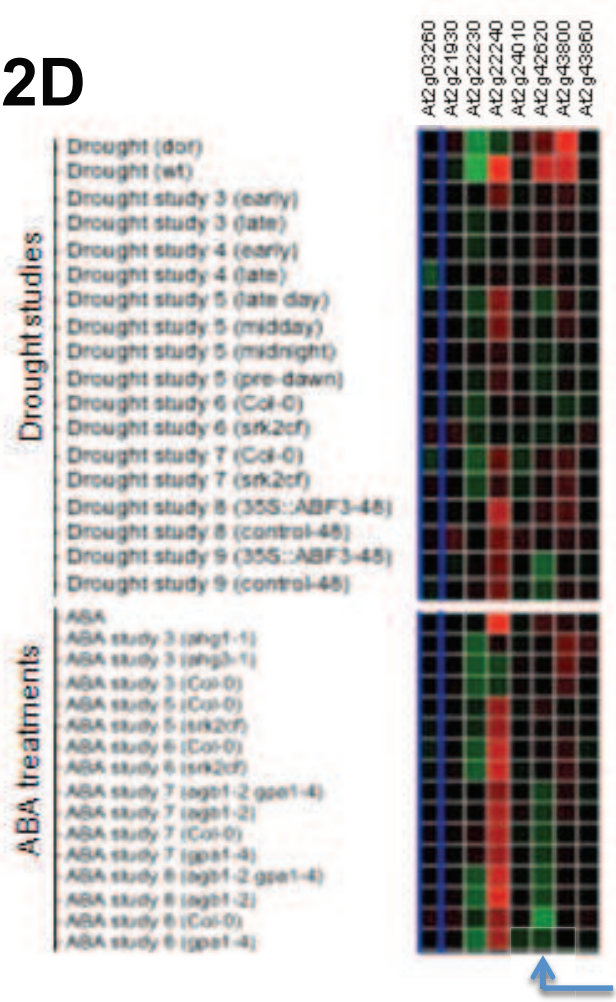
variable	position	LOD	Qvalue	ExpVar	gene	gene Annotation	expression level in the different organs
SLAwater deficit	**2 _ 611200**	**11.1**	**0.000**	**7.0**	**AT2G02320**	**ATPP2-B7 (Phloem protein 2-B7); carbohydrate binding**	
RDWwater deficit	2 _ 991056	10.1	0.081	6.2	AT2G03260	EXS family protein / ERD1/XPR1/SYG1 family protein	sperm cells,root cells
respAREA	**2 _ 9348577**	**15.0**	**0.002**	**9.8**	**AT2G21930**	**F-box family protein**	sperm cells,cotyledons
RSwell-watered	2 _ 9451187	9.4	0.092	4.5	AT2G22230	beta-hydroxyacyl-ACP dehydratase	leaf and root primordia
RSwell-watered	2 _ 9452821	9.4	0.092	4.5	AT2G22240	inositol-3-phosphate synthase isozyme 2 / myo-inositol-1-phosphate synthase 2 / MI-1-P synthase 2 / IPS 2	sperm cells
respAREA	**2 _ 10217046**	**12.8**	**0.008**	**5.2**	**AT2G24010**	**SCPL23 (serine carboxypeptidase-like 23); serine carboxypeptidase**	
respTRL	2 _ 13025977	10.7	0.053	6.7			
RDWwater deficit	**2 _ 14846319**	**14.9**	**0.000**	**8.7**	**AT2G35240**	**plastid developmental protein DAG**	
respTRL	2 _ 14964850	10.5	0.053	7.1			
LeafNbwell-watered	**2 _ 17755076**	**11.5**	**0.000**	**0.5**	**AT2G42620**	**MAX2 (MORE AXILLARY BRANCHES 2); ubiquitin-protein ligase**	seed coat
SDWwell-watered	2 _ 18147312	10.9	0.089	4.6	AT2G43800	formin homology 2 domain-containing protein / FH2 domain-containing protein	
FT LDVwell-watered	2 _ 18163622	11.1	0.087	11.4	AT2G43860	polygalacturose	

See legend on Chromosome 5

2C


At2g03260
At2g21930
At2g22230
At2g22240
At2g24010
At2g42620
At2g43800
At2g43860
callus
cell culture / primary cell
sperm cell
protoplast
root cell
root culture
seedling
inflorescence
rosette
juvenile leaf
adult leaf
petiole
senescent leaf
hypocotyl
leaf primordia
stem
axillary bud
axillary shoot
shoot apex
roots
lateral root
root tip
meristematic zone
elongation zone
root hair zone
endodermis
endodermis+cortex
epid. atrichoblasts
lateral root cap
stele

2D


At2g03260
At2g21930
At2g22230
At2g22240
At2g24010
At2g42620
At2g43800
At2g43860
Drought studies
Drought (dor)
Drought (wt)
Drought study 3 (early)
Drought study 3 (late)
Drought study 4 (early)
Drought study 4 (late)
Drought study 5 (late day)
Drought study 5 (midday)
Drought study 5 (midnight)
Drought study 5 (pre-dawn)
Drought study 6 (Col-0)
Drought study 6 (srk2cf)
Drought study 7 (Col-0)
Drought study 7 (srk2cf)
Drought study 8 (35S::ABF3-48)
Drought study 8 (control-48)
Drought study 9 (35S::ABF3-48)
Drought study 9 (control-48)
ABA treatments
ABA
ABA study 3 (ahg1-1)
ABA study 3 (ahg3-1)
ABA study 3 (Col-0)
ABA study 5 (Col-0)
ABA study 5 (srk2cf)
ABA study 6 (Col-0)
ABA study 6 (srk2cf)
ABA study 7 (aigb1-2 gpa1-4)
ABA study 7 (aigb1-2)
ABA study 7 (Col-0)
ABA study 7 (gpa1-4)
ABA study 8 (aigb1-2 gpa1-4)
ABA study 8 (aigb1-2)
ABA study 8 (Col-0)
ABA study 8 (gpa1-4)

models for root variables was lower than for shoot variables. QTL were detected on the five chromosomes, with a roughly identical repartition of the QTL on each chromosome.

One of the main results of this analysis is the relative continuity in the allelic effect along the five chromosomes. At the top of the chromosome 1, for all the QTL detected, positive effects were associated with alleles of Sha, whereas Bay alleles had a positive effect for all the QTL detected at the bottom of chromosome 1. Bay alleles also had a positive effect on the value of almost all the variables for which QTL were detected on chromosome 3 and 5, whereas Sha alleles were associated with increased values of all variables for QTL detected on chromosome 2 and 4. A second main result of this analysis is the great importance of a few numbers of regions per chromosome, which were involved in the control of several variables. Interestingly, those regions were generally specific to one growth condition (well watered, water deficit), or to the response to water deficit.

On the chromosome 1 (Fig. 1A), four regions appeared: the first region is located around the markers F21M12 and IND4992, and is mainly involved in the control of plant growth under stress, or in response to water deficit conditions, with in particular a very strong effect on Root/shoot response to water deficit. A second region located at the marker MSAT108193 was detected, and was completely "root specific", since Sha alleles increased the value of Root dry weight, Total root length, Root/shoot and Specific root length under well watered conditions, and in water deficit conditions for Root/shoot. Two other regions are involved in the control of global plant growth at the bottom of chromosome 1, around the F5I14-MSAT1.13 region, and at MSAT1.5. In these regions, Bay alleles increased the value of both shoot and root variables, with stronger on shoot variables. Three QTL that explained more than 20% of the variance were detected for Shoot dry weight under water deficit conditions (main effect QTL), for Rosette area response to water deficit and for Specific leaf area response to water deficit.

On chromosome 2 (Fig. 2A), three regions that had very strong effects on all the variables were also detected, around the MSAT2.38, IND628, and CZSOD2 markers. Around these markers, Sha alleles increased the values of variables. Very strong QTL were detected for Root dry weight, Total root length, Shoot dry weight and Rosette area at MSAT 2.38 either in well watered or in water deficit conditions, whereas the response of shoot and root variables to water deficit was mainly controlled by QTL at IND628 and CZSOD2 markers. At the bottom of the chromosome 2, a QTL that explains more than 20 % of the variance of Shoot dry weight and Rosette area under well-watered conditions was detected at the MSAT 2.22.

Bay alleles had a positive effect on the QTL located on the chromosome 3 (Fig. 3A), except for one important

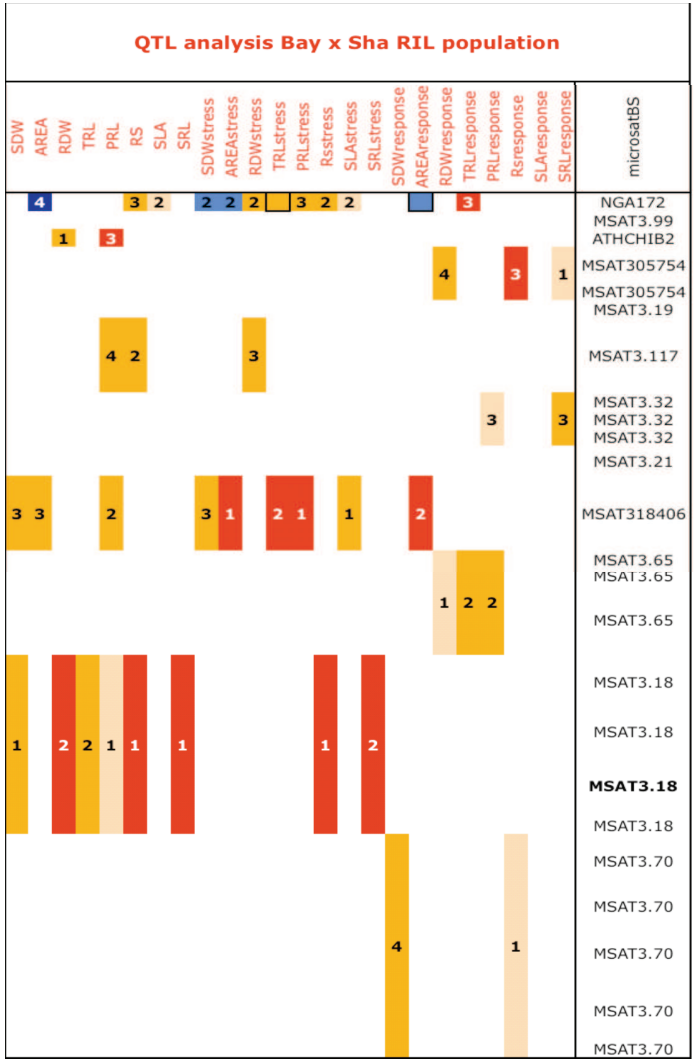
region at the top of the chromosome, near the NGA172 marker. In this region, the effects of QTL associated with shoot variables (Shoot dry weight and Rosette area) were associated with Sha alleles, whereas Bay alleles increased the value of root variables. The same effect of Bay alleles on root variables was observed for QTL located at the following markers (MSAT3.99, AthCHIB2, MSAT305754) with very important QTL related to the Root/shoot response to water deficit in particular. Another important region was detected near the MSAT318406, with a QTL that explain more than 20% of the variance of Rosette area, Total root length, and Primary root length under water deficit, and of the response of Rosette area to water deficit conditions (23% of explained variance). Lower on the chromosome 3, near the MSAT3.18, several QTL were detected, with Bay alleles increasing the value of shoot and root variables, mainly under well-watered conditions, but two QTL explaining more than 20% of the variance (both in interaction with the MSAT5.9 region on chromosome 5) were also detected for Root/shoot and Specific root length under water deficit conditions.

On chromosome 4 (Fig. 4A), QTLs were grouped around the MSAT4.15 marker. An epistatic QTL explaining 21% of the Rosette area variation under well watered in interaction with the NGA172 region on chromosome 3 was detected. Epistatic QTL for Specific leaf area under well watered conditions, and for Shoot dry weight, Total root length and Specific leaf area under water deficit conditions were also detected. Noteworthy, a main effect QTL explaining 12% of the variance of Rosette area response was found in this region.

The QTL explaining the largest part of trait variance on chromosome 5 were observed in five distinct regions (Fig. 5A). The first one is located at the NGA249 marker, and is involved in the control of Shoot dry weight and Rosette area under well-watered conditions in interaction with the MSAT2.22 on chromosome 2. At marker NGA151, a QTL controlling both Shoot dry weight and Primary root length response to water deficit was discovered in interaction with marker IND4992 on chromosome 1. Progressing on the chromosome 5, we found a region (NGA139) specifically involved in the control of shoot and root response to water deficit, with an important epistatic QTL associated with Total root length and Specific leaf area response, and to a lesser extent, to Shoot dry weight, Root dry weight, and Root/shoot response to water deficit. A root specific region was found near the MSAT5.9 marker, with epistatic QTL involved in the control of Root dry weight, Total root length, and Root/shoot under well watered conditions, and Root/Shoot and Specific root length under water deficit conditions. Finally, at the bottom of chromosome 5 (K9I9 marker), several QTL were detected, that were mainly associated with plant growth under water deficit conditions, in interaction with other genomic regions, such as the MSAT318406 region on the chromosome 3, for the control of Rosette area, Primary root length and Specific leaf area under water deficit. Positive effects of QTL on these variable values were associated with the presence of Sha alleles at this K9I9 marker.

Chromosome 3

3A

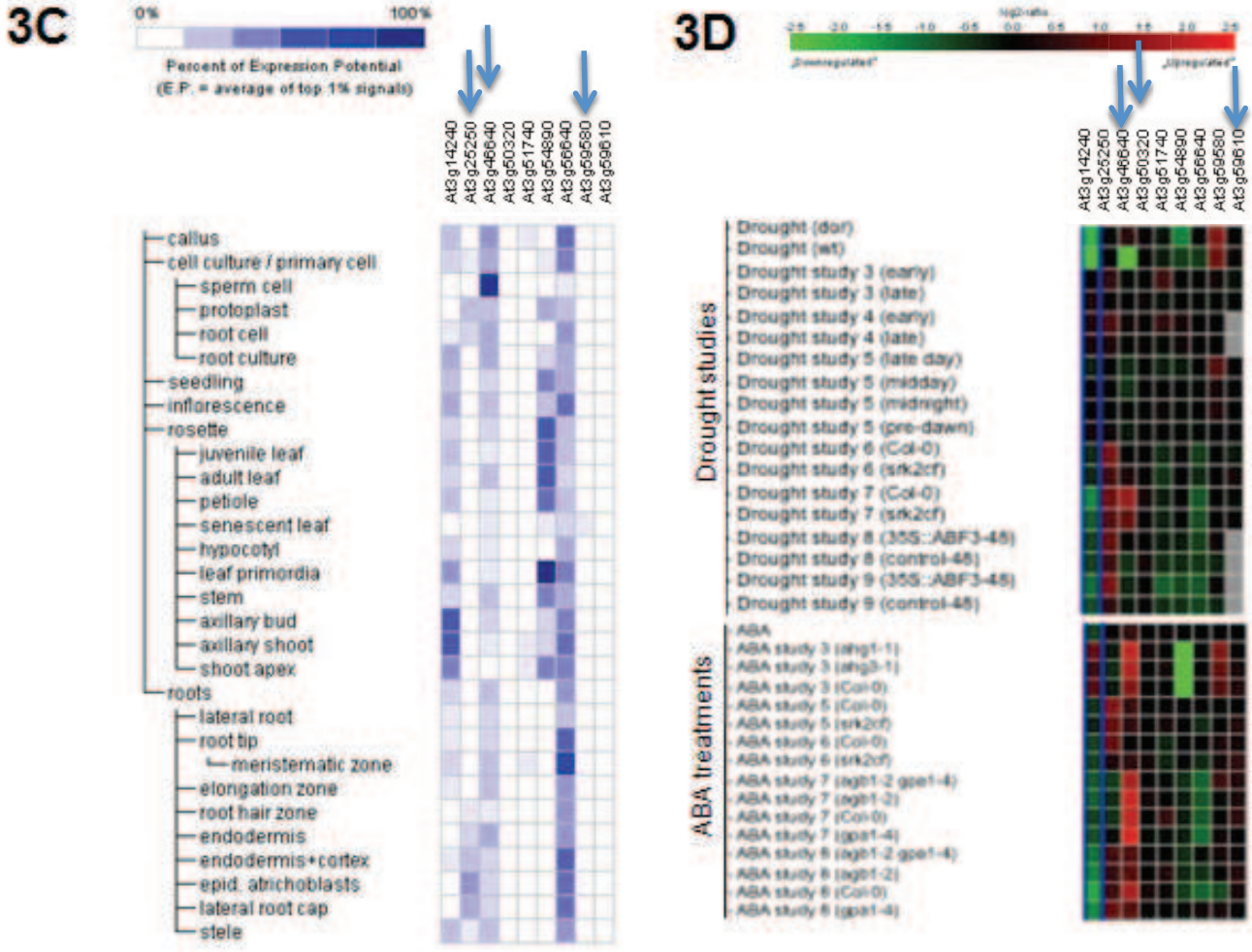


3B

GWA analysis

variable	position	LOD	Qvalue	ExpVar	gene	gene Annotation	expression level in the different organs
AREAwater deficit	3 _ 2520825	12.3	0.031	6.4			
RSwell-watered	3 _ 4743548	9.6	0.092	6.3	AT3G14240	subtilase family protein	shoot apex, ascission zone
respSRL	**3 _ 5107901**	**11.5**	**0.000**	**4.3**			
respTRL	3 _ 7881224	10.2	0.053	5.0			
RSwell-watered	**3 _ 9197443**	**13.4**	**0.014**	**6.9**	**AT3G25250**	**AGC2-1 (OXIDATIVE SIGL-INDUCIBLE1); kise**	
respTRL	3 _ 9784927	10.9	0.053	5.7			
respTRL	3 _ 12648056	11.4	0.053	4.7			
SDWwater deficit	3 _ 14774571	11.9	0.047	6.1			
FT LDwell-watered	**3 _ 17185050**	**11.8**	**0.000**	**4.2**	**AT3G46640**	**PCL1 (PHYTOCLOCK 1); DNA binding / transcription factor**	seeds, sperm cells
RSwell-watered	3 _ 18654580	9.6	0.092	6.0	AT3G50320	unknown protein	
RDWwater deficit	3 _ 19188282	11.6	0.042	5.7			
SDWwater deficit	3 _ 19189478	12.9	0.032	6.5	AT3G51740	IMK2 (INFLORESCENCE MERISTEM RECEPTOR-LIKE KISE 2); ATP binding / kise/ protein serine/threonine kise	
SRLwell-watered	**3 _ 20337151**	**13.2**	**0.008**	**1.3**	**AT3G54890**	**LHCA1; chlorophyll binding**	leaf primordia, rosette
RDWwater deficit	3 _ 20980556	12.7	0.020	8.0	AT3G56640	exocyst complex subunit Sec15-like family protein	
respAREA	**3 _ 20980556**	**17.2**	**0.000**	**8.9**	**AT3G56640**	**exocyst complex subunit Sec15-like family protein**	pollen, seedlings, xylem cells
respTRL	3 _ 21030118	10.5	0.053	6.4			
AREAwater deficit	**3 _ 22012236**	**14.7**	**0.003**	**7.6**	**AT3G59580**	**RWP-RK domain-containing protein**	rosette, inflorescence
SDWwater deficit	3 _ 22012236	10.6	0.079	5.6	AT3G59580	RWP-RK domain-containing protein	
AREAwater deficit	**3 _ 22019713**	**14.7**	**0.003**	**7.6**	**AT3G59610**	**F-box family protein / jacalin lectin family protein**	
SDWwater deficit	3 _ 22019713	10.6	0.079	5.6	AT3G59610	F-box family protein / jacalin lectin family protein	
respSLA	3 _ 23048931	12.1	0.037	8.6			

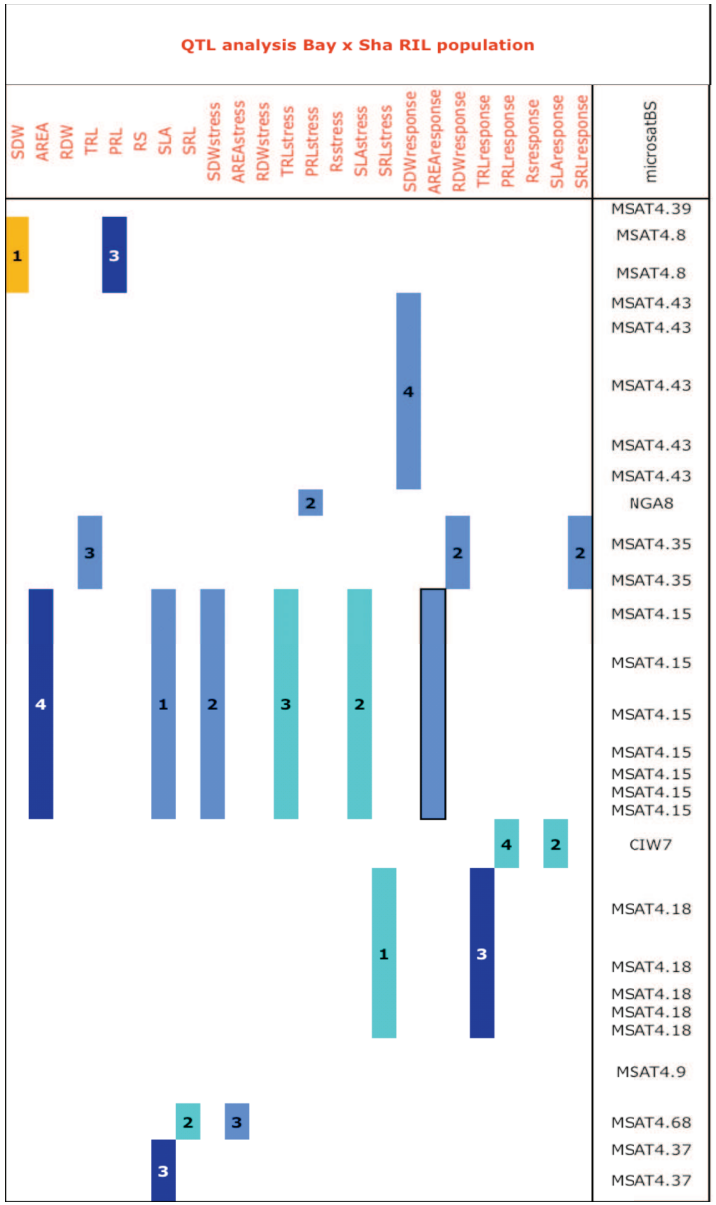
See legend on Chromosome 5


3C
0%
100%
Percent of Expression Potential
(E.P. = average of top 1% signals)
At3g14240
At3g25250
At3g46640
At3g50320
At3g51740
At3g54890
At3g56640
At3g59580
At3g59610
callus
cell culture / primary cell
sperm cell
protoplast
root cell
root culture
seedling
inflorescence
rosette
juvenile leaf
adult leaf
petiole
senescent leaf
hypocotyl
leaf primordia
stem
axillary bud
axillary shoot
shoot apex
roots
lateral root
root tip
meristematic zone
elongation zone
root hair zone
endodermis
endodermis+cortex
epid. atrichoblasts
lateral root cap
stele
3D
Drought studies
Drought (dor)
Drought (wt)
Drought study 3 (early)
Drought study 3 (late)
Drought study 4 (early)
Drought study 4 (late)
Drought study 5 (late day)
Drought study 5 (midday)
Drought study 5 (midnight)
Drought study 5 (pre-dawn)
Drought study 6 (Col-0)
Drought study 6 (srk2cf)
Drought study 7 (Col-0)
Drought study 7 (srk2cf)
Drought study 8 (35S::ABF3-48)
Drought study 8 (control-48)
Drought study 9 (35S::ABF3-48)
Drought study 9 (control-48)
ABA treatments
ABA
ABA study 3 (ahg1-1)
ABA study 3 (ahg3-1)
ABA study 3 (Col-0)
ABA study 5 (Col-0)
ABA study 5 (srk2cf)
ABA study 6 (Col-0)
ABA study 6 (srk2cf)
ABA study 7 (agb1-2 gpa1-4)
ABA study 7 (agb1-2)
ABA study 7 (Col-0)
ABA study 7 (gpa1-4)
ABA study 8 (agb1-2 gpa1-4)
ABA study 8 (agb1-2)
ABA study 8 (Col-0)
ABA study 8 (gpa1-4)
At3g14240
At3g25250
At3g46640
At3g50320
At3g51740
At3g54890
At3g56640
At3g59580
At3g59610

4A

4B

Chromosome 4

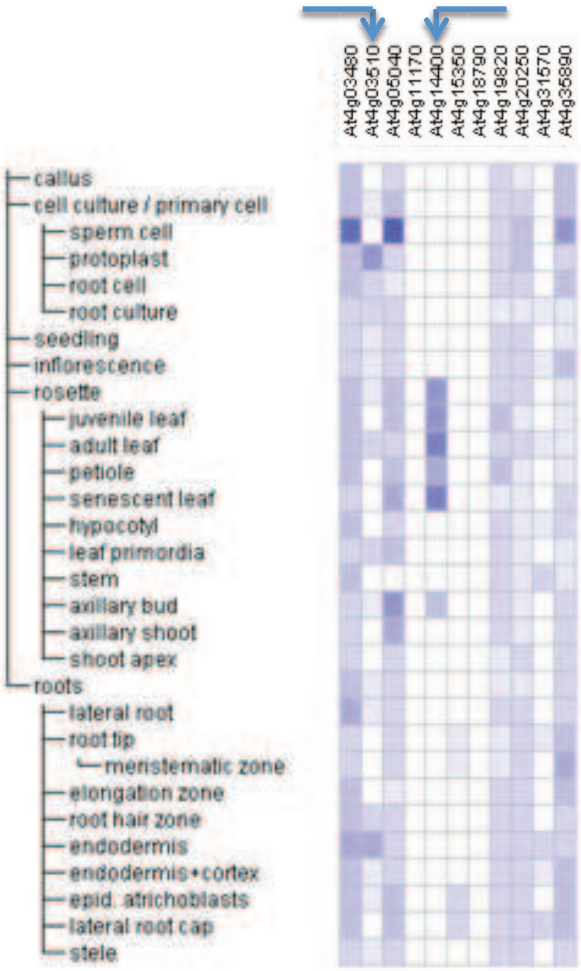


GWA ANALYSIS

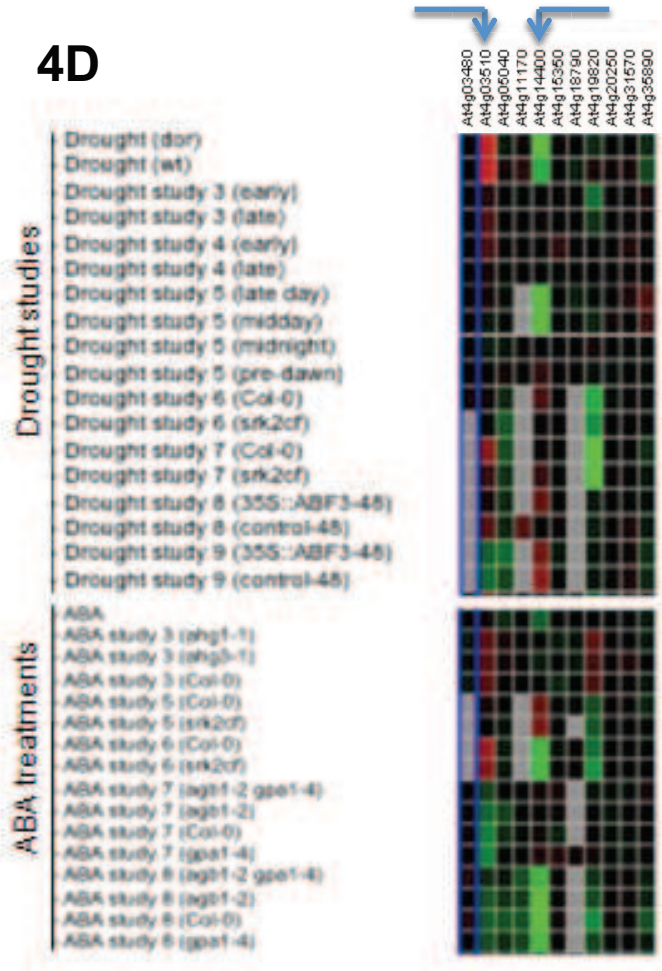
variable	position	LOD	Qvalue	ExpVar	gene	gene Annotation	expression level in the different organs
FT LDwell-watered	4 _ 199812	10.8	0.104	13.8			
respRS	**4 _ 494900**	**10.6**	**0.000**	**6.1**	**AT4G01160**	**BTB/POZ domain-containing protein**	
respTRL	4 _ 1055517	11.2	0.053	9.0			
RDWwell-watered	4 _ 1547716	13.5	0.012	8.0	AT4G03480	ankyrin repeat family protein	
RDWwell-watered	**4 _ 1558040**	**16.3**	**0.000**	**9.0**	**AT4G03510**	**RMA1 (Ring finger protein with Membrane Anchor 1); protein binding / ubiquitin-protein ligase/ zinc ion binding**	
respPRL	**4 _ 1744937**	**11.7**	**0.000**	**3.9**			
RDWwell-watered	4 _ 2580311	12.1	0.037	6.7	AT4G05040	ankyrin repeat family protein	
respTRL	**4 _ 5427593**	**12.7**	**0.000**	**6.4**			
PRLwater deficit	4 _ 6811043	11.4	0.077	3.6	AT4G11170	disease resistance protein (TIR-NBS-LRR class)	
RDWwater deficit	4 _ 7706537	10.9	0.067	7.0	AT4G13266	unknown protein	
respSLA	4_8296955	11.3	0.091	8.1	AT4G14400	ACD6 (ACCELERATED CELL DEATH 6); protein binding	
SLAwater deficit	4_8297198	10.5	0.042	6.1	AT4G14400	ACD6 (ACCELERATED CELL DEATH 6); protein binding	rosette, cauline leaves
respAREA	4_8298111	9.2	0.081	7.9	AT4G14400	ACD6 (ACCELERATED CELL DEATH 6); protein binding	
respSLA	**4 _ 8764497**	**12.5**	**0.000**	**7.5**	**AT4G15350**	**CYP705A2 (cytochrome P450**	
AREAwell-watered	4 _ 8826651	11.7	0.087	8.8			
RSwell-watered	4 _ 8933225	10.0	0.092	5.4			
respAREA	**4 _ 8960621**	**14.8**	**0.002**	**8.8**			
RSwell-watered	**4 _ 10037409**	**16.3**	**0.001**	**9.9**	**AT4G18110**	**zinc finger (C3HC4-type RING finger) family protein**	
LeafNbwater deficit	**4 _ 10319235**	**13.8**	**0.000**	**6.6**	**AT4G18790**	**NRAMP5 (NRAMP metal ion transporter 5); metal ion transmembrane transporter**	stamen
FT LDwell-watered	4 _ 10768200	11.0	0.104	1.7	AT4G19820	glycosyl hydrolase family 18 protein	
SRLwater deficit	**4 _ 10939704**	**9.9**	**0.000**	**7.4**	AT4G20250	unknown protein	
respTRL	4 _ 12208456	11.1	0.053	6.2			
RSwell-watered	4 _ 12341689	9.7	0.092	6.2			
PRLwater deficit	4 _ 14604738	11.5	0.077	8.6	AT4G29860	EMB2757/TAN (EMBRYO DEFECTIVE 2757); nucleotide binding	
SRLwell-watered	**4 _ 15305343**	**14.1**	**0.005**	**5.4**	**AT4G31570**	**unknown protein**	
SRLwell-watered	**4 _ 16260390**	**13.3**	**0.008**	**6.1**			
respRDW	**4 _ 16999346**	**10.7**	**0.000**	**6.4**	**AT4G35890**	**La domain-containing protein**	stamen

See legend on Chromosome 5

4C


At4g03480
At4g03510
At4g05040
At4g11170
At4g14400
At4g15350
At4g18790
At4g19820
At4g20250
At4g31570
At4g35890
callus
cell culture / primary cell
sperm cell
protoplast
root cell
root culture
seedling
inflorescence
rosette
juvenile leaf
adult leaf
petiole
senescent leaf
hypocotyl
leaf primordia
stem
axillary bud
axillary shoot
shoot apex
roots
lateral root
root tip
meristematic zone
elongation zone
root hair zone
endodermis
endodermis+cortex
epid. atrichoblasts
lateral root cap
stele

4D


At4g03480
At4g03510
At4g05040
At4g11170
At4g14400
At4g15350
At4g18790
At4g19820
At4g20250
At4g31570
At4g35890
Drought studies
Drought (dor)
Drought (wt)
Drought study 3 (early)
Drought study 3 (late)
Drought study 4 (early)
Drought study 4 (late)
Drought study 5 (late day)
Drought study 5 (midday)
Drought study 5 (midnight)
Drought study 5 (pre-dawn)
Drought study 6 (Col-0)
Drought study 6 (srk2cf)
Drought study 7 (Col-0)
Drought study 7 (srk2cf)
Drought study 8 (35S::ABF3-48)
Drought study 8 (control-48)
Drought study 9 (35S::ABF3-48)
Drought study 9 (control-48)
ABA treatments
ABA
ABA study 3 (ahg1-1)
ABA study 3 (ahg3-1)
ABA study 3 (Col-0)
ABA study 5 (Col-0)
ABA study 5 (srk2cf)
ABA study 6 (Col-0)
ABA study 6 (srk2cf)
ABA study 7 (agb1-2 gpa1-4)
ABA study 7 (agb1-2)
ABA study 7 (Col-0)
ABA study 7 (gpa1-4)
ABA study 8 (agb1-2 gpa1-4)
ABA study 8 (agb1-2)
ABA study 8 (Col-0)
ABA study 8 (gpa1-4)

GWA mapping of plant growth under different water regimes

We conducted genome-wide association (GWA) mapping of the variables that were used for the QTL analysis in a collection of 88 accessions, using the phenotypic data described in the previous chapters, and adding flowering time measurements (in long days, or in long days with vernalization).

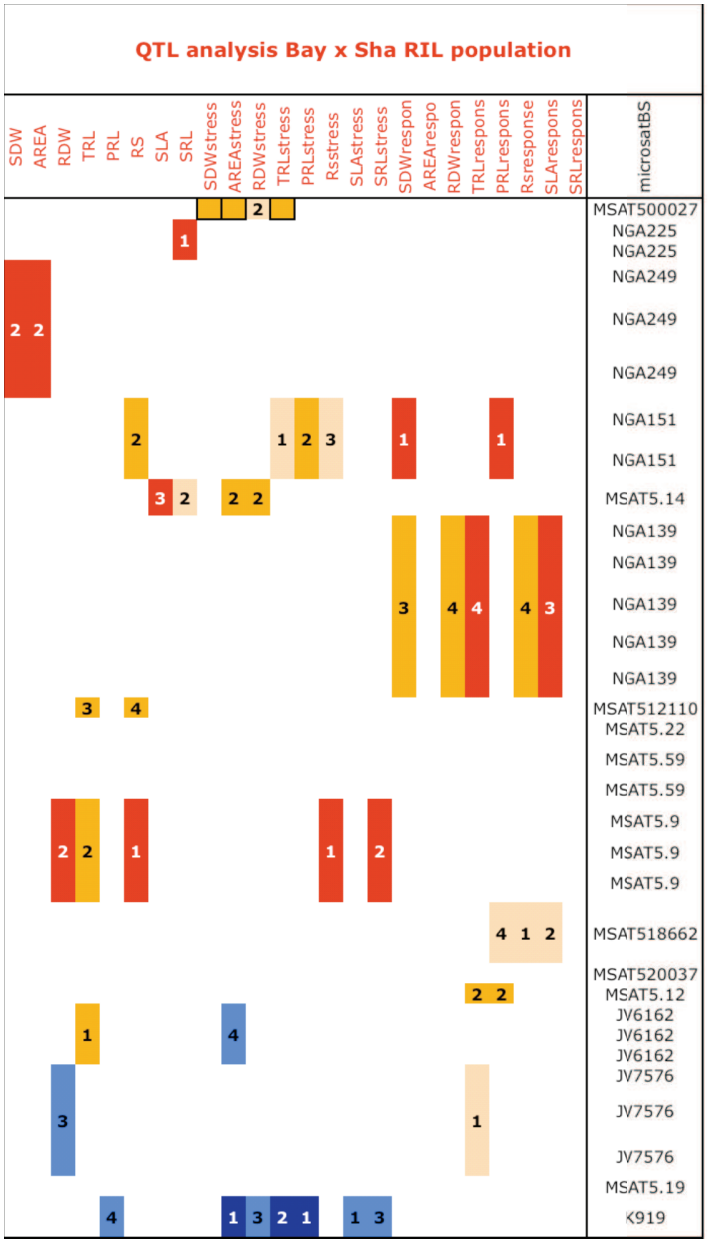
A total of 114 significant associations (*i.e.* with a Q-value of *False Discovery rate* <0.1) were detected for the eight variables studied, under well-watered, water deficit, and in response to water deficit conditions (Fig.1B, 2B, 3B, 4B, 5B). 50% (69) of these SNP were in coding sequences. 10 SNP on average were associated with the variation of each variable, and these SNP explained on average 6% of the trait variance, with a LOD of 12. The maximal LOD (19.6) corresponded to a SNP associated with Primary root length on the chromosome 5. As for the QTL analysis, the most significant SNP detected will be described for each chromosome, progressing from top to bottom. At the same time, they will be compared to the QTL detected for the same region in the QTL analysis, and the genes associated with these regions, if any, will be identified. Finally, using data available in the databases (ATH1 in Genevestigator), expression profiles of these genes in the different organs of the plant (rosette, roots, seed, flower) (Fig.1C, 2C, 3C, 4C, 5C), and in response to different growth conditions related to drought (soil water deficit, ABA treatment) (Fig.1D, 2D, 3D, 4D, 5D) will be presented, chromosome by chromosome.

Ten highly significant (Qvalue <0.01, shown **in bold in Fig 1B**) associations were detected on the chromosome 1 (Fig. 2A). One SNP located at the position 1_445336 accounted for 4.8% of the Specific root length variation under well-watered conditions (LOD =16.9), and was located in the gene AT1G02270, encoding an endonuclease. Three SNP were detected for the response of the Rosette area and Shoot dry weight to water deficit, and for Specific leaf area under well-watered conditions in the same region (around 1_4 450 000), and two of them were located in different genes, AT1G12805 encoding a nucleotide binding protein, and the other AT1G13390 gene for an unknown protein. A SNP associated with the Rosette area response to water deficit was detected at the position 1_6696977, with a LOD of 16.1. Another SNP associated with total root length response to water deficit was located in the same region. Interestingly, we also found QTL associated with the same trait in this region in the QTL analysis BayxSha, near the MSAT108193. Going down on the chromosome 1, three SNP were detected with a LOD score higher than 12, for primary root length under water deficit (1_13

5A

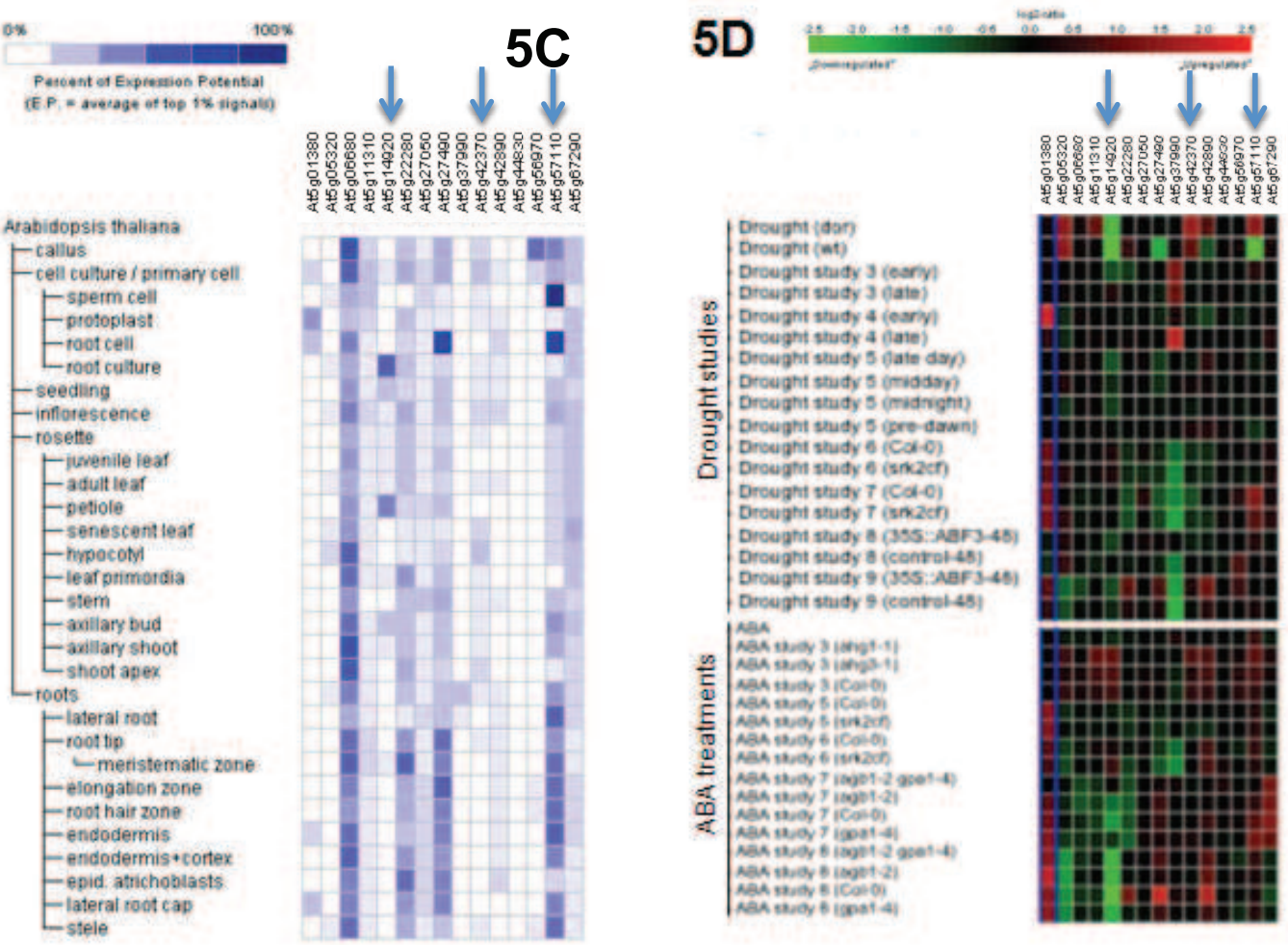
5B

Chromosome 5

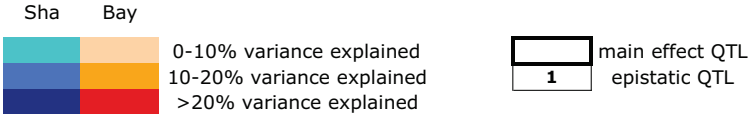


GWA analysis

variable	position	LOD	Qvalue	ExpVar	gene	gene Annotation	expression level in the different organs
SDWwater deficit	5 _ 11755	10.6	0.079	5.4			
respAREA	5 _ 157245	12.3	0.010	4.7	AT5G01380	transcription factor	protoplasts
SDWwell-watered	**5 _ 1576662**	**12.0**	**0.000**	**7.4**	**AT5G05320**	**monooxygenase**	
AREAwater deficit	5 _ 1587684	11.0	0.047	7.3			
respSLA	5 _ 2055081	11.0	0.080	3.5	AT5G06680	SPC98 (SPINDLE POLE BODY COMPONENT 98)	
RSwell-watered	5 _ 3608211	10.2	0.089	7.1	AT5G11310	pentatricopeptide (PPR) repeat-containing protein	seeds
respAREA	5 _ 4826809	11.4	0.040	6.3	AT5G14920	gibberellin-regulated family protein	
respAREA	**5 _ 7375002**	**12.2**	**0.011**	**7.1**	**AT5G22280**	**unknown protein**	root, seeds
SRLwell-watered	5 _ 7379992	11.7	0.031	5.5			
SDWwater deficit	5 _ 7420326	10.5	0.079	6.4			
AREAwell-watered	**5 _ 9520549**	**12.3**	**0.000**	**7.0**	**AT5G27050**	**AGL101; transcription factor**	
RDWwater deficit	5 _ 9704450	10.2	0.081	4.4	AT5G27490	integral membrane Yip1 family protein	roots, root cells
respTRL	5 _ 15134383	10.6	0.053	3.9	AT5G37990	S-adenosylmethionine-dependent methyltransferase	
RDWwater deficit	5 _ 15364518	10.7	0.070	6.6	AT5G38386	F-box family protein	
PRLwell-watered	**5 _ 16939987**	**19.6**	**0.000**	**8.8**	**AT5G42370**	**unknown protein**	
SDWwell-watered	5 _ 16939987	11.3	0.089	5.2			
TRLwell-watered	**5 _ 17194533**	**10.8**	**0.000**	**4.6**	**AT5G42890**	**sterol carrier protein 2 (SCP-2) family protein**	
RDWwater deficit	5 _ 18105433	10.5	0.076	5.3	AT5G44840	glycoside hydrolase family 28 protein / polygalacturose (pectise) family protein	
AREAwater deficit	5 _ 21654684	11.6	0.040	7.1			
respAREA	**5 _ 21654684**	**13.2**	**0.007**	**6.3**			
SDWwater deficit	5 _ 21654684	12.2	0.045	7.0			
SRLwell-watered	5 _ 21896890	10.9	0.046	7.2			
FT LDVwell-watered	5 _ 23045053	11.7	0.087	10.1	AT5G56970	CKX3 (CYTOKININ OXIDASE 3); cytokinin dehydrogese	
RSwell-watered	**5 _ 23116869**	**16.9**	**0.000**	**10.3**	**AT5G57110**	**ACA8 (AUTOINHIBITED CA2+ - ATPASE**	roots, root cells
SRLwell-watered	5 _ 24117724	10.6	0.049	7.2			
RDWwater deficit	5 _ 26846389	10.9	0.067	3.5	AT5G67290	FAD-dependent oxidoreductase family protein	seeds



Common legend to Figures 1-5: For each of the five chromosomes: **A**. **Results of the QTL analysis in the Bay x Sha RIL population, for eight growth and biomass partitioning variables** (SDW, Shoot dry weight, AREA, Rosette area, RDW, Root dry weight, TRL, Total root length, PRL, Primary root length, RS, Root/shoot ratio, SLA, Specific leaf area, SRL, Specific root length), under well-watered, water deficit (stress), or in response to water deficit conditions (response).The 69 microsatellites markers of the Bay xSha genetic map are indicated. When Sha alleles increase the trait value, the QTL is blue (orange when Bay alleles increase the trait value). The darker the color is, the higher the variance explained by the QTL is. Main effects QTL are black framed, and epistatic ones are indicated with the same number for both QTL in epistasis. **B**. **Results of the genome wide association study on the 88 accessions**, for the same variables than in the QTL analysis, and for Leaf number under well-watered, water deficit, and in response to water deficit, and for Flowering time under long day well-watered conditions (FT LD) and Flowering time under long day well-watered with a prolonged cold treatment that mimics vernalization process that occurs into the wild (FT LDV). Only the significant associations are presented (q-value of *False Discovery Rate* <0.1). The most significant associations (q-value of *False Discovery Rate* < 0.01) are in bold characters. For each significant association detected, the SNP position, the LOD score, the percentage of variance explained by the association, and the Q-value of *False Discovery Rate* are indicated. The gene in which the SNP detected is located is indicated (number and annotation). A summary of the results of the expression profiles of these genes in the different organs/tissues (see C) is given to facilitate the interpretation of the figure. **C. Heat map of expression profiles of the genes detected in the different plant organs/tissues**. **D. Heat map of the expression profiles of the genes detected in response to drought conditions or ABA treatments**, in different studies reported in the literature. (C,D, the number of genes indicated is lower than the number of genes detected in the GWA studies, because expression data of some of these genes were not available in the Genevestigator database. When information were not available, the cases are grey colored).



458 534), for the rosette area response (1_ 18 232 779) and for Root/shoot under water deficit. The later SNP detected was located in a gene (At1g52130) encoding an F-box protein. Three SNP controlling flowering time variation under long day with vernalization were also detected at the bottom of chromosome 1, corresponding to the region around the F5I14 and MSAT 1.13 markers, that were shown to have a strong effect on several variables in the QTL analysis. The SNP detected in this region all encode different genes. In this region, a gene coding for a vacuolar invertase (At1g62660) was identified, and its expression profiles indicates that it is specifically expressed in roots (Fig. 1C), and is differentially expressed according to drought (downregulation) and ABA treatments (upregulation) (Fig. 1D).

Five highly significant associations were detected on chromosome 2 (Fig. 2B). The first one controlled the specific leaf area under water deficit conditions (LOD = 11.1) in both QTL and GWA analysis, and is located in a gene encoding a carbohydrate binding protein (At2g02320). Unfortunately, no information about the expression of this gene was available. Two SNP accounting for the variation of rosette area response to water deficit were identified, and colocalized with several QTL controlling root and shoot traits in the RIL population. These two SNP were located in two different genes (At2g21930 and At2g24010) encoding an F-box family protein and a serine carboxypeptidase respectively (2B). A SNP associated with the root dry weight variation under water deficit (2_ 14846319) was detected and the corresponding gene encodes a plastid developmental protein. This SNP colocalized with a QTL controlling the variation of the root/shoot ratio in the QTL analysis. Three SNP controlling the variation of shoot traits (Leaf number, shoot dry weight and flowering time under well-watered conditions) were detected at the bottom of the chromosome 2 in both QTL and GWA studies (MSAT 2.22), and were associated with three different genes, one of them (At2g42620) encoding an ubiquitin ligase called *MORE-AXILLARY-BRANCHES-2,* that is specifically expressed in the stele (Fig. 2C), and shows little variation in response to ABA (Fig. 2D).

A region specifically controlling root variables (root/shoot well-watered, specific root length and total root length responses to water deficit) was identified at the top of chromosome 3, in both QTL (MSAT 305754, MSAT3.19, MSAT3.117, MSAT3.32) and GWA analyses (positions 3_4 743 548 to 3_12 648 056) (Fig. 3B). One gene (At3g25250), encoding an oxidative-signal inducible kinase was expressed root tissues (Fig. 3C) and apparently upregulated by drought conditions (Fig. 3D) was detected in this large region. Another interesting SNP was associated with flowering time variation in long days (3_17 185 050). Several QTL were detected at this locus in the BayxSha population, with very strong effects on shoot and root growth. This SNP was associated with a transcription factor (At3g46640) called *PHYTOCLOCK1*, expressed in seeds and sperm or proliferating cells (Fig. 3C), and strongly upregulated in response to ABA treatments (Fig. 3D). Another region of the chromosome

3 also affected root and shoot variables in both QTL (MSAT 3.18) and GWA analysis (positions 3_20 337 151 to 3_20 980 556). A SNP associated with the rosette area response to water deficit (3_20 980 556) displayed a very high LOD score (17.2) and explained a significant part of the variance of this trait (9%). At the very bottom of this chromosome, a region specifically controlling shoot traits (shoot dry weight and specific leaf area responses to water deficit) in the RIL population (near MSAT 3.70) colocalized with SNP associated with shoot traits (rosette area and shoot dry weight under water deficit conditions, and specific leaf area response to water deficit), some of them displaying high LOD score values (14.7). The two SNP associated with rosette area response corresponded to genes encoding a RWP-RK domain protein (At3g59580) slightly upregulated in response to drought conditions or ABA treatments, and an F-box protein, poorly expressed, and showing no variation with drought or ABA.

Nine SNP controlling root variables (root dry weight, primary and total root length, root/shoot) under different conditions (well watered, water deficit and in response to water deficit) were detected at the top of chromosome 4 (Fig. 4B). This region was associated with a QTL of shoot dry weight response to water deficit in the QTL analysis (Fig. 4A). One of these SNP had a very high LOD score (16.3), and the corresponding gene (At4g03510) encodes a Ring finger protein RMA1. This gene is expressed in leaves, and moderately upregulated in response to drought or ABA (Fig. 4C and 4D). A very interesting region was identified in the middle of chromosome 4, both in QTL analysis (MSAT 4.15) and in GWA study (around 4_8 300 000), and was associated with the variation of shoot variables such as specific leaf area, and rosette area, under water deficit, and in response to water deficit. Sha alleles increased the values of these variables in the QTL analysis, and noteworthy, in the GWA studies, Bay-0 and Shahdara had different alleles at one specific SNP (4_ 8 298 111), with Sha alleles increasing the value of rosette area response to water deficit. This SNP was located in a gene called *ACD6,* encoding a transmembrane ankyrin repeat protein, specifically expressed in rosettes and cauline leaves (Fig. 4C), and downregulated in several rought experiments (Fig. 4D). Another SNP (4_ 8 764 497) associated with specific leaf area response to water deficit identified in this region, a little bit far away on the chromosome 4, encodes a cytochrome P450 (At4g15350). A SNP associated with the Root/shoot variation under well-watered conditions was detected (4_ 10 037 409), with a high LOD score value (16.3), and was located in a gene (At4g18110) encoding a zinc-finger protein. A SNP associated with specific root length variation under water deficit conditions was detected (4_10 939 704) and colocalized with QTL for the same variable in the RIL population (near the MSAT 4.18). Finally, SNP controlling specific root length well-watered and root dry weight response to water deficit were identified at the very bottom of the chromosome 4, with averaged LOD scores of 12 (4B).

Three regions controlling growth response to water deficit were identified at the top of the chromosome 5 (Fig. 5B). The first SNP controlling shoot dry weight under well-watered conditions colocalized with QTL for the same variable in the RIL population, at the NGA249. In the NGA151 region, controlling several root and shoot traits in the QTL analysis, and especially shoot dry weight response to water deficit, one SNP was also associated with rosette area response to water deficit (5_4 826 809). This SNP was located in a gene (At5g14920) encoding a gibberellin regulated family protein that appeared to be downregulated in response to drought or ABA treatments (Fig. 5D). Around the NGA 139 marker, two SNP associated with variation of rosette area were detected (5_7 375 002 and 5_9 520 549). The latter is located in a gene encoding an agamous-like transcription factor (At5g27050). A very significant SNP (LOD score of 19.6), associated with primary root length under well-watered conditions in the GWA study (5_16 939 987) and to other root variables in the QTL study was identified, corresponding to a gene encoding an unknown protein (At5g42370) that was not differentially expressed in the different organs and in response to drought/ABA treatments (Fig. 5C and 5D). Two other SNP were identified at the bottom of the chromosome 5. The first one (5_21 654 684) was associated with the rosette area response to water deficit, and colocalized with a QTL controlling the rosette area under water deficit conditions in the RIL population (JV6162). The last one (5_23 116 869) was associated with the variation of root/shoot under well watered conditions with a very high LOD score (16.9), and corresponded to a gene expressed in roots (5C) encoding an ATPase *ACA8.*

Discussion

Genomic regions controlling either shoot or root growth were detected in both QTL and GWA analyses

Shoot and root growth are strongly interdependent through physiological connections related to carbon, mineral, and water exchange (see "Context" part, Poorter and Nagel, 2000). At the genetic level, under optimal growth conditions, root and shoot traits can also controlled by similar regions, in several species (see Chapter 1; Hund *et al.,* 2004; Cui *et al.,* 2008). Here, QTL specific of either shoot or root growth variables were identified. For instance, at the bottom of chromosome 2, near the MSAT 2.22 (around 2_18150000 for GWA), QTL specific of Shoot dry weight, Rosette area, and Leaf number were detected under well watered conditions. Two other regions specifically controlling shoot growth traits were identified, on the chromosome 4 and at the top of chromosome 5. On chromosome 4, this region was located near the MSAT4.15 marker, corresponding to a

region containing the ACD6 gene (Rate *et al.,* 1999; see end of this discussion section). This region was associated with the control of Rosette area and Specific leaf area under well watered conditions, but also to Shoot dry weight and Specific leaf area under water deficit conditions, and to the maintenance of Rosette area under water deficit conditions (main effect QTL), both for QTL and GWA analysis. No root specific QTL were detected in this region. On chromosome 5, QTL controlling Shoot dry weight and Rosette area under well watered conditions were detected near the NGA249 marker in both analyses.

Root specific QTL were also identified. For instance, near the MSAT108193 marker, QTL controlling root dry weight, total root length, root/shoot and specific root length were identified, under well watered conditions, water deficit conditions, and in response to water deficit conditions. Another root specific region was detected near the MSAT3.117, on the chromosome 3.

Interestingly, genomic regions affecting shoot and root growth variables together (F5I14, MSAT2.38, MSAT3.18, NGA172) identified in this study were also detected in previous hydroponic experiments (see Chapter1), with similar allelic effect. At the bottom of chromosome 1 (F5I14), and at the middle of chromosome 3 (MSAT3.18), Bay alleles were associated with plant growth, whereas shoot and growth are increased when Sha alleles were present on chromosome 2 (MSAT2.38). Unfortunately, Bay-0 and Shahdara carried the same alleles for almost all the SNP detected in the GWA study, which did not allow distinguishing these effects.

QTL specific to water deficit conditions affect root growth

When the environmental conditions are modified such as water deficit (chap 2) or mineral supply (eg Lopez-Bucio *et al.,* 2003), the strong root-shoot correlation is altered. For instance, under low phosphate, correlation between shoot dry weight and seminal root length in maize was moderate (Zhu *et al.,* 2006). In Arabidopsis, low nitrate conditions led to a lack of common QTL between root and shoot variables (Rauh *et al.,* 2002). Soil water deficit is known to uncouple shoot and root growth by changes in carbon metabolism or water fluxes (Muller *et al.,* 2011). Therefore, one of the hypothesis of the genetic analysis presented here was that water deficit could uncouple the strong overlap between shoot and root growth QTLs. Our analysis confirmed this hypothesis, since genomic regions controlling growth variables specifically under water deficit conditions were detected.

Most of the regions specifically identified under water deficit conditions, or in response to water deficit conditions,

were associated with root growth variation. Two root specific region were identified at the top of chromosome 3 in the RIL population (MSAT305754, MSAT3.32), and in the GWA study. These regions were also identified in other studies, with QTL of Lateral root length (Loudet *et al.,* 2005), or water and nitrate content variation (Loudet *et al.,* 2003). Another region specific of root growth under water deficit was found at the MSAT 3.65 marker. On chromosome 4 (MSAT4.35), QTL detected in both QTL and GWA analysis were associated with root growth variation.

Five candidate genes affecting tolerance to water deficit identified in QTL and GWA studies

Ten regions on average were associated with plant growth tolerance to soil water deficit in both QTL and GWA. Five of these regions were common to both analyses. The first common region is located at the top of chromosome 1, and encodes a nucleotide binding protein (At1g12805). A second common region responsible for plant growth response to water deficit was detected on chromosome 2, near the CZSOD2 marker, and covers two genes involved in this response, the At2g21930 gene, encoding an F-box protein that is expressed in sperm cells only, and the At2g24010 that encodes a serine carboxy-peptidase, expressed in all the organs. The role of these two enzyme families on plant response to water deficit has been previously reported. F-Box family genes are required for panicle, leaf, and seed development (Durfee *et al*. 2003; Woo *et al*. 2001) and are regulated by light and temperature stress (Oono *et al.,* 2006; Calderon-Villalobos *et al.* 2007). Several F-box proteins have been reported to be involved in the ABA pathway (AtTLP9, Ko *et al.,* 2006) and in response to drought stress (Qin *et al.,* 2009), and another F-box gene detected in this study and controlling shoot growth at the bottom of chromosome 2, *ORE9* (Woo *et al.,* 2001) is induced by drought conditions. Carboxypeptidases are enzymes responsible for the release of free amino acids by hydrolyzing a peptidic bond, and have been shown to be overexpressed under drought conditions (Kawasaki *et al.,* 2000).

A third region controlling plant tolerance to drought in both QTL and GWA analyses is located at the bottom of chromosome 3, near the MSAT3.70 marker. This region covers a gene encoding a Receptor like protein kinase (RWP-RK), with little expression level. This gene family is known to be involved in nitrogen sensing and metabolism (Castaings *et al.,* 2009), and seems to be slightly overexpressed by drought conditions and ABA treatments.

The fourth region affecting plant tolerance to drought in both studies is located at the middle of the chromosome 4 (MSAT 4.15). This region covers a gene called *ACD6* for *accelerated cell death 6* that encodes a transmembrane ankyrin protein (Rate *et al.,* 1999) mainly expressed in rosette and cauline leaves. This gene is responsible for a trade-off between plant growth and susceptibility to a wide range of pathogens (Todesco *et al.,* 2010). A specific allele of ACD6, that differs from the reference allele of Col-0, strongly enhances resistance to a broad range of pathogens, but at the same time slows the production of new leaves and greatly reduces the biomass of mature leaves. This allele segregates at intermediate frequency throughout the worldwide range of Arabidopsis thaliana, consistent with this allele providing substantial fitness benefits despite its marked impact on growth. In our QTL study, Sha allele at the marker MSAT4.15, close to ACD6, contributed to plant growth maintenance (Rosette area, Shoot dry weight and Specific leaf area) under drought conditions. Interestingly, Bay-0 and Shahdara accessions had different alleles of ACD6 in the GWA study, again with Sha allele maintaining plant growth under drought conditions. Sha allele of ACD6 was as divergent from the Col-0 reference allele as it was from a specific strain of *Arabidopsis lyrata*, and could have played a role in the evolution of the species (Todesco *et al.,* 2010). Noteworthy, ACD6 gene was observed to be downregulated in several accessions or mutants under drought conditions and in response to ABA in the literature.

The last genomic region involved in plant growth variation under drought conditions was located at the top of chromosome 5, encompassing a large genomic region (NGA151 and NGA139), with multiple QTL in both QTL and GWA studies. The GWA study enabled to identify two genes related to plant tolerance to drought. The first one encodes a gibberellin-regulated family protein. Gibberellins have been convincingly shown to play a prominent role in growth regulation under optimal, but also, under stress conditions (Achard *et al.,* 2006; Ubeda-Tomas *et al.,* 2009), and especially under drought conditions, by repressing the levels of DELLA proteins, known to inhibit growth, via interactions with ABA and ethylene pathways (Achard *et al.,* 2006). A survey of the literature showed that this gibberellin-regulated gene is downregulated under drought conditions, and in response to ABA treatment. The second gene identified in this region is an *agamous-like* gene, encoding a MADS box protein responsible for floral transition in Arabidopsis (Lehti-Siu *et al.,* 2005). These genomic regions controlling plant response to water deficit in both the RIL population and the collection of accessions reveal genes that are very conserved, even throughout widespread Arabidopsis accessions that have evolved to adapt to a range of environmental conditions.

Other regions responsible for variation of plant growth under drought conditions either in the RIL population or in the collection of accessions were also identified. These regions were located near the MSAT 1.13 (chromosome1), IND628 (chromosome2), NGA172 (chromosome3) and MSAT4.43 (chromosome4) for the RIL

population, and near the MSAT108193 (chromosome1), MSAT3.18 (chromosome3), and JV6162 (chromosome5) for the accessions. Contrary to the common regions previously described, identifying specific regions according to the allelic variants could highlight a possible selection of these alleles through evolutionary processes, for these accessions being more adapted to specific environmental conditions.

Two regions related to plant growth response to water deficit were identified specifically in the QTL analysis. At the bottom of the chromosome1, near the F5I14 and MSAT 1.13 markers, and around the MSAT 318406 on chromosome 3, strong QTL controlling shoot and root growth, and their response to drought conditions were detected. Interestingly, in the GWA study, QTL were identified for flowering time in these two regions, and not for growth response to drought, highlighting the strong linkage between flowering time and growth patterns, especially under drought conditions. The F5I14 region was previously detected as being involved in the control of shoot and root growth traits in hydroponics (see Chapter1). A gene coding for a vacuolar invertase (Sergeeva *et al.,* 2006), involved in root elongation, and specifically expressed in root cells has been identified in this region. This gene appeared to be downregulated under drought conditions.

Conclusion

Our analysis showed that as in the case of hydroponics, several QTLs common to root and shoot growth were detected. However, these were rarer than in hydroponics, suggesting hydroponics could amplify the source-limitation of root growth. possibly with revealed substantial overlap between genomic regions identified through classical QTL mapping and GWA. However, several QTLs were root or shoot specific, especially under drought conditions, suggesting drought partly uncouples root and shoot growth. Several QTLs pointed towards interesting candidate regions. In particular, a root specific vacuolar invertase was associated with flowering time. Moreover, ACD6 was very consistently associated with a series of variable, in particular SLA and SLA response to soil water deficit, making it a good candidate for drought tolerance (see chap 2). A functional approach is now needed to confirm some of these QTLs (experiments are in progress) and start the functional analysis of the relationship between their function and drought tolerance.

Chapter 5 / General conclusions and perspectives

Coordination between shoot and root growth with or without soil water deficit

- At the onset of the present work, previous studies had highlighted the strong coupling between root and shoot growth at the inter-specific level but evidence for coupling at the intra-specific level were sparser. At several occasions, our study has highlighted the strong linkage between root and shoot growth, translated both through correlation (chap 2) and common genetic models (chap 1 and 4). The later conclusion holds for both the genetic models build using 2 alleles (Bay-0 x Sha) or a range of alleles (88 accessions). The coordination was very high in hydroponics and apparently higher than in soil conditions suggesting that hydroponics represent a potentially biased device in which mechanical constraints are low and root growth essentially source limited. It would have been valuable to see if such tight relationships are weaker under higher C availability (eg high light or high CO_2).
- In soil-based substrate, genetic models for shoot and root growth variables were also strongly overlapping. However, several shoot or root specific regions were identified. Moreover, soil water deficit tended to uncouple root and shoot genetic models allowing thus to identify regions responsible for root growth maintenance under water deficit, independently of shoot growth. This result is in line with the idea that root growth tend to be sink rather than source limited upon water deficit.

Growth maintenance is favoured by a series of traits, including biomass partitioning

- One of our hypothesis was that root-shoot ratio would contribute to stress tolerance. Our results tend to exclude a strong role of this ratio, at least under our conditions in which the soil volume was limited. This could also be a reason why the specific root length was not related to tolerance. By contrast, our results have highlighted the

strong contribution of the specific leaf area. Tolerant genotypes where those with high SLA able to be strongly decreased upon stress, thus maintaining dry weight accumulation despite reduced surface expansion.

- Size or not size effect ? In line with other studies (ongoing meta-analysis by Denis Vile at LEPSE), drought tolerance through growth maintenance is negatively correlated with size. This result is also visible under N deficiency (O Loudet, pers comm) and could represent a general ecological rule that would thus deserve a renewed and concerted analysis.

Growth maintenance under drought is related to climate aridity

- Within regions displaying the largest range of climate, growth maintenance was related to climate aridity as evaluated through climatic water balance, especially in summer. A tendency was detected between tolerance and vernalization suggesting tolerant accessions could be more often summer annual while sensitive would be more often behaving as winter annuals.

GWA identified candidate genes for growth maintenance under drought

- Specific regions for root and shoot growth under well water or water stress conditions as well as regions responsible for controlling response to soil water deficit were identified both using classical QTL determination and GWA. This result suggest that drought tends to uncouple root from shoot growth.
- Few but promising regions were detected associated with growth maintenance. Among them, several transcription factors (AGL, F-box…) were detected as well as ACD6, previously described as responsible for a trade-off between plant growth and susceptibility to a wide range of pathogens known (Todesco *et al.,* 2010). The gene list can be intuitively extended to a cell wall root invertase INV, previously reported as being involved in root growth control and a carboxy-peptidase, involved in amino acids reprocessing during stress. The later could then be associated with osmotic adjustment under stress since amino acids have been shown to be involved in this response (Hummel *et al.,* 2011)

What next ?

Short term perspectives

Among the data collected, a limited number remain to be analysed. This is the case of the zenithal images taken daily during the experiments. They could provide an estimate of relative expansion rate of the rosette and thus an additional variable to link in our analysis.

In this line, several analysis remain to be done. (i) using residual or PCA coordinates such as those performed in Chap 1 in genetic studies. This could further contribute to identifying root or shoot specific regions. (ii) Linking climate based tolerance gradients such as those identified in chap 3 to biomass partitioning variables (Root-shoot, Specific leaf area and Specific root length) to further explore if natural selection has operated through common leverage effect in different regions

An experiment using ACD6 mutant, complemented lines and constitutive overexpressors is ongoing in order to validate the major QTL on chr 4.

Another QTL of interest is located at the top of chr3, and controls root-shoot partitioning in all experiments performed. SNPs in two genes have been detected and the importance of these two genes would deserve dedicated experiments, at least using available SALK mutants.

Mid-term perspectives

Other strategies are escape and increased WUE. This could be addressed through dedicated experiments in which growth in followed non-destructively to preserve plants for flowering recordings. This could extend the analysis of MacKay *et al.,* (2003) showing a pleiotropic link between escape and WUE.

An analysis similar to that performed here but on a much narrower geographical panel would provide a vast amount of information on the climatic sources of trait variation. This can be rapidly envisaged since collections are available, in particular in those most promising regions, Spain and Central Asia. This could be done in collaboration with Carlos Alonso-Blanco and Karl Schmidt respectively since they have performed an extensive sampling in these regions. Recent studies in Spain are in line with this idea (Pico *et al.,* 2009; Montesinos *et al.,* 2010)

In some on-going or already performed PHENOPSIS studies in the Montpellier group, an increasing proportion are considering the same variables than the ones focused on in our study (studies by D Vile, R Valluru notably) in various genetic panels or sub-panels. The central importance of SLA could thus be further explored on such extended dataset.

Other research programs in the Montpellier group aim to relate growth patterns to functional traits such as photosynthesis, conductance, C metabolism. Similar approaches are performed in various labs (eg at IPK Gatersleben R Meyer, T Altmann). We could use a structured panel along a gradient of tolerance (Spain and Central Asia) in order to explore their main metabolic and functional feature in relation with candidate processes involved (C metabolism, hydraulic network, respiration…)

We have used a raw protocol for evaluating local climates which does not take into account either local climatic particularities (related to slopes, cloudiness), or edaphic characterization. This could be done using model aided characterization of climatic constraints as recently shown using the crop model APSIM (Chenu *et al.,* 2011)

Long-term perspectives

This thesis has open the door to further characterization of an ambitious characterization of functional variables associated with selection of a wild species in front of water deficit. Currently, discussions are on-going in the group to envisage the following steps of that work. Clearly, 2 directions can be taken. (i) extending the accession panel to perform more powerfull stats and avoid false positive hits. (ii) focusing on detailed geographic regions that share the same history in term of settlement and similar genetic structure. This would be very helpful to detect drivers of local adaptation to environmental constraints, the Holy Grail widely chased by several groups worldwide.

Appendix / Description of the genotypes used in each chapter

	RIL		Acc20		Acc80		Acc8	
informations and genotypic data	Loudet et al., 2002		Clark et al., 2007		1001genomes.org, Cao et al., 2011		Clark et al., 2007	
Chapter1								
Chapter2								
Chapter3								
Chapter4								
	165 RIL	130 RIL		origin		origin		origin
			BAY0	Germany	BAK2	Caucasia	SHA	CentralAsia
			BOR4	East Europa	BAK7	Caucasia	BOR4	EastEuropa
			BR0	East Europa	DOG4	Caucasia	BR0	EastEuropa
			BUR0	Ireland	ISTISU1	Caucasia	EST1	EastEuropa
			C24	Spain	KASTEL1	Caucasia	C24	Spain
			COL0	USA	LAG22	Caucasia	FEI0	Spain
			CVI0	Cape Verde	LERIK13	Caucasia	TS1	Spain
			EST1	East Europa	NEMRUT1	Caucasia	BAY0	Germany
			FEI0	Spain	VASH1	Caucasia		
			GOT7	Germany	XAN1	Caucasia		
			LER	Germany	YEG1	Caucasia		
			LOV5	Sweden	ICE150	CentralAsia		
			NFA8	England	ICE152	CentralAsia		
			RRS10	USA	ICE153	CentralAsia		
			RRS7	USA	ICE60	CentralAsia		
			SHA	Central Asia	ICE61	CentralAsia		
			TAMM2	Finland	ICE70	CentralAsia		
			TS1	Spain	ICE73	CentralAsia		
			TSU1	Japan	ICE75	CentralAsia		
			VAN0	Canada	SHA	CentralAsia		
					BOR4	EastEuropa		
					BR0	EastEuropa		
					DEL10	EastEuropa		
					EST1	EastEuropa		
					ICE1	EastEuropa		
					ICE21	EastEuropa		
					ICE29	EastEuropa		
					ICE33	EastEuropa		
					ICE36	EastEuropa		
					ICE63	EastEuropa		
					ICE7	EastEuropa		
					KOCH1	EastEuropa		
					ICE127	Russia		
					ICE130	Russia		
					ICE134	Russia		
					ICE138	Russia		
					ICE71	Russia		
					ICE72	Russia		
					ICE102	SouthItaly		
					ICE104	SouthItaly		
					ICE106	SouthItaly		
					ICE107	SouthItaly		
					ICE111	SouthItaly		
					ICE112	SouthItaly		
					ICE119	SouthItaly		
					ICE120	SouthItaly		
					ICE91	SouthItaly		
					ICE92	SouthItaly		
					ICE93	SouthItaly		
					ICE163	SouthTyrol		
					ICE169	SouthTyrol		
					ICE173	SouthTyrol		
					ICE181	SouthTyrol		
					ICE212	SouthTyrol		
					ICE213	SouthTyrol		
					ICE216	SouthTyrol		
					ICE226	SouthTyrol		
					ICE228	SouthTyrol		
					ICE79	SouthTyrol		
					ICE97	SouthTyrol		
					ICE98	SouthTyrol		
					AGU1	Spain		
					C24	Spain		
					CDM0	Spain		
					DON0	Spain		
					FEI0	Spain		
					ICE49	Spain		
					ICE50	Spain		
					LEO1	Spain		
					MER6	Spain		
					PED0	Spain		
					PRE6	Spain		
					PRO0	Spain		
					QUI0	Spain		
					TS1	Spain		
					VIE0	Spain		
					EY152	Tubingen		
					HKT24	Tubingen		
					NIE12	Tubingen		
					RU3127	Tubingen		
					STAR8	Tubingen		
					TUSB302	Tubingen		
					TUSCHA9	Tubingen		
					TUV12	Tubingen		
					TUWA12	Tubingen		
					WALHASB4	Tubingen		
					BAY0	Germany		

References

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP. 2006. Integration of plant responses to environmentally activated phytohormonal signals. *Science*, **311**:91-94.

Aguirrezabal LAN, Deleens E, Tardieu F. 1994. Root elongation rate is accounted for by intercepted PPFD and source-sink relations in field and laboratory-grown sunflower. *Plant, Cell and Environment* **17**: 443-450.

Alexandre CM, Hennig L. 2008 FLC or not FLC: the other side of vernalization. *J Exp Bot.* **59**:1127-35.

Allen RG, Pereira LS, Raes D, Smith M. 1998. Crop evapotranspiration-guidelines for computing crop water requirements. FAO irrigation and drainage paper 56. Rome:Food and agriculture organization of the United nations.

Alonso-Blanco C, Peeters AJM, Koornneef M, Lister C, Dean C, van den Bosch N, Pot J, Kuiiper MTR. 1998. Development of an AFLP based linkage map of Ler, Col, and Cvi Arabidopsis thaliana ecotypes and construction of a Ler/Cvi Recombinant Inbred Lines population. *The plant journal* **14**:259-271.

Alonso-Blanco C, Koornneef M. 2000. Naturally occurring variation in Arabidopsis: an underexploited resource for plant genetics. *Trends Plant Sci* **5**: 22–29.

Amasino RM, Michaels SD. 2010. The timing of flowering. *Plant Physiol* **154**:516-20.

Amasino RM. 2005. Vernalization and flowering time. *Curr Opin Biotechnol*. **16**:154-8.

Araus JL, Slafer GA, Reynolds MP, Royo C. 2002. Plant breeding and drought in C3 cereals: what should we breed for? *Ann Bot.* **89:** 925-40.

Aranzana MJ, Kim S, Zhao K, Bakker E, Horton M, Jakob K, Lister C, Molitor J, Shindo C, Tang C, Toomajian C, Traw B, Zheng H, Bergelson J, Dean C, Marjoram P, Nordborg M. 2005. Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. *PLoS Genet*. 1:e60.

Asch F, Dingkuhn M, Sow A and Audebert A. 2005. Drought-induced changes in rooting patterns and assimilate partitioning between root and shoot in upland rice. *Field Crops Research* **93**:223-236.

Aschmann H., 1973. Distribution and peculiarity of Mediterranean ecosystems. In: Di Castri F. & Mooney H.A. (ed.), Mediterranean ecosystems: origin and structure. Springer Verlag, New York: 11-19.

Ashraf M. 2010. Inducing drought tolerance in plants: Recent advances. *Biotechnology Advances* **28:**169-183

Asins MJ. 2002. Present and future of quantitative trait locus analysis in plant breeding. *Plant breeding* **121**:281-291.

Atwell S, Huang YS, Vilhjálmsson BJ, Willems G, Horton M, Li Y, Meng D, Platt A, Tarone AM, Hu TT, Jiang R, Muliyati NW, Zhang X, Amer MA, Baxter I, Brachi B, Chory J, Dean C, Debieu M, de Meaux J, Ecker JR, Faure N, Kniskern JM, Jones JD, Michael T, Nemri A, Roux F, Salt DE, Tang C, Todesco M, Traw MB, Weigel D, Marjoram P, Borevitz JO, Bergelson J, Nordborg M. 2010. Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. *Nature*. **3**:627-

31.

Azhiri-sighari T, Yamauchi A, Yamoshita A, Wade LJ. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering II. Root growth. *Plant production science*. **3**:180-188.

Balaguer L, Martinez-Ferri E, Valladares F, Perez-Corona ME, Baquedano FJ, Castillo FJ, Manrique E. 2002. Population divergence in the plasticity of the response of Quercus coccifera to the light environment. *Functional ecology*. **15**:124-135.

Balasubramanian S, Schwartz C, Singh A, Warthmann N, Kim MC, Maloof JN, Loudet O, Trainer GT, Dabi T, Borevitz JO, Chory J, Weigel D. 2009. QTL mapping in new arabidopsis thaliana advanced intercross-recombinant inbred lines. *PLoS ONE* **4**: e4318.

Barakat A, Matassi G, Bernardi G. 1998. Distribution of genes in the genome of Arabidopsis thaliana and its implications for the genome organization of plants. *Proceedings of the national academy of sciences USA* **95**:10044-10049.

Barnaud A, Lacombe T, Doligez A. 2006. Linkage disequilibrium in cultivated grapevine, Vitis vinifera L. *Theor Appl Genet*. **112**:708-16.

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature*. **427**:164-7.

Bäurle I, Dean C. 2006. The timing of developmental transitions in plants. *Cell.* **125**:655-64.

Beck JB, Schmuths H, Schaal BA. 2008. Native range genetic variation in Arabidopsis thaliana is strongly geographically structured and reflects Pleistocene glacial dynamics. *Mol Ecol*. **17**:902-15.

Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate / a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**: 289-300.

Bergelson J, Roux F. 2010.Towards identifying genes underlying ecologically relevant traits in Arabidopsis thaliana. *Nat Rev Genet*. **11**:867-79.

Bevan M, Bancroft I, Mewes HW, Martienssen R, McCombie R. 1999. Clearing a path through the jungle: Progress in Arabidopsis genomics. *Bioessays* **21**:110-120.

Beven K. 1979. A sensitivity analysis of the Penman-Monteith actual evapotranspiration estimates. *Journal of hydrology* **44**:169-190.

Bikard D, Patel D, Le Metté C, Giorgi V, Camilleri C, Bennett MJ, Loudet O. 2009. Divergent evolution of duplicate genes leads to genetic incompatibilities within A. thaliana. *Science* **323**:623-6.

Bingham IJ, Stevenson EA. 1993. Control of root growth: effects of carbohydrates on the extension, branching and rate of respiration of different fractions of wheat roots. *Physiologia Plantarum* **88**: 149-158.

Bingham IJ, Panico A, Stevenson EA. 1996. Extension rate and respiratory activity in the growth zone of wheat roots: Time-course for adjustments after defoliation. *Physiologia Plantarum* **98**:201–209.

Blum AJ. 1996. Crop response to drought and the interpretation of adaptation. *Plant Growth Regul* **20**:135–48.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants- an economic analogy. *Anuual review of ecology and systematics.* **16**:363-392.

Bogeat-Triboulot MB, Brosché M, Renaut J, Jouve L, Le Thiec D, Fayyaz P, Vinocur B, Witters E, Laukens K, Teichmann T, Altman A, Hausman JF, Polle A, Kangasjärvi J, Dreyer E. 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in Populus euphratica, a poplar growing in arid regions. *Plant*

Physiol. **143**:876-92.

Bois B, Pieri P, van Leeuwen C, Gaudillere JP. 2005. Sensitivity analysis of the Penman-Monteith evapotranspiration formula and comparison of empirical methods used in viticulture soil water balance. In: XIV International GESCO viticulture congress, Geisenheim Germany 23-27 Oct 2005.

Bois B, Pieri P, van Leeuwen C, Wald L, Huard F, Gaudillere JP, Saur E. 2008. Using remotely sensed solar radiation data for reference evapotranspiration estimation at a daily time step. *Agricultural and forest meteorology* **148**:619-630.

Bolanos J, Edmeades GO, Martinez L. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in drought-adaptive physiological and morphological traits. *Field crops research* **31**:269-286

Bomblies K, Yant L, Laitinen RA, Kim ST, Hollister JD, Warthmann N, Fitz J, Weigel D. 2010. Local- scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of Arabidopsis thaliana. *PLoS Genet* **6**:e1000890.

Borevitz JO, Nordborg M. 2003. The impact of genomics on the study of natural variation in Arabidopsis. *Plant Physiol*.**132**:718-25..

Bouchabke O, Chang F, Simon M, Voisin R, Pelletier G, Durand-Tardif M. 2008. Natural variation in Arabidopsis thaliana as a tool for highlighting differential drought responses *PLoS One*. **3:**e1705.

Bouche N, Bouchez D. 2001. Arabidopsis genes knock-out: Phenotypes wanted. *Current opinion in plant biology* **4**:111-117.

Boudry P, McCombie H, van Dijk H. 2002. Vernalization requirement of wild beet (Beta vulgaris ssp maritima): Among population variation and its adaptative significance. *Journal of ecology* **90**:693- 703.

Boyer JS. 1970. Leaf enlargement and metabolic rates in corn, soybean and sunflower at various leaf water potential. *Plant physiology* **46**: 233-235.

Brachi, B. et al. 2010. Linkage and association mapping of Arabidopsis thaliana flowering time in nature. *PLoS Genet*. 6.

Bradford KJ, Hsiao TC.1982**.** Stomatal behavior and water relations of waterlogged tomato plants. *Plant Physiol.* **70**:1508-13.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in genetics* **13**:115-155.

Bruce WB, Edmeades GO, Barker TC. 2002. Molecular and physiological approaches to maize improvement for drought tolerance. *Journal of experimental botany* **53**:13-25.

Brouwer R. 1962. Distribution of dry matter in plants. *Netherlands Journal of Agricultural Science* **10**:361-376.

Caicedo A, Stinchcombe JR, Olsen KM, Schmitt J, PuruggananMD. 2004. Epistatic interaction between Arabidopsis FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. *Proc Natl Acad Sci USA* **101**:15670–15675.

Calderon-Villalobos LI, Nill C, Marrocco K, Kretsch T, Schwechheimer C. 2007. The evolutionarily conserved Arabidopsis thaliana F-box protein AtFBP7 is required for efficient translation during temperature stress. *Gene* **392**:106–116.

Cao J., Schneeberger K., Ossowski S., Gunther T., Bender S., Fitz J., Koenig D., Lanz C., Stegle O., Lippert C., Muller J., Alonso-Blanco C., Borgwardt K., Schmid K.J., and Weigel D. 2011. Phase I of the 1001 Genomes Project for Arabidopsis thaliana, submitted to Nature genetics.

Castaings L, Camargo A, Pocholle D, Gaudon V, Texier Y, Boutet-Mercey S, Taconnat L, Renou JP,

Daniel-Vedele F, Fernandez E, Meyer C, Krapp A. 2009. The nodule inception-like protein 7 modulates nitrate sensing and metabolism in Arabidopsis. *Plant J*. **57**:426-35.

Carbonell EA, Asins MJ. 1996. Statistical models for the detection of genes controlling quantitative traits loci expression. *Jain SM, Sopory SK, Weilleux RE eds in In vitro haploid production in higher plants in Kluwer academic publishers, the Netherlands* **2**:255-285.

Carlborg O, Haley CS. 2004. Epistasis: too often neglected in complex trait studies? *Nature Review Genetics* **5**: 618-625.

Cattivelli L., Rizza F., Badeck FW., Mazzucotelli E., Mastrangelo AM, Francia E., Mare`C.,Tondelli A., Stanca M. 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. *Field Crop Research*, **105**:1-14.

Chang C, Bowman JL, Dejohn AW, Lander ES, Meyerowitz EM. 1988. Restriction fragmnet length polymorphism linkage map for Arabidopsis thaliana. *PNAS* **85**:6856-6860.

Chapin FS, McNaughton SJ. 1989. Lack of compensary growth under phosphorus deficiency in grazing-adapted grasses from the Serengeti plains. *Oecologia* **79**:551-557.

Chapman SC. 2007. Use of crop models to understand genotype by environment interactions for drought in real-world and simulated plant breeding trials. *Euphytica* **161**:195-208.

Charmet G. 2000. Power and accuracy of QTL detection: Simulations studies of one QTL models. *Agronomy* **20**:309-323.

Chase K., Adler F. R. and Lark K. G. 1997. Epistat : a computer program for identifying and testing interactions between pairs of quantitative trait loci. *Theoretical and Applied Genetics* **94**:724-730

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CP, Osório ML, Carvalho I, Faria T, Pinheiro C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Ann Bot*. **89:**907-16.

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

Chenu K, Chapman SC, Hammer GL, McLean G, Salah HB, Tardieu F. 2008. Short-term responses of leaf growth rate to water deficit scale up to whole-plant and crop levels: an integrated modelling approach in maize. *Plant Cell Environ*.**31**:378-91.

Chenu K, Chapman SC, Tardieu F, McLean G, Welcker C, Hammer GL. 2009. Simulating the yield impacts of organ-level quantitative trait loci associated with drought response in maize: a "gene-to-phenotype" modeling approach. *Genetics*. **183**:1507-23.

Cheplick GB. 2003. Evolutionary significance of genotypic variation in developmental reactions norms for a perrenial grass under competitive stress. *Evolutionary ecology* **17**:175-196.

Chevalier F, Pata M, Nacry P, Doumas P, Rossignol M. 2003. Effects of phosphate availability on the root system architecture: large-scale analysis of the natural variation between Arabidopsis accessions. *Plant, Cell & Environment* **26**:1839-1850.

Chimenti CA, Pearson J, Hall AJ. 2002. Osmotic adjustments and yield maintenance under drought in sunflower. *Field crops research* **75**:235-246.

Christophe A, Moulia B, Varlet-Grancher C. 2006. Quantitative contributions of blue light and PAR to the photocontrol of plant morphogenesis in Trofolium repens (L.). *Journal of Experimental Botany* **57**: 2379-2390.

Christophe A, Letort V, Hummel I, Cournède PH, (de) Reffye P, Lecoeur J. 2008. Model-based analysis

of the dynamics of trophic competition in Arabidopsis thaliana during its development. *Functional Plant Biology* **35**:1147-1162.

Clark RM., Schweikert G., Toomajian C., Ossowski S., Zeller G., Shinn P., Warthmann N., Hu T., Fu G., Hinds D., Chen H., Frazer K., Huson D., Schölkopf B., Nordborg M., Rätsch G., Ecker J., Weigel D. 2007. Common Sequence Polymorphisms Shaping Genetic Diversity in Arabidopsis thaliana. *Science*. **317**:338-342

Claassen MM, Shaw RH. 1970. Water deficit effects on corn. 2. Grain components. *Agronomy journal* **62**:652-655.

Cody ML, Mooney HA. 1978. Convergence Versus Nonconvergence in Mediterranean-Climate Ecosystems. *Annual review of ecology and systematics* **9**:265-321.

Coleman JS, McConnaughay KDM, Ackerly DD. 1994. Interpreting phenotypic variation in plants. *Trends in Ecology and evolution*. **9**:187-191.

Coleman JS, McConnaughay KDM. 1995. A non functional interpretation of a classifical optimal-partitioning example. *Functional ecology* **9**:951-954.

Cookson SJ, Granier C. 2006. A dynamic analysis of the shade-induced plasticity in Arabidopsis thaliana rosette leaf development reveals new components of the shade-adaptative response. *Ann Bot.* **97**:443- 52.

Cooper M, van Eeuwijk FA, Hammer GL, Podlich DW, Messina C. 2009. Modeling QTL for complex traits: detection and context for plant breeding. *Current Opinion in Plant Biology* **12**: 231-240.

Cornwell WK, Grubb PJ. 2003. Regional and local patterns in plant species richness with respect to resource availability. *Oikos* **100**:417-428.

Costa França MG, Pham Thi AT, Pimentel C, Pereyra Rossiello RO, Zuily-Fodil Y, Laffray D. 2000. Differences in growth and water relations among Phaseolus vulgaris cultivars in response to induced drought stress. *Environ Exp Bot.* **43**:227-237.

Coupland G. 1995. Genetic and environmental control of flowering time in Arabidopsis. *Trends Genet* **11**: 393–397.

Courtois B, McLaren G, Sinha PK, Prasad K, Yadav R, Shen L. 2000. Mapping QTLs associated with drought avoidance in upland rice. *Molecular breeding* **6**:55–66.

Craufurd PQ, Wheeler TR, Ellis RH, Summerfiled RJ, Williams JH. 1999. Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. *Crop science* **39**:136-142.

Cui K, Huang J, Xing Y, Yu S, Xu C, Peng S. 2008. Mapping QTLs for seedling characteristics under different water supply conditions in rice (Oryza sativa). *Physiologia Plantarum* **132**:53-68.

Darvasi A, Soller M. 1994. Optimum spacing of genetic markers for determining linkage between marker loci and quantitative trait loci. *Theoretical and applied genetics* **89**:351-357.

Davies WJ, and Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual review of plant physiology and plant molecular biology*. **42:**55-76.

Debaeke P., Aboudrare A.. 2004. Adaptation of crop manadgement to water limited environments. *European journal of agronomy* **21**:433-446.

de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X. 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends in Plant Science* **12**:474- 481.

De Vienne D. 1998. Les marqueurs moléculaires en génétique et biotechnologies végétales. Paris INRA

editions.

De Vienne D, and Causse M. 1998. La cartographie et la caractérisation des locus contrôlant la variation des caractères quantitatifs in:"Les Marqueurs moléculaire en génétique et biotechnologies végétales". Ed. INRA, Paris.

DeWitt TJ. 1998. Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a fresh water snail. *Journal of evolutionary biology* **11**:465-481.

DeWitt TJ, Scheiner SM. 2004. Phenotypic plasticity:Functional and conceptual approaches. Oxford University press.

Deyn GBD, Cornelissen JHC, Bardgett RD. 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters* **11**:516-531.

Diaz S, Cabido M, Casanoves F. 1998. Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science* **9**:113–122.

Doerge RW. 2002. Mapping and analysis of quantitative trait loci in experimental populations. *Nat Rev Genet.* **3**:43-52.

Dong H, Kong X, Luo Z, Li W, Xin C. 2010. Unequal salt distribution in the root zone increases growth and yield of cotton. *European journal of agronomy* **33**:285-292.

Dorn, L. et al . 2000. Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution* **54**:1982–1994.

Drouet JL, Pagès L. 2006. GRAAL-CN: a model of growth, architecture and allocation for carbon and nitrogen dynamics within whole plants formalised at the organ level. *Ecological Modelling* **206**: 231-249.

Durfee T, Roe JL, Sessions RA, Inouye C, Serikawa K, Feldmann KA, Weigel D, Zambryski PC. 2003. The F-box-containing protein UFO and AGAMOUS participate in antagonistic pathways governing early petal development in Arabidopsis. *Proc Natl Acad Sci U S A* **100**:8571–8576.

East EM. 1916. Studies on size inheritance in Nicotiana. *Genetics* **1:** 164-167.

Ehrenreich, I. M. et al. 2009. Candidate gene association mapping of Arabidopsis flowering time. *Genetics* **183**:325–335.

Edwards MD, Stuber CW, Wendel JF. 1987. Molecular marker facilitated investigations of QTL in maize. I. Number, genomic distribution and types of gene action. *Genetics* **116**:113-125.

Eissenstat DM, Achor DS. 1991. Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. *New Phytologist* **141:**309–321.

El-Lithy ME, Clerkx EJ, Ruys GJ, Koornneef M, Vreugdenhil D. 2004. quantitative trait locus analysis of growth-related traits in a new arabidopsis recombinant inbred population. *Plant Physiology*. **135**: 444-458.

Engelbrecht BM, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP. 2007. Drought sensitivity shapes species distribution patterns in tropical forests. *Nature*. **447**:80-2.

Enquist BJ, Niklas KJ. 2002. Global Allocation Rules for Patterns of Biomass Partitioning in Seed Plants. *Science* **295**:1517-1520.

Erice G., Louahlia S., Irigoyen JJ., Sanchez-Diaz M., Avice JC. 2010 Biomass partitioning, morphology and water status of 4 alfalfa genotypes submitted to progressive drought and recovery. *Journal of plant physiology* **167**:114-120.

Ericsson T 1995. Nutrient, Growth and Allocation, Growth and shoot:root ratio of seedlings in relation to nutrient availability. *Plant and Soil* **168**:205-214.

Farrar JF, Jones CL. 1986. Modification of respiration and carbohydrate status of barley roots by selective pruning. *New Phytologist* **102**: 513-521.

Farrar JF, Gunn S. 1998. Allocation: Allometry, acclimation-and alchemy? *In Lambers H, Poorter H, van Vuuren MMI eds. Inherent variation in plant growth. Leiden, the Netherlands: Backhuys,* 183-198.

Fett-Neto AG, Fett JP, Viera Goulart LV, Pasquali G, Termignoni R, Ferreira AG. 2001. Distinct effects of auxin and light on adventitious root development in Eucalyptus saligna and Eucalyptus globulus. *Tree Physiology* **21**:457–464.

Flint-Garcia, S.A., Thornsberry, J.M., Buckler, E.S. 2003. Structure of linkage disequilibrium in plants. *Annu. Rev. Plant Biol.* **54**:357-374.

Fournier-Level A, Lacombe T, Le Cunff L, Boursiquot JM, This P. 2009. Evolution of the VvMybA gene family, the major determinant of berry colour in cultivated grapevine (Vitis vinifera L.). *Heredity.* **104**:351-62.

Franks SJ. 2011. Plasticity and evolution in drought avoidance and escape in the annual plant Brassica rapa. *New phytologist* **190**:249-257.

Frary A., Nesbitt TC, Grandilb S, Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD. *2000. fw2.2: A quantitative trait locus key to the evolution of tomato fruit size. Science 298:85-88.*

Freixes S, Thibaud M, Tardieu F, Muller B. 2002. Root elongation and branching is related to local hexose concentration in Arabidopsis thalianaseedlings. *Plant, Cell & Environment* **25**:1357-1366.

Gallais, A., 1989 Théorie de la sélection en amélioration des plantes. Ed. Masson, Paris.

Gansel X, Muños S, Tillard P, Gojon A. 2001. Differential regulation of the NO3– and NH4+ transporter genes AtNrt2.1 and AtAmt1.1 in Arabidopsis: relation with long-distance and local controls by N status of the plant. *The Plant Journal* **26**:143–155.

Garcia, L. V. 2003. Controlling the false discovery rate in ecological research. *Trends Ecol. Evol*. **18**: 553-554.

Garcia, L. V. 2004. Escaping the Bonferroni iron claw in ecological studies. *Oikos* **105**: 657-663.

Gazzani S, Gendall AR, Lister C, Dean C. 2003. Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. *Plant Physiol* **132**:1107–1114.

Gerald FJN, Lehti-Shiu MD, Ingram PA, Deak KI, Biesiada T, Malamy JE. 2006. Identification of quantitative trait loci that regulate Arabidopsis root system size and plasticity. *Genetics* **172**:485-98.

Girin T, El kafafi E, Widiez T, Erban A , Hubberten HM, Kopka J, Hoefgen R, Gojon A, Lepetit M. 2010. Identification of Arabidopsis mutants impaired in the systemic regulation of root nitrate uptake by the nitrogen status of the plant. *Plant physiology* **153**:1250-1260.

Giuliani S, Sanguineti MC, Tuberosa R, Bellotti M, Salvi S, Landi P. 2005. Root-ABA1, a major constitutive QTL, affects maize root architecture and leaf ABA concentration at different water regimes. *Journal of Experimental Botany* **56**:3061-70.

Givnish TJ. 2002**.** Ecological constraints on the evolution of plasticity in plants. *Evolutionary ecology* **16**:213-242.

Glazier AM, Nadeau JH, Aitman TJ. 2002. Finding genes that underlie complex traits. *Science*. **298**:2345-2349.

Goffinet B, Mangin B. 1998. Comparing methods to detect more than one QTL on a chromosome.

Theoretical and applied genetics **46**:300-319.

Gonzalez AJ, Gianoli E. 2004. Morphological plasticity in response to shading in three convolvulus species of different ecological breadth. *Acta oecologica* **26**:185-190.

Goudrian J, van Laar HH. 1994. Modeling potential crop growth processes. Kluwer Academic Dordrecht, the Netherlands.

Granier C, Tardieu F. 1999. Water deficit and spatial pattern of leaf development. Variability In responses can Be simulated using a simple model of leaf development. *Plant Physiol*. **119**:609-620.

Granier C, Massonnet C, Turc O, Muller B, Chenu K, Tardieu F. 2002. Individual leaf development in Arabidopsis thaliana: a stable thermal-time-based programme. *Ann Bot.* **89**:595-604.

Granier C, Aguirrezabal L, Chenu K, Cookson SJ, Dauzat M, Hamard P, Thioux JJ, Rolland G, Bouchier-Combaud S, Lebaudy A, Muller B, Simonneau T, Tardieu F. 2006. PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in Arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytol*.**169**:623-35.

Grant D, Cregan P, Shoemaker RC. 2000. Genome organization in dicots: genome duplication in Arabidopsis and synteny between soybean and Arabidopsis. *Proc Natl Acad Sci U S A*. **97**:4168-73.

Grechi I, Hilbert G, Milin S, Robert T, Gaudillere JP. 2007. Effect of light and nitrogen supply on internal C:N balance and control of root to shoot biomass allocation in grapevine. *Environmental and experimental botany* **59**:139-149.

Grime, J. P. 1979. Plant strategies and vegetation processes. 222pp.

Gupta PK, Rustgi S, Kulwal PL. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Mol Biol*. **57**:461-85.

Hagenblad, J. & Nordborg, M. 2002. Sequence variation and haplotype structure surrounding the flowering time locus FRI in Arabidopsis thaliana. *Genetics* **161**:289–298.

Haley CS, Knott SA. 1992. A simple regression method for mapping quantitative trait morphology to light reduction in maize genotypes. *Crop science* **41**:363-371.

Hammer GL, Dong Z, McLean G, Doherty A, Messina C, Schussler J, Zinselmeier C, Paszkiewicz S, Cooper M. 2009. can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. corn belt ? *Crop Science* **49**: 299-312.

Hannah MA, Wiese D, Freund S, Fiehn O, Heyer AG, Hincha DK. 2006. Natural genetic variation of freezing tolerance in Arabidopsis. *Plant Physiol*.**142**:98-112.

Hao Z., Li X., Liu X., Xie C., Li M., Zhang D. and Zhang S. 2009. Meta-analysis of constitutive and adaptive QTL for drought tolerance in maize. *Euphytica*, **174**: 165-177.

Hargreaves GH, Samani ZA. 1982. Estimating potential evapotranspiration. *Journal of irrigation and drainage engineering* **108**:225-230.

Hausmann NJ, Juenger TE, Sen S, Stowe KA, Dawson TE, Simms EL. 2005. Quantitative trait loci affecting delta^{13}C and response to differential water availability in *Arabidopsis thaliana*. *Evolution*. **59**:81-96.

Heilmeier H, Erharda M, Wartinger A, Brinckmann E, Horn R and Schulze ED. 2001. Biomass partitioning in response to soil drought: A pot experiment with Prunus dulcis trees during four years. *Basic and Applied Ecology* **2**:165-175.

Henderson I, Shindo C, Dean C. 2003. The need for winter in the switch to flowering. *Annu Rev Genet* **37**:

371–392.

Ho LC. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Molecular Biology* **39**: 355-378.

Hochholdinger F, Tuberosa R. 2009. Genetic and genomic dissection of maize root development and architecture. *Current Opinion in Plant Biology* **12**:172–177.

Hoffmann MH. 2002. Biogeography of Arabidopsis thaliana (L.) Heynh. (Brassicaceae). *Journal of biogeography* **29**: 125–134.

Holland JB. 2007. Genetic architecture of complex traits in plants. *Curr Opin Plant Biol*. **10**:156-61.

Hopkins R, Schmitt J, Stinchcombe JR. 2008. A latitudinal cline and response to vernalization in leaf angle and morphology in Arabidopsis thaliana (Brassicaceae). *New Phytol*. 179:155-64.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W and Zimmermann P. 2008. Genevestigator V3: a reference expression database for the meta-analysis of transcriptomes. *Advances in Bioinformatics*

Hsiao TC. 1973. Plant responses to water stress. *Annual Review of Plant Physiology* **24**: 519-570.

Hsiao TC., Acevedo E. 1974. Plant response to water deficits, water use efficiency, and drought resistance. *Agricultural meteorology*. **14**. 59-84.

Hulme PE. 2005. Adapting to climate change: Is there scope for ecological management in the face of a global threat? *Journal of applied ecology* **42**:784-794.

Hummel I, Pantin F, Sulpice R, Piques M, Rolland G, Dauzat M, Christophe A, Pervent M, Bouteillé M, Stitt M, Gibon Y, Muller B. 2010. Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiology* **154**:357-72.

Hund A, Fracheboud Y, Soldati A, Frascaroli E, Salvi S, Stamp P. 2004. QTL controlling root and shoot traits of maize seedlings under cold stress. *Theoretical and Applied Genetics* **109**: 618-629.

IPCC 2001. Climate change 2001, IPCC 3rd assesment report.

Iwata H, Ebana K, Uga Y, Hayashi T, Jannink J. 2010. Genome-wide association study of grain shape variation among Oryza sativa L. germplasms based on elliptic Fourier analysis. *Molecular Breeding* **25:** 203-215.

Jansen RC, Stam P.1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* **136**:1447-1455.

Johannsen W. 1909. Elemente des exakten Erblichkeitsliehre. *Fisher. Jena*.

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C. 2000. Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science* **290**: 344–347.

Joshi J, Schmid B, Caldeira MC, Dimitrakopoulos PG, Good J, Harris R, Hector A, Huss-Danell K, Juenger T, Sen S, Stowe K, Simms E. 2005. Epistasis and genotype-environment interaction for quantitative trait loci affecting flowering time in Arabidopsis thaliana. *Genetica* **123:** 87-105.

Jumpponen A, Minns A 2001. Local adaptation enhances performance of common plant species. *Ecology Letters* **4**: 536–544.

Kage H, Kochler M and Stützel H. 2004. Root growth and dry matter partitioning of cauliflower under drought stress conditions: measurement and simulation. *European Journal of Agronomy* **20**:379-394.

Kalapos T, van den Boogaard R, Lambers H. 1996. Effect of soil drying on growth, biomass allocation and

leaf gas exchange of two annual grass species. *Plant and soil* **185**:137-149.

Kamoshita, A., Wade, L., Ali, M., Pathan, M., Zhang, J., Sarkarung, S., Nguyen, H.. 2002. Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions. *Theoretical and Applied Genetics* **104**: 880-893.

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, Daly MJ, Eskin E. 2008. Efficient control of population structure in model organism association mapping. *Genetics*. **178**:1709-23.

Kang HM, Sul JH, Service SK, Zaitlen NA, Kong SY, Freimer NB, Sabatti C, Eskin E. 2010. Variance component model to account for sample structure in genome-wide association studies. *Nat Genet*. **42:**348-54.

Kao CH, Zeng ZB, Teasdale RD. 1999. Multiple interval mapping for quantitative trait loci. *Genetics* 152:1203-1216.

Kao CH. 2004. Multiple-interval mapping for quantitative trait loci controlling endosperm traits. *Genetics.* **167**:1987-2002.

Karcher D., Richardson MD., Hignight K., Rush D. 2008. Drought tolerance of tall Fescue populations selected for high RS ratios and summer survival *Crop Science* **48**:771–777.

Kawasaki S, Miyake C, Kohchi T, Fujii S, Uchida M, Yokota A. 2000. Responses of wild watermelon to drought stress: accumulation of an ArgE homologue and citrulline in leaves during water deficits. *Plant Cell Physiol*. **41**:864-73.

Kenis K, Keulemans J. 2007. Study of tree architecture of apple (Malus × domestica Borkh.) by QTL analysis of growth traits. *Molecular Breeding* **19:**193-208.

Kim S, Plagnol V, Hu TT, Toomajian C, Clark RM, Ossowski S, Ecker JR, Weigel D, Nordborg M. 2007. Recombination and linkage disequilibrium in Arabidopsis thaliana. *Nat Genet.* **39**:1151-5.

Knapp SJ, Bridges WC. 1990. Using molecular markers to estimate quantitative trait locus parameters: Power and genetic variances for unreplicated and replicated progeny. *Genetics* **126**:769-777.

Ko JH, Yang SH, Han KH. 2009. Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. *Plant J*. **47**:343-55.

Kobayashi Y. and Weigel D. 2007. Move on up, it's time for change—mobile signals controlling photoperiod-dependent flowering. *Genes and development* **21**: 2371-2384.

Koch, M.A. et al. . 2001. Comparative genomics and regulatory evolution: conservation and function of the Chs and Apetala3 promoters. *Mol. Biol. Evol*. **18**:1882–1891.

Koornneef M, van Eden J, Hanahrt C, Stam P, Braaksma FJ, Feenstra WJ. 1983. The linkage map of Arabidopsis thaliana L. Heyn. *Journal of heridity*.**74**:265-272.

Koornneef M, Alonso-Blanco C, Vreugdenhil D. 2004. Naturally occurring genetic variation in Arabidopsis thaliana. *Annu Rev Plant Biol* **55**:141–172.

Koornneef M, Hanhart CJ, Van der Veen JH. 1991. A genetic and physiological analysis of late flowering mutants in Arabidopsis thaliana. *Mol Gen Genet* **229:** 57–66.

Kover PX, Valdar W, Trakalo J, Scarcelli N, Ehrenreich IM, Purugganan MD, Durrant C, Mott R. 2009. A Multiparent Advanced Generation Inter-Cross to fine-map quantitative traits in Arabidopsis thaliana. *PLoS Genet.* **5**:e1000551.

Kowalski, S. P., Lan, T. H., Feldmann, K. A. & Paterson, A. H. 1994. QTL mapping of naturally-occurring variation in flowering time of Arabidopsis thaliana. *Mol. Genet. Genomics* **245**:548–555.

Kroymann J. and Mitchell-Olds T. 2005. Epistasis and balanced polymorphism influencing complex trait variation. *Nature* **435**: 95-98.

Lacointe A. 2000. Carbon allocation among tree organs : a review of basic processes and representation in functional-structural tree models. *Annals of Forest Science* **57**: 521-533.

Lafarge T., De Ressac M, Tardieu F. 1998. Elongation rate of sorghum leaves has a common response to meristem temperature in diverse African and European environmental conditions. *Field crops research* **58**:69-79.

Lafitte HR, Yongsheng G, Yan S, Li ZK. 2007. Whole plant responses, key processes and adaptation to drought stress. The case of rice. *Journal of experimental botany* 58:169-175.

Lander ES, Botstein D. 1989. Mapping medeleian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**:185-199.

Lander ES, Schork NJ. 1994. Genetic dissection of complex traits. *Science* **265**:2037-48.

Langlade NB, Feng X, Dransfield T, Copsey L, Hanna AI, Thebaud C, Bangham A, Hudson A, Coen E. 2005. Evolution through genetically controlled allometry space. *Proceedings of the National Academy of Sciences* **102:**10221-10226.

Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B. 2006. A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. *Theoretical and Applied Genetics* **113:**1131-1146.

Laplaze L, Benkova E, Casimiro I, Maes L, Vanneste S, Swarup R, Weijers D, Calvo V, Parizot B, Herrera-Rodriguez MB, Offringa R, Graham N, Doumas P, Friml J, Bogusz D, Beeckman T, Bennett M. 2007. Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell*. **19**:3889-900**.**

Leblon B, Guerif M 1992. A root biomass partitioning function for use in models which predict total biomass from absorbed photosynthetically active radiation (PAR). *European journal of agronomy* **1**:139-146.

Lebon E, Pellegrino A, Louarn G, Lecoeur J. 2006**.** Branch development controls leaf area dynamics in grapevine (Vitis vinifera) growing in drying soil. *Ann Bot*. **98**:175-85.

Lecoeur J., Wery J., Turc O., Tardieu F. 1995. Expansion of pea leaves subjected to short water deficit-cell number and cell size are sensitive to stress at different periods of leaf development. *J Exp Bot*. **46** 1093-1101.

Le Corre V, Roux F, Reboud X. 2002. DNA polymorphism at the FRIGIDA gene in Arabidopsis thaliana: extensive nonsynonymous variation is consistent with local selection for flowering time. *Mol Biol Evol* **19**:1261–1271.

Le Corre V. 2005. Variation at two flowering time genes within and among populations of Arabidopsis thaliana: Comparison with markers and traits. *Molecular ecology* **14**:4181-4192.

Lehti-Shiu MD, Adamczyk BJ, Fernandez DE. 2005. Expression of MADS-box genes during the embryonic phase in Arabidopsis. *Plant Mol Biol*. **58**:89-107.

Lei Y., Yin C and Li C., 2006. Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of Populus przewalskii. *Physiologia Plantarum* **127**:182–191.

Lempe J, Balasubramanian S, Sureshkumar S, Singh A, Schmid M, Weigel D. 2005. Diversity of flowering responses in wild Arabidopsis thaliana strains. PLoS Genet. 1:109-18.

Li B, Suzuki JI, Hara T. 1998. Latitudinal variation in plant size and relative growth rate in Arabidopsis

thaliana. *Oecologia* **115**: 293–301.

Li J, Xie Y, Dai A, Liu L, Li Z. 2009. Root and shoot traits responses to phosphorus deficiency and QTL analysis at seedling stage using introgression lines of rice. *Journal of Genetics and Genomics* **36**:173–183.

Liao H, Yan X, Rubio G, Beebe SE, Blair MW, Lynch JP. 2004. Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. *Functional Plant Biology* **31**: 959–970.

Lister C, Dean C. 1993. Recombinant Inbred Line for mapping RFLP and phenotypic markers in Arabidopsis thaliana. *The plant journal* **4**:745-750.

Liu G, Freschet GT, Pan X, Cornelissen JH, Li Y, Dong M. 2010. Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. *New Phytol*. **188**:543- 53.

Liu F, Stützel H. 2004. Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (Amaranthus spp.) in response to drought stress. *Scientia Horticulturae* **102**:15-27.

Lockhardt JA. 1965. An analysis of irreversible plant cell elongation. *Journal of theoretical biology* **8**:264- 275.

Lopez-Bucio J, Hernandez-Abreu E, Sanchez-Calderon L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L. 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the arabidopsis root system. *Plant Physiology* **129**: 244-256.

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L. 2003. The role of nutrient availability in regulating root architecture. *Curr Opin Plant Biol*. **6**:280-7.

Loudet O, Chaillou S, Camilleri C, Bouchez D, Daniel-Vedele F. 2002. Bay-0× Shahdara recombinant inbred line population: a powerful tool for the genetic dissection of complex traits in Arabidopsis. *Theoretical and Applied Genetics* **104**: 1173-1184.

Loudet O, Gaudon V, Trubuil A, Daniel-Vedele F. 2005. Quantitative trait loci controlling root growth and architecture in Arabidopsis thaliana confirmed by heterogeneous inbred family. *Theoretical and Applied Genetics* **110**: 742-753.

Lynch JP. 2007. Roots of the second green revolution. *Australian Journal of Botany* **55**: 493.

Lynch, M. and Conery, J.S. 2000. The evolutionary fate and consequences of duplicate genes. *Science* **290:**1151–1155.

Mackay I, Powell W. 2007. Methods for linkage disequilibrium mapping in crops. *Trends Plant Sci*. **12**:57-63.

MacKay TFC, Stone EA, Ayrolles JF. 2009 The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics* **10:** 565-577.

MacMillan K, Emrich K, Piepho H, Mullins C, Price A. 2006. Assessing the importance of genotype x environment interaction for root traits in rice using a mapping population. I: a soil-filled box screen. *Theoretical and Applied Genetics* **113**: 977-986.

Malamy J. 2005. Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell & Environment* **28**: 67-77.

Malmberg RL. 2005. Epistasis for Fitness-Related Quantitative Traits in Arabidopsis thaliana Grown in the Field and in the Greenhouse. *Genetics* **171**: 2013-2027.

Maloof J. 2003. QTL for plant growth and morphology. *Current opinion in plant biology* **6**:85-90.

Manschadi AM, Hammer GL, Christopher JT, DeVoil P. 2008. Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (Triticum aestivum L.). *Plant and Soil* **303**: 115–129.

Marcelis L, Heuvelink E, Goudriaan J. 1998**.** Modelling of biomass production and yield of horticultural crops : a review. *Scientia Horticulturae* **74**: 83-111.

Mariac C, Jehin L, Saïdou AA, Thuillet AC, Couderc M, Sire P, Jugdé H, Adam H, Bezançon G, Pham JL, Vigouroux Y. 2011. Genetic basis of pearl millet adaptation along an environmental gradient investigated by a combination of genome scan and association mapping. *Mol Ecol*. **20**:80-91.

Marron N, Dreyer E, Boudouresque E, Delay D, Petit JM, Delmotte FM, Brignolas F. 2003. Impact of successive drought and rewatering cycles on growth and specific leaf area of two Populus x Canadensis (Moench) clones, " dorskamp" and " Louisa_ avanzo". *Tree Physiology* **23**:1225-1235.

Mathews KL, Malosetti M, Chapman S, McIntyre L, Reynolds M, Shorter R, van Eeuwijk F. 2008. Multi-environment QTL mixed models for drought stress adaptation in wheat. *Theor Appl Genet*. **117**:1077-91.

Mauricio R. 2001**.** Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nat Rev Genet*. **2**:370-81.

McConnaughay KDM and Coleman JS. 1998. Can plants track changes in nutrient availability via changes in biomass partitioning? *Plant and soil* **202**:201-209.

McKay, J.K. et al. 2001. Local adaptation despite absence of marker diversity in the rare Sapphire Rockcress. *Proc. R. Soc. London B Biol. Sci*. **268**:1715–1721.

McKay JK, Richards JH, Mitchell-Olds T. 2003. Genetics of drought adaptation in Arabidopsis thaliana: I. Pleiotropy contributes to genetic correlations among ecological traits. *Mol Ecol* **12**:1137–1151.

McKay JK, Richards JH, Nemali KS, Sen S, Mitchell-Olds T, Boles S, Stahl EA, Wayne T, Juenger TE. 2008. Genetics of drought adaptation in Arabidopsis thaliana II. QTL analysis of a new mapping population, KAS-1 x TSU-1. *Evolution*. **62**:3014-26.

McMullen, M. D. et al. 2009. Genetic properties of the maize nested association mapping population. *Science* **325**:737–740.

Mei HW, Li ZK, Shu QY, Guo LB, Wang YP, Yu XQ, Ying CS, Luo LJ. 2005. Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two backcross populations. *Theoretical and Applied Genetics* **110**:649-59.

Meinke DW, Cherry JM, Dean C, Rounsley SD, Koornneef M. 1998. Arabidopsis thaliana: a model plant for genome analysis. *Science* **282:**662-679.

Metcalf CJ, Mitchell-Olds T. 2009. Life history in a model system: opening the black box with Arabidopsis thaliana. *Ecol Lett*. **12**:593-600.

Mezmouk S, Dubreuil P, Bosio M, Décousset L, Charcosset A, Praud S, Mangin B. 2011. Effect of population structure corrections on the results of association mapping tests in complex maize diversity panels. *Theor Appl Genet*.**122**:1149-60.

Michaels SD, Amasino RM. 1999. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11**: 949–956.

Michaels SD, He Y, Scortecci KC, Amasino RM. 2003. Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of a summer-annual flowering behavior in Arabidopsis. *Proc Natl Acad Sci USA* **100**: 10102–10107.

Minchin PEH, Lacointe A. 2005. New understanding on phloem physiology and possible consequences for modelling long-distance carbon transport. *New Phytologist* **166**: 771-779.

Mingo DM, Theobauld JS, Bacon MA, Davies WJ, Dodd IA. 2004. Biomass allocation in tomato

(Lycopersicum esculentum) plants grown under partial root zone drying: enhancement of growth. Functional plant biology 31:971-978.

Mitchell-Olds T., 2001. Arabidopsis thaliana and its wild relatives: a model system for ecology and evolution. *TRENDS in Ecology & Evolution* **16**:693-700.

Mitchell-Olds T. 2010. Complex-trait analysis in plants. *Genome Biol*. **11**:113.

Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Le Thiec D, Bréchet C, Brignolas F. 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of Populus deltoides x Populus nigra. *New Phytol*. **169**:765-77.

Monneveux P. and Belhassen E. 1996. The diversity of drought adaptation in the wide. *Plant Growth Regulation* **20**: 85-92.

Montesinos A, Tonsor SJ, Alonso-Blanco C, Picó FX. 2009. Demographic and genetic patterns of variation among populations of Arabidopsis thaliana from contrasting native environments**.** *PLoS One*. **4**:e7213.

Montesinos-Navarro, A., Wig, J., Xavier Pico, F. and Tonsor, S. J. 2010. Arabidopsis thaliana populations show clinal variation in a climatic gradient associated with altitude. *New Phytologist* **189,** 282:294.

Morgan T. 1911. Random segregation versus coupling in Mendelian inheritance. Science **34**: 384.

Mouchel CF., Briggs GC, Hardtke CS. 2004. Natural genetic variation in Arabidopsis identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. *Genes and Development* **18**, 700-714.

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M, Gibon Y. 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *J Exp Bot*. **62**:1715-29.

Muller B, Stosser M, Tardieu F. 1998. Spatial distributions of tissue expansion and cell division rates are related to irradiance and to sugar content in the growing zone of maize roots. *Plant, Cell & Environment* **21**: 149-158.

Muller B, Reymond M, Tardieu F. 2001.The elongation rate at the base of a maize leaf shows an invariant pattern during both the steady-state elongation and the establishment of the elongation zone. *J Exp Bot*. **52**:1259-68.

Muller B, Bourdais G, Reidy B, Bencivenni C, Massonneau A, Condamine P, Rolland G, Conéjéro G, Rogowsky P, Tardieu F. 2007. Association of specific expansins with growth in maize leaves is maintained under environmental, genetic, and developmental sources of variation. *Plant Physiol.* **143:**278-90.

Müller I., Schmid B., Weiner J. 2000. The effect of nutrients availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspectives in plant ecology, evolution, and systematics* **3**:115-127.

Mündermann L, Erasmus Y, Lane B, Cohen E, Prusinkiewicz P. 2005. Quantitative modelling of *Arabidopsis* development. *Plant Physiology* **139**:960–968.

Myles S, Peiffer J, Brown PJ, Ersoz ES, Zhang Z, Costich DE, Buckler ES. 2009. Association mapping: critical considerations shift from genotyping to experimental design. *Plant Cell*. **21**:2194-2202.

Nasrallah, M.E. et al. 2000. Arabidopsis species hybrids in the study of species differences and evolution of amphiploidy in plants. *Plant Physiol*. **124**:1605–1614.

Neale DB, Savolainen O. 2004. Association genetics of complex traits in conifers. *Trends Plant Sci* **9**:325-

30.

Nemri A, Atwell S, Tarone AM, Huang YS, Zhao K, Studholme DJ, Nordborg M, Jones JD. 2010. Genome-wide survey of Arabidopsis natural variation in downy mildew resistance using combined association and linkage mapping. *Proc Natl Acad Sci U S A*. **107:**10302-7.

New M, Lister D, Hulme M, Makin I. 2002. A high-resolution data set of surface climate global land areas. *Climate research* **21**:1-25.

Niklas KJ., 1994, Plant allometry, the scalling of forms and process, First edition. *The American journal of botany* **81**:134-144.

Nooryazdan H., Seriezs H., Bacilieri R., David J., Berville AJ. 2011. Construction of a crop-wild hybrid population for broadening genetic diversity in cultivated sunflower and first evaluation of its combining ability: the concept of neodomestication. *Euphytica* **178**:159-175.

Nordborg M, Bergelson J. 1999. The effect of seed and rosette cold treatment on germination and flowering time in some Arabidopsis thaliana (Brassicaceae) ecotypes. *Am J Bot* **86**: 470–475.

Nordborg M. 2000. Linkage disequilibrium, gene trees and selfing: an ancestral recombination graph with partial self-fertilization. *Genetics* **154**:923-9**.**

Nordborg M, Borevitz JO, Bergelson J, Berry CC, Chory J, Hagenblad J, Kreitman M, Maloof JN, Noyes T, Oefner PJ, Stahl EA, Weigel D. 2002**.** The extent of linkage disequilibrium in Arabidopsis thaliana. *Nat Genet*. **30**:190-3.

Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, Zheng H, Bakker E, Calabrese P, Gladstone J, Goyal R, Jakobsson M, Kim S, Morozov Y, Padhukasahasram B, Plagnol V, Rosenberg NA, Shah C, Wall JD, Wang J, Zhao K, Kalbfleisch T, Schulz V, Kreitman M, Bergelson J. 2005. The pattern of polymorphism in Arabidopsis thaliana. *PLoS Biol*. **3**:e196.

Nordborg M, Weigel D. 2008. Next-generation genetics in plants. *Nature* **456**:720-3.

Ochoa IE, Blair MW, Lynch JP. 2006. QTL analysis of adventitious root formation in common bean under contrasting phosphorus availability. *Crop Science* **46**:1609-1621.

Olien WC, Lakso AN. 1986. Effect of rootstock on apple (*Malus domestica*), tree water relations. *Physiologia plantarum* **67**:421-430.

Oono Y, Seki M, Kim JM, Takashi K, Umezawa T, Kuroda H, Matsui M, Shinozaki K. 2006. Functional analysis of Arabidopsis F-box proteins involved in drought stress response. *Plant cell and physiology* **47**:102

Orr HA. 2006. The population genetics of adaptation on correlated fitness landscapes: the block model. *Evolution* **60**:1113-24.

Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I. 2007. Specific root length as an indicator of environmental change. *Plant Biosystems* **141**: 426.

Padilla FM, Miranda JD, Jorquera MJ, Pugnaire FI. 2009. Variability in amount and frequency of water supply affects roots but not growth of arid shrubs. *Plant Ecol* **204**:261–270.

Palaisa K, Morgante M, Tingey S, Rafalski A. 2004. Long-range patterns of diversity and linkage disequilibrium surrounding the maize Y1 gene are indicative of an asymmetric selective sweep. Proc Natl Acad Sci U S A. 101:9885-90.

Parent B, Conejero G, Tardieu F. 2009. Spatial and temporal analysis of non-steady elongation of rice leaves. *Plant Cell Environ*. **32**:1561-72.

Parent B, Suard B, Serraj R, Tardieu F. 2010. Rice leaf growth and water potential are resilient to evaporative demand and soil water deficit once the effects of root system are neutralized. *Plant Cell Environ*. **33**:1256-1267.

Parmesan C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics* **37**:637-669

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD. 1988. Resolution of quantitative traits into medeleian factors by using a complete linkage map of restriction fragments length polymorphisms. *Nature* **335**:721-726

Patterson TB, Guy RD, Dang QL. 1997. Whole-plant nitrogen and water-relations traits, and their associated trade-off, in adjacent muskeg and upland boreal spruce species. *Oecologia* **110**:160–8.

Penning de Vries FW, van Laar HH. 1982. Simulation of plant growth and crop production. Center for agricultural publishing and documentation, Wageningen, the Netherlands, 308p.

Picó FX, Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C. 2008. Natural genetic variation of Arabidopsis thaliana is geographically structured in the Iberian peninsula. Genetics. 180:1009-21.

Pigliucci M, **Schlichting CD.** 1998. Reaction norms of Arabidopsis V- Flowering time controls phenotypic architecture in response to nutrient stress. *Journal of evolutionary biology* **11**:285-301.

Pigliucci M. 2001. Phenotypic plasticity: Beyond nature and nurture. Jones Hopkins University press**.**

Pigliucci M. 2003. Selection in a model system: ecological genetics of flowering time in Arabidopsis thaliana. *Ecology* **84**:1700–1712.

Platt A, Vilhjálmsson BJ, Nordborg M. 2010. Conditions under which genome-wide association studies will be positively misleading. *Genetics*. **186**:1045-52.

Poorter H, Rijn CPE, Vanhala TK, Verhoeven KJF, Jong YEM, Stam P, Lambers H. 2005. A genetic analysis of relative growth rate and underlying components in Hordeum spontaneum. *Oecologia* **142**: 360-377.

Poorter H, Nagel O. 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. *Functional Plant Biology*. **27**:1191.

Price AH, Tomos AD. 1997. Genetic dissection of root growth in rice (Oryza sativa L.). II: mapping quantitative trait loci using molecular markers. *Theoretical and Applied Genetics* **95**:143–152.

Price AH, Townend J, Jones MP, Audebert A, Courtois B. 2002. Mapping QTLs associated with drought avoidance in upland rice grown in the Philippines and West Africa. *Plant Mol Biol.* **48**:683-95.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945-59.

Pitzschke A. and Hirt H. 2010. New insights into an old story: Agrobacterium-induced tumour formation in plants by plant transformation. *The EMBO Journal* **29**:1021 - 1032.

Qaderi MM, Kurepin LV, Reid DM. 2006. Growth and physiological responses of canola (Brassica napus) to three components of global climate change: temperature, carbon dioxide and drought. *Physiologia Plantarum* **128**:710-721.

Qin Y, Ye H, Tang N, Xiong L. 2009. Systematic identification of X1-homologous genes reveals a family involved in stress responses in rice. *Plant Mol Biol.* **71**:483-96.

Qin H, Morris N, Kang SJ, Li M, Tayo B, Lyon H, Hirschhorn J, Cooper RS, Zhu X. 2010. Interrogating local population structure for fine mapping in genome-wide association studies. *Bioinformatics*. **26**:2961-8.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rafalski A. 2002. Applications of single nucleotide polymorphisms in crop genetics. *Curr Opin Plant Biol.* **5**:94-100.

Ramirez-Valiente, Valladares, Delgado, Granados S, Aranda I. 2011. Factors affecting cork oak growth under dry conditions: local adaptation and contrasting additive genetic variance within populations. *Tree genetics and genomes* **7**:285-295.

Rate DN, Cuenca JV, Bowman GR, Guttman DS, Greenberg JT. 1999. The gain-of-function Arabidopsis *acd6* mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth. *Plant Cell*. **11**:1695-708.

Rauh B., Basten C., Buckler E. 2002. Quantitative trait loci analysis of growth response to varying nitrogen sources in Arabidopsis thaliana. *Theoretical and Applied Genetics* **104**:743-750.

Rebai A. Goffinet B, Mangin B. 1995. Comparing power of different methods for QTL detection. *Biometrics* **51**:87-99.

Reiter RS, Coors JG, Sussman MR, Gabelman WH. 1991. Genetic analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphisms. *Theoretical and Applied Genetics* **82**: 561–568.

Remington DL., Ungerer MC, Purugganan MD. 2001. Map-based cloning of quantitative trait loci: Progress and prospects. *Genet Res*, **78**:213-218.

Reymond, P. et al. .2000. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. *Plant Cell* **12**:707–720.

Reymond M, Muller B, Leonardi A, Charcosset A, Tardieu F. 2003. Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiol*. **131**:664-675.

Reymond M, Svistoonoff S, Loudet O, Nussaume L, Desnos T. 2006. Identification of QTL controlling root growth response to phosphate starvation in Arabidopsis thaliana. *Plant, Cell & Environment* **29**:115-25.

Reynolds HL, and D'Antonio C. 1996. The ecological significance of plasticity in root weight ratio in response to nitrogen: Opinion. *Plant and Soil* **185**:75-97.

Robin C, Hay MJM, Newton PCD, Greer DH. 1994. Effect of light quality (red : far red ratio) at the apical bud of the main stolon morphogenesis of Trifolium repens L. *Annals of Botany* **74**: 119-123.

Robinson D. 1986. compensatory changes in the partitioning of dry matter in relation to nitrogen uptake and optimal variations in growth. *Annals of Botany* **58**: 841-848.

Rolland F., Moore B. and Sheen J. 2002. Sugar Sensing and Signaling in Plants. *The Plant Cell*, **14**:185:205.

Rosenberg, N. A. et al. 2010. Genome-wide association studies in diverse populations. Nature Rev. Genet. 11:356–366.

Rowe HC, Kliebenstein DJ. 2008. Complex genetics control natural variation in Arabidopsis thaliana resistance to Botrytis cinerea. *Genetics* **180**:2237-2250.

Ryser P, Eek L. 2000. Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. *American Journal of Botany* **87**: 402- 411.

Sadok W, Naudin P, Boussuge B, Muller B, Welcker C, Tardieu F. 2007. Leaf growth rate per unit

thermal time follows QTL-dependent daily patterns in hundreds of maize lines under naturally fluctuating conditions. *Plant Cell Environ*.**30**:135-46.

Saïdou AA, Mariac C, Luong V, Pham JL, Bezançon G, Vigouroux Y. 2009. Association studies identify natural variation at PHYC linked to flowering time and morphological variation in pearl millet. *Genetics*. **182**:899-910.

Salah H, Tardieu F. 1997. Control of Leaf Expansion Rate of Droughted Maize Plants under Fluctuating Evaporative Demand (A Superposition of Hydraulic and Chemical Messages?). *Plant Physiol*. **114**:893-900.

Saldana A, Gianoli E, Lusk CH. 2005. Ecophysiological responses to light availability in three blechnum species (Pteridophyta blechnaceae) of different ecological breadth. *Oecologia* **145**:252-257.

Sanguineti M, S. Li, M. Maccaferri, S. Corneti, F. Rotondo, T. Chiari, R. Tuberosa. 2007. Genetic dissection of seminal root architecture in elite durum wheat germplasm. *Annals of Applied Biology* **151**: 291-305.

Santoni S., Faivre-rampant F, Prado E and Prat D. 2000. Molecular markers for analysis of genetic resources and plant breeding. Cahiers Agricultures 9: 311-327.

Sax K. 1923. The association of size differences with seed coat pattern and pigmantation in Phaseolus vulgaris. *Genetics* **8**:552-560.

Schenk, P.M. et al. .2000. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. *Proc. Natl. Acad. Sci. U. S. A*. **97**:11655–11660.

Schlichting CD, Smith H. 2002. Phenotypic plasticity: Linking molecular mechanisms with evolutionary outcomes. *Evolutionary ecology* **16:**189-211.

Schmid KJ, Törjék O, Meyer R, Schmuths H, Hoffmann MH, Altmann T. 2006. Evidence for a large- scale population structure of Arabidopsis thaliana from genome-wide single nucleotide polymorphism markers. *Theor Appl Genet*. **112**:1104-1114.

Sebastian RL, Kearsey MJ, King GJ. 2002. Identification of quantitative trait loci controlling developmental characteristics of Brassica oleracea L. *Theoretical and Applied Genetics* **104**: 601- 609.

Sergeeva LI, Keurentjes JJB, Bentsink L, Vonk J, van der Plas LHW, Koornneef M, Vreugdenhil D. 2006. Vacuolar invertase regulates elongation of Arabidopsis thaliana roots as revealed by QTL and mutant analysis. *Proc Natl Acad Sci USA* **103**:2994-2999.

Setter TL, Yan J, Warburton M, Ribaut JM, Xu Y, Sawkins M, Buckler ES, Zhang Z, Gore MA. 2010. Genetic association mapping identifies single nucleotide polymorphisms in genes that affect abscisic acid levels in maize floral tissues during drought. *J Exp Bot*. **62**:701-16.

Shao HB, Chu LY, Jaleel CA, Zhao CX. 2008. Water-deficit stress-induced anatomical changes in higher plants. *C R Biol*. **331**:215-25.

Sharbel TF, Haubold B, Mitchell-Olds T. 2000. Genetic isolation by distance in Arabidopsis thaliana: biogeography and postglacial colonization of Europe. *Mol Ecol.* **9**:2109-18.

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES. 2000. The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). *Proc Natl Acad Sci USA* **97**:3753–3758.

Sherrard ME, Maherali H. 2006. The adaptive significance of drought escape in Avena barbata, an annual grass. *Evolution*. **60**:2478-89.

Shindo C, Bernasconi G, Hardtke CS. 2007. Natural genetic variation in Arabidopsis: tools, traits and

prospects for evolutionary ecology. *Ann Bot*. **99:**1043-54.

Shindo C. 2005. Role of FRIGIDA and FLOWERING LOCUS C in Determining Variation in Flowering Time of Arabidopsis. *Plant physiology* **138**:1163-1173.

Shipley B, Peters RH 1990. A test of the Tilman model of plant strategies: relative growth rate and biomass partitioning. *The American naturalist,* 136:139-153.

Simpson GG, Dean C. 2002. Arabidopsis, the Rosetta stone of flowering time? *Science* **296**: 285–289.

Skirycz A, Vandenbroucke K, Clauw P, Maleux K, De Meyer B, Dhondt S, Pucci A, Gonzalez N, Hoeberichts F, Tognetti VB, Galbiati M, Tonelli C, Van Breusegem F, Vuylsteke M, Inzé D. 2011. Survival and growth of Arabidopsis plants given limited water are not equal. *Nat Biotechnol*. **29**:212-4.

Sobeih W, Dodd IC, Bacon MA, Grierson D, Davies WJ. 2004. Long distance signals regulating stomatal condutance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjectted to partial root zone drying. *Journal of experimental botany* **55**:2353-2363.

Spollen WG, LeNoble ME, Samuels TD, Bernstein N, Sharp RE. 2000. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiol.* **122**:967-76.

Stanton ML, Roy BA, Thiede DA. 2000. Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution*, **54**:93-111.

Stenøien H, Fenster CB, Tonteri A, Savolainen O. 2005. Genetic variability in natural populations of Arabidopsis thaliana in northern Europe. *Mol Ecol* **14**:137–148

Stenoien HK, Fenster CB, Kuittinen H, Savolainen O. 2002. Quantifying latitudinal clines to light responses in natural populations of Arabidopsis thaliana (Brassicaceae). American Journal of Botany **89**:1604–1608.

Stinchcombe JR, Caicedo AL, Hopkins R, Mays C, Boyd EW, Purugganan MD, Schmitt J. 2005. Vernalization sensitivity in Arabidopsis thaliana (Brassicaceae): the effects of latitude and FLC variation. *American Journal of Botany* **92**:1701–1707.

Stinchcombe JR, Weinig C, Ungerer M, Olsen KM, Mays C, Halldorsdottir SS, Purugganan MD, Schmitt J. 2004. A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene FRIGIDA. *Proc Natl Acad Sci USA* **101**:4712–4717.

Stitwell RC, Fox CW. 2007. Environmental effect on sexual size dimorphism of a seed-feeding bettle. *Oecologia* **153**:273-280.

Storz JF, Kelly JK. 2008. Effects of spatially varying selection on nucleotide diversity and linkage disequilibrium: insights from deer mouse globin genes. *Genetics* **180**:367-79.

Streisfeld MA, Kohn JR. 2005. Contrasting patterns of floral and molecular variation across a cline in Mimulus aurantiacus. *Evolution* **59**:2548-2559.

Sultan ES. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**: 537-542.

Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T. 2007. Root tip contact with low-phosphate media reprograms plant root architecture. *Nature Genetics* **39**: 792 – 796.

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. 2005. Ethylen inhibits abscissic acid

induced stomatal closure in Arabidopsis. *Plant Physiology* **138**:2337-2343.

Tardieu F. et al. 2006. Expertise sècheresse INRA**.**

Tardieu F., Granier C., Muller B., 1999. Modelling leaf expansion in a fluctuating environment: are changes in specific leaf area a consequence of changes in expansion rate? *New phytol.* **143:**33-43.

Tardieu F, Muller B, Reymond M. 2003. Leaf growth regulation under drought: combining ecophysiological modelling, QTL analysis and search for mechanisms. *J Exp Bot*. **54** 18-19.

Tardieu F, Tuberosa R. 2010. Dissection and modelling of abiotic stress tolerance in plants. *Curr Opin Plant Biol*. **13**:206-12.

Tardieu F, Granier C, Muller B. 2011. Water deficit and growth. Co-ordinating processes without an orchestrator? *Curr Opin Plant Biol*. **14**:1-7.

Teale WD, Paponov IA, Palme K. 2006. Auxin in action: signalling, transport and the control of plant growth and development. *Nature Reviews Molecular Cell Biology* **7**:847-859.

Tezara W, Colombo R, Coronel I, Marín O. 2011. Water relations and photosynthetic capacity of two species of Calotropis in a tropical semi-arid ecosystem. *Ann Bot*.**107**:397-405.

Thaler P, Pagès L. 1996. Root apical diameter and root elongation rate of rubber seedlings (Hevea brasiliensis) show parallel responses to photoassimilate availability. *Physiologia Plantarum* **97:**365–371.

Thaler P, Pagès L. 1998. Modelling the influence of assimilate availability on root growth and architecture. *Plant and Soil* **201**:307–320.

Thompson,J.D., Gibson,T.J., Plewniak,F., Jeanmougin,F. and Higgins,D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24**:4876-48.

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler ES. 2001. Dwarf8 polymorphisms associate with variation in flowering time. *Nat Genet* **28**:286-9.

Thornley JHM.1972. A balance quantitative model for root:shoot ratios in vegetative plants. *Annals of botany* **36**:431-441.

Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q, Flint-Garcia S, Rocheford TR, McMullen MD, Holland JB, Buckler ES. 2011. Genome-wide association study of leaf architecture in the maize nested association mapping population. *Nat Genet*. **43**:159-62.

Tisné S, Reymond M, Vile D, Fabre J, Dauzat M, Koornneef M, Granier C. 2008. Combined genetic and modeling approaches reveal that epidermal cell area and number in leaves are controlled by leaf and plant developmental processes in Arabidopsis. *Plant Physiol*. **148**:1117-27.

Tisné S, Schmalenbach I, Reymond M, Dauzat M, Pervent M, Vile D, Granier C. 2010. Keep on growing under drought: genetic and developmental bases of the response of rosette area using a recombinant inbred line population. *Plant, Cell & Environment* **33**:1875-87.

Todesco M, Balasubramanian S, Hu TT, Traw MB, Horton M, Epple P, Kuhns C, Sureshkumar S, Schwartz C, Lanz C, Laitinen RA, Huang Y, Chory J, Lipka V, Borevitz JO, Dangl JL, Bergelson J, Nordborg M, Weigel D. 2010. Natural allelic variation underlying a major fitness trade-off in Arabidopsis thaliana. *Nature*. **465**:632-6.

Trevaskis B, Hemming MN, Dennis ES, Peacock WJ. 2007. The molecular basis of vernalization-induced flowering in cereals. *Trends Plant Sci*.**12**:352-7.

Troughton A 1956. Studies on the growth of young grass plants with special reference to the relationship

between the shoot and root systems. *Journal of the british grassland society* **11**:56-65.

Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S. 2002. Mapping QTLs regulating morpho-physiological traits and yield: Case studies shortcomings and perspectives in drought stressed maize. *Annals of Botany* **89**:941-943.

Tuberosa R, Salvi S. 2006. Genomics-based approaches to improve drought tolerance of crops.*Trends Plant Sci.* **11**:405-12.

Turner NC. 1997. Further progress in crop water relations. *Advances in agronomy*. **58**:293-338.

Ubeda-Tomas S, Federici F, Casimiro I, Beemster GTS, Bhalerao R, Swarup R, Doerner P, Haseloff J, Bennett MJ. 2009. Gibberellin signalling in the endodermis controls Arabidopsis root meristem size. *Curr Biol*, **19**:1194-1199.

Utz HF, Melchinger AE. 1994. Comparison of different approaches to Interval mapping of quantitative trait loci in 9th Eucarpia (section Biometric plant breeding, van Ooijen JW, Jansen RC eds) 195-204.

Valladares F, Sanchez-Gomez D, Zavala MA. 2006. Quantitative estimation of phenotypic plasticity: Bridging the gap between the evolutionary concept and its ecological applications. *Journal of ecology* **94**:1103-1116.

Vamerali T, Saccomani M, Bona S, Mosca G, Guarise M, Ganis A. 2003. A comparison of root characteristics in relation to nutrients and water stress in two maize hybrids. *Plant and soil* **255**:157-167.

van den Boogaard R, de Boer M, Veneklaas EJ, Lambers H. 2006. Relative growth rate, biomass allocation pattern and water use efficiency of three wheat cultivars during early ontogeny as dependent on water availability. *Physiologia plantarum* **98**:493-504.

van Kleunen M, Fisher M. 2005. Constraints on the evolution of adaptative phenotypic plasticity in plants. *New phytologist* **166**:49-60.

van der Weele CM, Spollen WG, Sharp RE, Baskin TI. 2000. Growth of Arabidopsis thaliana seedlings under water deficit studied by control of water potential in nutrient-agar media. *J Exp Bot*. **51**:1555-62.

van Eeuwijk F., Bink M., Chenu K. and Chapman SC. 2010. Detection and use of QTL for complex traits in multiple environments. *Curr. Opinion in Plant Biol*. **13**:193–205

Vartanian N, Marcotte L, Giraudat J. 1994. Drought Rhizogenesis in Arabidopsis thaliana (Differential Responses of Hormonal Mutants). *Plant Physiol*. **104**:761-767.

Verhoeven, K. J. F., Simonsen, K. L. and McIntyre, L. M. 2005. Implementing false discovery rate control: increasing your power. *Oikos* **108**: 643-647.

Verslues PE, Bray EA. 2006. Role of abscisic acid (ABA) and Arabidopsis thaliana ABA-insensitive loci in low water potential-induced ABA and proline accumulation. *J Exp Bot*. **57**:201-12.

Vlad D, Rappaport F, Simon M, Loudet O. 2010. Gene transposition causing natural variation for growth in Arabidopsis thaliana. *Plos Genetics*. **6**:5.

Vocanson A., Roger-Estrade J., Boizard H., and Jeuffroy MH. Effects of Soil Structure on Pea (Pisum sativum L.) Root Development According to Sowing Date and Cultivar. *Plant and Soil* **281**:121-135.

Voisin AS, Reidy B, Parent B, Rolland G, Redondo E, Gerentes D, Tardieu F, Muller B. 2006. Are ABA, ethylene or their interaction involved in the response of leaf growth to soil water deficit? An analysis using naturally occurring variation or genetic transformation of ABA production in maize. *Plant Cell Environ*. **29**:1829-40.

Wang Z. Quebedeaux B., Stutte GW. 1995. Osmotic adjustment: Effect of water stress on carbohydrates in leaves stems and roots of apple. *Australian journal of plant physiology*. **22:**747-754

Wang W, Vinocur B, Shoseyov O, Altman A. 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci.* **9**:244-52.

Wardlaw IF. 1990. The control of carbon partitioning in plants. *New Phytologist* **116:**341-381.

Weigel D. Mott R. 2009. The 1001 Genomes Project for *Arabidopsis thaliana*. *Genome Biology*, **10**:107.

Weisshuhn K, Auge H, Prati D. 2011. Geographic variation in the response to drought in nine grassland species. *Basic and applied ecology* **12**:21-28.

Welcker C, Boussuge B, Bencivenni C, Ribaut JM, Tardieu F. 2007. Are source and sink strengths genetically linked in maize plants subjected to water deficit? A QTL study of the responses of leaf growth and of Anthesis-Silking Interval to water deficit. *J Exp Bot*. **58**:339-349.

Werner T, Motyka V, Laucou V, Smets R, van Onckelen H, Schmülling T. 2003. Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alteration indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *The plant cell* **15**:2532-2550.

Wilson B. 1988. A Review of Evidence on the Control of Shoot: Root Ratio, in Relation to Models. *Ann Bot* **61**: 433-449.

Woo HR, Chung KM, Park JH, Oh SA, Ahn T, Hong SH, Jang SK, Nam HG. 2001. ORE9, an F-box protein that regulates leaf senescence in Arabidopsis. *Plant Cell* **13**:1779–1790.

Wright, G.C., and Nageswara Rao RC. 1994. Peanut water relations. p. 281–325. In J. Smartt (ed.) The groundnut crop. Chapman & Hall, London.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M. 2004. The worldwide leaf economic spectrum**.** *Nature.* **428**:821-827.

Xiong LZ, Liu KZ, Dai KX, Xu CG and Zhang Q. 1999. Identification of genetic factors controlling domestication-related traits of rice using an F2 population of a cross between Oryza sativa and O. rufipogon. *Theoretical and applied genetics*. **98**:244-251.

Xiong L, Wang RG, Mao G, Koczan JM. 2006. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant physiology* **142**: 1065.

Yin C, Wang X, Duan B, Luo J, Li C. 2005. Early growth, dry matter allocation and water use efficiency of two sympatric species as affected by water stress. *Environmental and Experimental Botany* **53**:315-322.

Yu J, Buckler ES. 2006. Genetic association mapping and genome organization of maize. *Curr Opin Biotechnol* **17**:155-60.

Yu, J., Holland, J. B., McMullen, M. D. & Buckler, E. S. 2008. Genetic design and statistical power of nested association mapping in maize. *Genetics* **178:**539–551.

Zhang, J. and Davies, W. J. 1987. Increased synthesis of ABA in partially dehydrated root tips and ABA transport from roots to leaves. *J. exp. Bot*. **38**: 2015–2023.

Zhang, X., Richards, E. J. & Borevitz, J. O. 2007. Genetic and epigenetics dissection of cis regulatory variation. *Curr. Opin. Plant Biol*. 10, 142–148.

Zhang Z, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM, Buckler ES. 2010. Mixed linear model approach adapted for genome-wide association studies. *Nat Genet*. **42**:355-60.

Zhang X, Zang R and Li C. 2004. Population differences in physiological and morphological adaptations of Populus davidiana seedlings in response to progressive drought stress. *Plant Science* **166**:791-797.

Zhang WP, Shen XY, Wu P, Hu B, Liao CY. 2001. QTLs and epistasis for seminal root length under a different water supply in rice (Oryza sativa L.). *Theoretical and Applied Genetics* **103**:118–123.

Zhao D., Reddy KR, Kakani DG, Reddy DR. 2005. Nitrogen deficiency efficiency effect of plant growth, leaf photosynthesis, and hyperspectral reflectance proprieties of sorghum. *European journal of agronomy* **22**: 391-403.

Zhao J, Paulo MJ, Jamar D, Lou P, van Eeuwijk F, Bonnema G, Vreugdenhil D, Koornneef M. 2007. Association mapping of leaf traits, flowering time, and phytate content in Brassica rapa. Genome. **50**:963-73.

Zhen Y, Ungerer MC. 2008. Clinal variation in freezing tolerance among natural accessions of Arabidopsis thaliana *New Phytologist* **177**:419-427.

Zheng ZB, Kao CH, Basten CJ. 1999. Estimating the genetic architecture of quantitative traits. *Genetic research* **74**:279-289.

Zheng ZB. 1994. Precision mapping of Quantitative trait loci. *Genetics* **136**:1457-1468.

Zheng BS, Yang L, Zhang WP, Mao CZ, Wu YR, Yi KK, Liu FY, Wu P. 2003. Mapping QTLs and candidate genes for rice root traits under different water-supply conditions and comparative analysis across three populations. *Theor Appl Genet*. **107**:1505-15.

Zhu J, Mickelson S, Kaeppler S, Lynch J. 2006. Detection of quantitative trait loci for seminal root traits in maize (Zea mays L.) seedlings grown under differential phosphorus levels. Theoretical and Applied *Genetics* **113**:1-10.

Zhu C., Gore M., Buckler ES., and Yu J. 2008. Status and Prospects of Association Mapping in Plants. *The Plant Genome* **1:**5–20.

Zinselmeier C. Jeong BR, Boyer JS. 1999. Starch and the control of kernel number in maize at low water potentials. *Plant physiology* **121**:25-36.

Growth maintenance under water deficit mainly results from the maintenance of water uptake at the root level, and assimilates production by leaves. To optimize both processes, plant need to adjust organ growth and biomass allocation patterns between roots and shoots (root/shoot ratio), but also within the organs, through specific leaf area and specific root length variations. The main objectives of this study were (i) to evaluate the impact of growth and biomass allocation patterns modifications on growth maintenance under drought conditions, (ii) to rely the genotypic responses to water deficit conditions and the climatic features of the natural environment in which they evolved, and (iii) to identify the key genetic regions responsible for shoot and root growth variation in response to water deficit conditions. We used different sets of genotypes, a population of recombinant inbred lines, and different sets of accessions of Arabidopsis thaliana, collected in a wide range of environments. An analysis of the allometric relationships between shoot and root growth related variables under both well watered and water deficit conditions allowed to highlight the importance of specific leaf area plasticity to maintain plant growth under water deficit. A detailed climatic characterization of the natural habitats of the accessions studied, combined to the evaluation of growth response to water deficit in these accessions allowed connecting low climatic water balance to better tolerance to water deficit conditions in specific regions, suggesting that this climatic feature could have shaped the evolution of genotypes in certain regions. Finally, using these two sets of genotypes, joint linkage and linkage disequilibrium analysis were performed on growth related traits under well watered and water deficit conditions. Some genetic regions involved in the control of root and shoot related traits were strongly coupled, especially in well watered experiments, but we managed to identify root specific regions using calculated variables that takes global plant growth as a cofactor. Under water deficit, the regions controlling root and shoot growth were less associated, and very strong QTL were detected, specifically associated to one or the other part. Genomic regions associated to growth response to water deficit were also detected, and the accuracy of association mapping enabled to identify target genes that could be play a role in growth maintenance under drought.