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SUMMARY

- Understanding the physiological and genetic bases of plant performance under drought is an important challenge in the context of global climate change.
- Changes in leaf area caused by drought were analysed in a population of recombinant inbred lines derived from a cross between two *Arabidopsis thaliana* accessions, L*er* and An-1, at two day-lengths. Quantitative trait loci (QTLs) controlling responses of leaf production and expansion to drought were identified and characterised by dynamic and cellular processes.
- The analysis presented here revealed that at least in short days, the maintenance or increase in rosette area in a few recombinant inbred lines was mainly due to changes in leaf production caused by a combination of three favorable alleles from both L*er* and An-1. In addition, genetic evidence that the maintenance of rosette area in An-1 is due to the low leaf area of An-1 in well-watered conditions has been reported. Finally, a favorable allele for the response of individual leaf expansion to water deficit originating from L*er* has been identified.
- This work is an example of the promising of quantitative genetics to give insight into the processes underlying plant performance, disentangle their complex relationships, and identify corresponding QTLs.

KEY WORDS: Arabidopsis, drought, quantitative trait loci, leaf expansion, leaf production

Introduction

Plant responses to soil water deficit have been described for years in the literature in terms of molecular, biochemical, physiological and developmental changes (see reviews from Passioura, 1996; Bray, 2004; Wery, 2005; Shinozaki & Yamaguchi-Shinozaki, 2007). This interest comes from the fact that in the actual agricultural context, water is an important factor limiting crop productivity. Furthermore, the occurrence of dry periods and the distribution of arid zones around the world have certainly been an important selective factor driving plant species biogeography. Reduction in leaf area and regulation of stomatal conductance are early responses to water deficit (Westgate & Boyer, 1985; Boyer, 1970). Maintained or increased root growth in response to drought has been reported in a number of species (Van der Weele *et al.*, 2000; Granier *et al.*, 2006) as well as early flowering (Passioura, 1996). All these co-ordinated responses allow plants to reduce transpiration rate, conserve water, explore new zones in the soil and to reproduce before dry conditions occur. However, because of inherent contradictions in their cascade effects, they cannot be directly related to drought tolerance (Tardieu, 2005). For example, reductions in leaf area, regulation of stomatal conductance and early flowering are often associated to a water conservation strategy which may have a positive impact in a few situations but may also cause a decrease in cumulated photosynthetic activity during the plant cycle and as such limit biomass production. Similarly, delay of senescence progression reported in response to drought can be seen as an advantage in some cases, with a lengthening of the photosynthetic activity over the plant cycle, but a disadvantage in others with an increase in water consumption (Borrell *et al.*, 2001; Rivero *et al.*, 2007). Genetic differences in one of these responses could certainly optimise plant performance under specific drought situations. However, this strongly depends upon the water deficit scenario including the severity of the deficit, the duration of the drought period and the position of the drought period in the plant cycle.

Genotypes with unaffected or increased whole plant leaf area could have an advantage in terms of plant performance under drought, at least in moderate or transitory water deficits scenarios (Tardieu, 2005). In order to perform a genetic engineering approach for the creation of genotypes with maintained or increased leaf area under drought treatments, it is important to identify which underlying processes are involved in the control of leaf growth and establish a hierarchy among them. In many analyses on the effect of environmental stresses, one considers that whole plant leaf area can be divided into two independent processes, leaf production and leaf expansion, and that stresses affect both independently (Yegappan *et al.*, 1982ab). However, many results are in support of a tight functional relation between both. For example, the number of cells within a leaf depends on the number of developing leaves (Wilson, 1966; Ter Steege *et al.*, 2005; Tisné *et al.*, 2008) while cell area within a leaf depends on flowering transition (Ashby, 1948; Cookson, Chenu & Granier, 2007; Tisné *et al.*, 2008).

The objective of the work presented here was to identify cellular or developmental strategies by which plants increase or maintain their leaf area in response to a moderate water deficit treatment, either via an increase in leaf production or an increase in leaf expansion. A QTL mapping approach and an original method for the evaluation of leaf expansion independently of leaf production were used with the aim of exploring the genetic architecture of several traits and identifying the relationships among them both in well-watered and water deficit conditions. The work present here was achieved on the model plant *Arabidopsis thaliana* because of the recent methodological concepts set up for reproducible phenotyping of leaf growth responses to drought and the genetic variability of leaf growth responses to drought found in this species (Granier *et al.*, 2006; Aguirrezabal *et al.*, 2006). A multi-scale dynamic phenotyping approach was used to identify events allowing stable leaf development when *Arabidopsis thaliana* plants are subjected to moderate drought treatments. In a preliminary work among a collection of 25 accessions, leaf production was shown to be less affected by drought than leaf expansion and, An-1 was identified as the only accession able to

maintain or increase its whole leaf area in response to drought (Aguirrezabal *et al.*, 2006). Therefore, a population of recombinant inbred lines issued from the cross between L*er* and An-1 was selected for this study with the idea to detect favourable alleles with an impact on individual leaf expansion in response to drought originating from An-1 (El-Lithy *et al.*, 2006).

MATERIAL AND METHODS

Plant material

For QTL mapping, 120 recombinant inbred lines (RILs) previously generated from a cross between Landsberg *erecta* (L*er)* and Antwerp (An-1) were used (El-Lithy *et al.*, 2006). SSLP markers were added on all the RILs to increase the density of markers on the genetic map and a new genetic map was generated using JoinMap4. Two RILs (RIL-103 and RIL-114) out of the 120 RILs were rejected from the analysis because of suspicious genotyping data. All the remaining RILs were grown together in four replicates with the two parental lines grown in eight replicates in each experiment.

Growth conditions

Plants were grown in four independent experiments in the PHENOPSIS automated phenotyping platform (Granier *et al.*, 2006). All micro-meteorological conditions were controlled to remain constant and homogeneous within the whole growth chamber during all four experiments (Granier *et al.*, 2006). Only the watering regime and day-length varied from one experiment to another (Table I). Light in the PHENOPSIS growth-chamber was provided by a bank of cool-white fluorescent tubes and HQi lamps. Day-length was maintained at 12 h or 16 h depending on the experiment. In all experiments, light was measured continuously at the plant level, using a photosynthetic photon flux density (PPFD) sensor (LI-190SB, LICOR, Lincoln, Nebraska, USA). Daily incident PPFD was calculated by multiplying day-length and mean instantaneous incident PPFD. Air temperature and relative humidity were measured every 20 s (HMP35A Vaisala Oy, Helsinki, Finland). All measurements of temperature, PPFD and relative humidity were averaged and stored every 600 s in a datalogger (Campbell Scientific, LTD-CR10 Wiring Panel, Shepshed, Leicestershire, England). Mean air vapour

pressure deficit (VPD) was calculated during the light period. For each experiment, mean micro-meteorological conditions are presented in Table I.

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 planting. Subsequent changes in pot weight were attributed to a change in soil water status. This allowed to calculate and adjust daily soil water content.

During Exp. 1 and 3, plants were grown at optimal soil water content (see Granier *et al.*, 2006). Soil water content was adjusted daily to 0.40 g H_2O g^{-1} dry soil from germination until the end of rosette leaf development (Table I). During Exp. 2 and 4, plants were grown in water deficit conditions from emergence of the first pair of leaves (stage 1.02, according to Boyes *et al.* 2001) until the end of rosette development. Soil water content was first adjusted daily to 0.35 g H_2O g^{-1} dry soil from germination until stage 1.02 and then, irrigation was stopped during a few days (4 to 5 depending on the genotype) to reach a soil water content of 0.20 g H_2O g^{-1} dry soil or 0.22 g H_2O g^{-1} dry soil respectively for experiment 2 and 4 respectively. Soil water content was maintained at this value until the end of rosette leaf development (Table I).

Growth measurements

Digital pictures of all individual pots were taken automatically on a daily basis during the experiments (Granier *et al.*, 2006). On these pictures, three times a week, stages of leaf development were scored for each individual plants as described in Boyes *et al.* (2001). At the end of the experiments, when plants had reached stage 6.00 (first flower open, Boyes *et al.*, 2001), the rosettes were cut, leaves were detached, laminas were separated from the petiole and stuck with double side adhesive on a sheet of paper in the order of their emergence on the rosette. The leaves were then scanned for further measurements. In addition, a transparent negative film of the adaxial epidermis of the sixth leaf was obtained after evaporation of a

varnish spread on the surface of the leaf. The sixth leaf was selected for cellular measurements as its whole development (from initiation to end of expansion) occurred during the water deficit period in Exp. 2 and 4.

Leaf area. Individual leaf area (LA_i, cm^2) was measured on the leaf scans with image analysis software (Bioscan-Optimas V 4.10, Edmonds, WA). Rosette area (RA, cm^2) was determined as the sum of the individual leaf blade area.

Leaf production. Leaf number (LN, leaves) was estimated at stage 6.00 by counting the number of leaves formed after the two cotyledons. Duration of vegetative phase (DV, days) was calculated as the time elapsed between the emergence of the first pair of leaves and the emergence of the first flower buds (corresponding respectively to stages 1.02 and 5.00 in Boyes *et al.*, 2001). Leaf emergence rate (LER, $leaves.day^{-1}$) was calculated as the ratio between the number of rosette leaves and the duration of the vegetative phase.

Calculation of the expansion index. To eliminate the effect of leaf number on individual leaf area, an expansion index was calculated for each genotype. A degree 2 polynomial was locally fitted to the relationships between rosette leaf number and leaf area for each nodal position using the *loess* function in R (degree of smoothing was set by alpha = 0.75), and the expansion index was calculated as the mean of the residuals to this fitted loess for leaves 3 to 6 of each plant.

Cellular development. Films of epidermal imprints of the sixth leaf were placed under a microscope (Leica, Leitz DM RB, Wetzlar, Germany) coupled to an image analyser. Epidermal cell area (CA, μm^2) was estimated by measuring 25 epidermal cell areas at four different zones on each leaf, near the base, near the tip and one on each side of the leaf with

image analysis software (Bioscan-Optimas V 4.10, Edmonds, WA). Mean epidermal cell area is the mean of these 100 cells. Epidermal cell number (CN) was estimated from epidermal cell density by counting the number of epidermal cells in three different zones on each leaf.

Quantification of the water deficit response. The response of each variable to water deficit was calculated for each RIL in each day-length condition as the ratio between the mean value in water deficit and the mean value in well-watered conditions.

QTL mapping

Composite interval mapping. QTLs were first identified using single interval mapping (SIM) in the software package MapQTL 5 (Van Ooijen J.W., 2007 MAPQTL®5, Software for the mapping of quantitative traits loci in experimental populations, Kyazma B.V., Wageningen, Netherlands). Cofactors were then selected using the automatic cofactor selection (ACS) chromosome per chromosome.

The selected cofactors were used in composite interval mapping (CIM). The cofactors for which no QTL was detected (LOD under a 95% LOD threshold < 2.4 estimated by performing permutation tests implemented in MapQTL®5 using at least 1000 permutations of the original data set) were removed successively.

Epistatic interactions. Detection and test of epistatic interactions between loci were performed using the software Epistat (Chase, Adler and Lark, 1997). Both epistatic interaction and QTL in main effects were statistically tested using the general linear model module of the statistical package of SPSS 11.0.1 for Windows (SPSS Inc., Chicago, USA). QTL models were composed of all statistically significant (p value < 0.05) main and interaction effects.

Statistical analyses

All statistical analyses were done using the computer package SPSS 11.0.1 for Windows (SPSS Inc., Chicago, USA) and the R software (R Development Core Team, 2007). Statistical differences between parental lines were tested by ANOVA in R. Broad sense heritabilities were estimated as the proportion of variance explained by between-line differences based on measurements of four plants per genotype. For each variable, the skewness of the frequency distributions was calculated to estimate their normality. To reduce the positive skewness of the distribution for rosette area, and leaf number (Supplemental Fig. 2), square root, log and reciprocal transformations were tested. A natural logarithmic transformation of the data approximated a normal distribution. The detection of QTL has been done with both non-transformed and transformed data and the two methods did not modify the QTL detection (not shown). The estimated additive genetic effect, the percentage of variance explained and the total variance explained by each QTL and all the QTLs of the QTL models were obtained using the statistical package of SPSS. Whether the relationships between leaf growth variables were affected by allelic segregation at specific loci was tested automatically using a script developed in R (R Development Core Team, 2007).

RESULTS

Modifications in the correlations between rosette area, number of rosette leaves and individual leaf area by drought.

Leaf production was quantified by the number of rosette leaves while leaf expansion was first quantified by individual leaf area at two given nodal positions: leaf 3 and leaf 6. In the four environmental conditions tested here (Table I), including optimal soil water content, water deficit treatments, short-day and long-day treatments (respectively 12 and 16 h day-length), rosette area was positively correlated to the number of rosette leaves (Table II). At both day-lengths, but only when plants were grown in drought conditions, rosette area was positively correlated to individual leaf areas (Table II). In optimal soil water content, both leaf 3 area and leaf 6 area were correlated to one another but only leaf 6 area was significantly, although weakly, correlated to rosette area (Table II). The number of rosette leaves was weakly and negatively correlated to individual leaf areas for plants grown in optimal soil water conditions whereas it was strongly and positively correlated to individual leaf areas for plants grown in the drought treatments (Table II).

An index was calculated to estimate individual leaf expansion independently of leaf production.

Leaf production and leaf expansion could not be considered as independent as shown by the correlations found between those variables and their modifications by the soil water status (Table II). For further analysis of this interaction, relationships between individual leaf area and the number of rosette leaves were examined for each nodal position in the four environmental conditions, and a loess adjustment was fitted to each relationship, as shown for leaves 3 and 6 (Fig. 1a-d). Consistent trends among the different leaves and the four different conditions were observed. For plants grown in well-watered conditions, individual leaf area at

a given nodal position decreased with increasing number of rosette leaves for rosettes with 5 to 15 leaves (Fig. 1a-c). However, for rosettes with more leaves (only in short days), individual leaf area was not affected by higher number of rosette leaves (Fig. 1a). For plants grown in water deficit, individual leaf area at a given nodal position increased with increasing number of rosette leaves for rosettes with 5 to 15 leaves (Fig. 1b-d). Again, for rosette with more leaves (only in short days) individual leaf area was no longer affected by higher number of rosette leaves (Fig. 1b). To eliminate the effect of the number of rosette leaves on the expansion of individual leaves, an index was calculated as the mean of the residuals to the fitted loess for leaves 3 to 6 of each plant (see "Materials and Methods"). This index quantifies the part of leaf area explained by leaf expansion itself, excluding the effect of leaf production. It is called the expansion index (EI) afterwards and is independent of the number of rosette leaves produced by the plant in all 4 conditions (Supplemental Fig. 1).

Variation in leaf growth variables in the two parental accessions grown in the four environmental conditions.

An-1 had a lower rosette area than L*er* in all four environmental conditions and this was due to lower values of both individual leaf area and number of rosette leaves (Fig. 2a, b; Fig. 3a, b). In short days, rosette area was reduced by a factor 6 for L*er* and by a factor 3 for An-1 when plants were grown under water deficit condition. For both accessions, the effect of water deficit was less pronounced in the long day experiment with a more moderate water deficit treatment: rosette area was reduced by a factor 1.2 for L*er* and was not significantly affected for An-1 (Fig. 3a). For both accessions in short days and for L*er* in long days, reductions in rosette area under influence of water deficit was related to reductions in individual leaf expansion (Fig. 2a, b). The number of rosette leaves was not affected by water deficit in the two accessions and for both day-lengths. Accordingly, in An-1 at both day-lengths and in L*er* in long days, water deficit treatments did not significantly affect the duration of the vegetative phase nor the leaf emergence rate (Fig. 2c-f). However, in L*er*

plants grown in short days, the water deficit treatment caused an increase in the duration of vegetative phase that was compensated for by a decrease in leaf emergence rate (Fig. 2c-f).

Epidermal cell area in leaf 6 was higher in An-1 than in L*er* in well-watered conditions at both day-lengths and in the water deficit treatment in long days (Fig 2g, h). In contrast, epidermal cell area did not differ between the two parental lines when plants were subjected to the water deficit treatment in short-days (Fig 2g). Water deficit caused a reduction in epidermal cell area in leaf 6 by a factor 2 for L*er* and by a factor 3.7 for An-1 in short days. In contrast, it was not significantly affected by the water deficit treatment in long days (Fig. 2g, h). Epidermal cell number in leaf 6 was much higher in L*er* than in An-1 in well-watered conditions at both day-lengths and in water deficit condition in long days. It was not affected by water deficit in An-1 for both day-lengths and was reduced by a factor 4.3 and 1.7 in L*er* for short days and long days respectively (Fig 2i, j).

Variation in rosette area, number of rosette leaves and expansion index within the population of RILs grown in four environmental conditions.

The range of variation in the whole population was higher in short days than in long days for the three variables (Fig. 3a-c; Supplemental Fig. 2). Water deficit treatment caused a decrease in mean rosette area in the population at both day lengths but it did not affect significantly the mean number of rosette leaves, even if the number of rosette leaves was increased in some lines under short day conditions (Fig. 3a, b). The broad sense heritabilities ranged from 0.74 to 0.94 for rosette area and the number of rosette leaves (Table III). For the expansion index heritabilities varied from 0.26 to 0.70 and were decreased by the water deficit treatment in both day-lengths conditions (Table III).

Genetic control of leaf growth responses to water deficit

As a first step, QTLs of the three leaf growth variables were detected in well watered and water deficit treatments and in short and long days (Fig. 4a, b). Among the 21 loci identified

using this method, ten corresponded to loci with QTLs identified both in the well-watered and water deficit conditions (Fig. 4a, b). Eight loci were identified with QTLs detected only in the well-watered treatment: three in short days controlling the expansion index, and five in long days controlling the expansion index for four of them and the number of rosette leaves for the last one (Fig. 4a, b). Only three loci were identified with QTLs detected only in the water deficit treatment: one in short days controlling the number of rosette leaves and two in long days controlling the expansion index (Fig. 4a, b).

As a second step, leaf growth responses to water deficit were quantified by the reductions of the three leaf growth variables at both day-lengths. Fourteen QTLs were detected for this set of three variables: nine in short days and five in long days (Fig. 5). They clustered at nine different loci and among them, six coincided with loci detected by the first method (Fig. 4a, b). Five loci controlled the response of rosette area to water deficit (RA-1, RA/LN-4, RA/EI-4, RA/LN-5 and RA-5) with epistatic interactions between RA/LN-4 and RA/LN-5 (Fig. 5). Among them, three were detected only in short days (RA-1, RA/LN-4 and RA/LN-5) while other two were detected at both day-lengths (RA/EI-4 and RA-5). Four loci controlled the response of the number of rosette leaves to water deficit (LN-2, RA/LN-4, RA/LN-5, LN-5) in short days. Two colocalized with QTLs previously detected for the response of rosette area to water deficit (RA/LN-4, RA/LN-5 while other two were in epistatic interaction (LN-2 and LN-5). Three loci controlled the response of the expansion index to water deficit (EI-1, EI-3 and RA/ EI-4). Two were only detected in long days (EI-1 and EI-3). EI-3 explained 12.5% of the genetic variance of the response of the expansion index to water deficit in long days. The third one was detected both in long and short days and it colocalized with a QTL for the response of rosette area to water deficit in long days (RA/ EI-4).

Detection of three interacting loci that affected the relationship between rosette area in well watered condition and in water deficit condition.

We tested whether the relationship between rosette area in the well watered treatment and in water deficit treatment was affected by allelic segregation at specific loci in short days as well as long days. Three markers, FRI on chromosome 4, SNP77 and SNP236 on chromosome 5 affected significantly the slope of the linear regression between the rosette area in well watered and water deficit treatments in short days, but not in long days (Supplemental Fig. 3). These three loci were previously identified as QTLs controlling the number of rosette leaves and rosette area in short days in well-watered as well as water deficit conditions (one on the top of chromosome 4 and two on the top of chromosome 5, Fig. 4a). Only two of them were also identified in long days with QTLs controlling the number of rosette leaves and rosette area both in well-watered and water deficit conditions (one on the top of chromosome 4 and one on chromosome 5, Fig. 4b). When variables were expressed in terms of responses to water deficit (see materials and methods), the locus on top of chromosome 4 was only identified in short days (RA/LN-4) with a positive effect of the L*er* allele both on rosette area and the number of rosette leaves. Only one locus was identified on chromosome 5, just between the two loci identified with absolute variables and also only in short days with a positive effect of the An-1 allele on the same variables (RA/LN-5, Fig. 4a and 5). Accordingly, the specific combination of three alleles with a L*er* allele around FRI and An-1 alleles at SNP77 and SNP236 conferred a lower reduction of rosette area in response to water deficit and this was specific for the short day experiments (Fig. 6a, b).

Relationships between these three loci and the responses of both leaf production and leaf expansion to water deficit.

Plants with the specific combination of the three alleles described above increased significantly their number of rosette leaves in response to water deficit, compared to plants without this combination in short days (water deficit response higher than 1.0, Fig. 7a). This increase in the number of rosette leaves in response to water deficit was due to a larger increase in the duration of the vegetative phase, while the response of leaf emergence rate to

water deficit did not differ significantly between plants with this specific combination of alleles and others (Fig. 7a). This effect was however only found in short days, as the response of neither the number of rosette leaves, of the duration of the vegetative phase nor the leaf emergence rate to water deficit differed between plants with or without this combination of three alleles in long days (Fig. 7d).

The specific combination of the three alleles also conferred a positive effect on the response of individual leaf expansion to water deficit in short days (Fig. 7c). Leaf 6 area was less reduced and this was due to a lower reduction in both epidermal cell area and epidermal cell number (Fig. 7c). In long days, the specific allelic combination did not affect significantly the response of leaf 6 area and epidermal cell area to water deficit but it allowed to maintain to some extent epidermal cell number in the leaf (Fig. 7d).

Characterisation of QTLs controlling the responses of rosette area and expansion index to water deficit

RA/EI-4 and RA-5 were identified as two clusters of QTLs, at SNP295 and nga76 respectively, that controlled the response of rosette leaf expansion to water deficit (Fig. 5). An An-1 allele at these respective markers had a positive effect on the response of rosette area or the expansion index to water deficit. Those two clusters were also identified as QTLs of rosette area and/or expansion index specific to the well watered treatment both in long and short days. Accordingly, at both day-lengths, the comparison of the allelic values of individual leaf area at these two markers revealed that in well watered conditions, individual leaf expansion was reduced in lines with the An-1 allele at SNP295 or nga76 compared to those with the L*er* allele (Fig. 8a-d). In contrast, in water deficit conditions, there was no effect of the allelic values at these markers on individual leaf areas (Fig. 8a-d). Leaf expansion of lines with the An-1 alleles at these markers was less reduced by water deficit only because individual leaf areas in well watered conditions were lower in lines with the An-1 allele than those with the L*er* ones (Fig. 8a-d).

EI-3, a QTL at SNP105, controlled the response of the expansion index to water deficit in long days with a positive effect of the L*er* allele at this marker (Fig. 5). This QTL clustered with QTLs for rosette area and number of rosette leaves both in well watered and water deficit conditions and both in short and long days (Fig. 4a, b). In long days, it also clustered with a QTL for the expansion index but only in the water deficit conditions (Fig. 4b). Accordingly, in short days, the allelic values at SNP105 did not affect individual leaf area neither in the well watered nor in the water deficit condition (Fig. 9a). In long days, the allelic values at SNP105 did not affect individual leaf area in the well-watered condition, but affected it significantly in water deficit conditions with a higher leaf area at each nodal position for RILs carrying L*er* alleles at SNP105 (Fig. 9b). At the cellular level, L*er* alleles at EI-3 conferred a lower decrease both in epidermal cell area and in epidermal cell number in long days whereas it did not affect the response of these two variables to water deficit in short days (Fig. 9c, d).

DISCUSSION

Leaf growth variables are differently affected by drought in the two parental lines.

The two parental accessions used in this study, L*er* and An-1, had previously been identified as displaying contrasted leaf growth in optimal conditions at the cellular, leaf and whole rosette levels (Tisné *et al.*, 2008). Results reported here indicate that both accessions also differ in their leaf growth responses when subjected to drought treatments.

At the whole rosette level, the number of rosette leaves was not affected by water deficit in L*er* and An-1 as previously reported for other Arabidopsis *thaliana* accessions (Aguirrezabal *et al.*, 2006) or other species (Yeggapan *et al.*, 1982a; Wery, 2005). However, in L*er* plants grown in short days, the soil water deficit treatment affected the duration of the vegetative phase without affecting the number of rosette leaves. This behavior seems to be peculiar since many studies using *Arabidopsis thaliana* have reported that leaf number as well as the date of bolting or flowering time are similarly affected by different treatments, including regimes of irrigation, light or day-length (Pigliucci *et al.*, 1995, Pigliucci & Kolodynska, 2002, Cookson, Chenu & Granier 2007). Consequently, in many studies, the total number of leaves is used to quantify the date of flowering (Alonso-Blanco *et al.*, 1998). Results presented here show that, at least for L*er* in short days and in response to a moderate water deficit treatment, flowering time and number of rosette leaves can be de-correlated.

At the cellular level, water deficit is known to affect both cell division and cell expansion within leaves in many species including Arabidopsis *thaliana*. Depending on the stage of application and the severity of drought, both cellular components can be differently affected (Yeggapan *et al.*, 1982b, Randall & Sinclair, 1988, Alves & Setter, 2004, Aguirrezabal *et al.*, 2006). Water deficit, here, was imposed during the whole growing period from the initiation of leaf 6 until the end of rosette expansion. Epidermal cell number was reduced by drought in L*er* leaves, but it was not affected in An-1 neither in short days nor in long days. In both

accessions, cell area was reduced by drought treatment in short days but not in long days. These results reveal an interaction between leaf growth responses to soil water deficit and day-lengths at the cellular level. However, it is difficult to make the distinction between a direct effect of day-length or an indirect effect via the number of leaves produced, as it has been shown that leaf production itself influences leaf expansion and cellular processes underlying leaf expansion (Wilson, 1966; Cookson, Chenu & Granier, 2007, Tisné *et al.*, 2008).

Both water deficit and day-length affect the relationships between leaf production and leaf expansion.

Evidence that leaf production and leaf expansion are linked is supported here by the results obtained for the population of recombinant inbred lines: in well watered conditions, leaf area at a given nodal position tends to be higher for plants with a reduced number of leaves. This was consistent with results obtained earlier by modifying day-length: increasing day-length reduces the number of rosette leaves and also causes an increase in individual leaf area at a given nodal position (Cookson, Chenu & Granier, 2007). However, results presented here show that this negative relationship between individual leaf area at a nodal position and the number of rosette leaves is not unique and differs among the different experimental treatments. The relationship remained negative, but differed slightly in well-watered conditions between the two experiments, suggesting that day-length could have a proper effect on it. In addition, the relationship was completely modified by soil water content since leaf area at a given nodal position was lower for plants with a reduced number of leaves in water deficit conditions. In many studies, the effects of environmental conditions on leaf production and leaf expansion are analyzed through the effects on the number of leaves as an indicator of leaf production, and the area of a leaf at a given nodal position as an indicator of leaf expansion (e.g. Yegappan *et al.*, 1982a, b; Aguirrezabal *et al.*, 2006). It is clear from our

results that this leads to a bias in the analyses when individual leaf expansion is compared in genotypes with different number of rosette leaves because part of individual leaf expansion depends on the number of leaves produced. The original method of loess adjustment proposed here allowed the estimation of a new variable quantifying the part of leaf expansion that is not related to leaf production. Thus the effects of drought were analyzed on both components of whole leaf area, thereby minimizing the interaction in a population of genotypes with a high range of variability in leaf number.

Short and long days revealed different QTLs involved in the response of leaf growth to drought.

An epistatic interaction between the top of chromosome 4 and the top of chromosome 5 controlled the response of leaf number and rosette area to water deficit only under short-day conditions. Each of the loci had an effect both in optimal and drought conditions, but the epistatic interaction was specific to the drought condition and conferred extreme phenotypes. Such an interaction was expected for traits like leaf number, which is tightly linked with flowering time as the pathways regulating these processes are known to have interacting determinants (Mouradov *et al.*, 2002). Moreover, epistasis have been often shown in *A. thaliana* to affect different traits in response to environmental fluctuations: flowering time in response to vernalization or day-length (Alonso-Blanco *et al.*, 1998), leaf $\delta^{13}C$ in response to drought (Hausmann *et al.*, 2005) or whole-plant growth in response to temperature (Alcázar *et al.*, 2009). In the L*er* x An-1 population, this interaction affected leaf production variables, with a cascade effect on leaf expansion, and it explained the main part of the rosette area response to water deficit in short-day conditions.

Long days modified substantially the response of leaf growth to water deficit: firstly, because the three-way interaction for leaf production had no effect at this day-length, and, secondly, because a long-day specific QTL, EI-3, controlled the response of leaf expansion to water deficit. This QTL was the only one detected in this study with a positive effect on leaf

expansion specific to the water deficit conditions. Two other QTLs, RA/EI-4 and RA-5, detected as responses of rosette area to water deficit, have an effect on leaf expansion only in the well watered treatment. Plants carrying the An-1 alleles at these two markers have smaller leaf area and number of leaf epidermal cells when grown in optimal conditions, while these QTLs were not associated with leaf area in water deficit conditions. These two QTLs could thus explain the An-1 phenotype which suffers from growth deficiency in optimal conditions while maintaining or even increasing its rosette area in response to moderate water deficit (Aguirrezabal *et al.*, 2006). In plants carrying L*er* alleles at EI-3, the maintenance of individual leaf area was related to a lower decrease in both cell area and cell number. It is noteworthy that this QTL could not have been detected if experiments had only been performed in short days. However, it is not possible to know whether this QTL is really long-day specific, or if it was masked in short-days conditions because of the very strong effect of the three way epistatic interaction described above. This epistatic interaction controlling leaf number affected indirectly most of the variance of leaf area in short days and this could explain why the expansion index calculated for the water deficit condition in short days had the lowest heritability and did not allow the detection of QTLs. Growing plants under different day lengths turned out to be highly efficient in the separation of the different processes that contribute to rosette area, namely leaf expansion itself, and the indirect effects of flowering time both on the number of leaves and on individual leaf expansion.

Perspectives in genetic engineering to increase or maintain leaf growth in drought conditions

A first strategy to maintain or increase rosette area in response to drought was related to a maintenance or an increase in the number of leaves associated with an increase in the duration of the vegetative phase. This response has rarely been reported in the literature and it contrasts with the strong selection for earlier flowering in dry environments reported in different species (Heschel & Riginos, 2005, Sherrard & Maherali, 2006). The loci on chromosomes 4

and 5 involved in this combination of alleles overlap with regions previously identified for flowering time in RIL populations with L*er* as one of the parental accession, and candidate genes have been proposed. In the L*er* x Cvi-0 RIL population, a strong epistatic interaction has often been detected in the top of chromosome 5, and has been attributed to polymorphism in *FLOWERING LOCUS C (FLC)* around SNP77 and *HUA2* around SNP236 (Alonso-Blanco *et al.*, 1998a; Juenger *et al.,* 2005). An-1 has been shown to contain an active allele of *FLC* which could be required in this interaction (El-Lithy *et al,.* 2006). For the SNP236 QTL, the polymorphism involved might be a *hua2.5* mutation as the parental accession L*er* carries this allele of the *HUA2* gene (Doyle *et al*., 2005). *HUA2* acts as a promoter of the floral repressor FLC in presence of a functional *FRI* allele; it confers a delayed flowering and several morphological defects (Wang *et al*., 2007). This conditional effect of *HUA2* on flowering time is consistent with the epistatic effects observed in the population used in this study. Plants carrying the L*er* allele at SNP236 marker produce less vegetative leaves and flower earlier. Under short-day conditions and in the water deficit treatment, all the QTLs found for rosette area and leaf number were epistatic with the QTL at SNP236 (Fig. 4a), conferring to this QTL a central role in the maintenance of expansion under drought via an adjustments of leaf production and flowering time. FRI, which is polymorphic between L*er* and An-1, is a candidate gene on chromosome 4, and it has been shown to condition *HUA2* effects on FLC. However, both parental accessions do not contain a functional allele of FRI (El-Lithy *et al.,* 2006).

A second strategy to maintain rosette area in response to drought is to maintain individual leaf expansion by controlling both cell expansion and cell division. EI-3 was the only locus detected for leaf expansion in response to water deficit which conferred an advantage independently of plant phenology. Many QTLs have been identified in the top of chromosome 3 in different RIL populations and for various traits and environments: flowering time and $\delta^{13}C$ (Juenger *et al.*, 2005) or rosette diameter (Ungerer *et al.*, 2002) in the L*er* x Cvi population, dry matter and nitrogen percentage in the Bay-0 x Sha population (Loudet *et al.*,

2003), rate of germination in the L*er* x Sha population (Clerkx *et al.*, 2004). In our study, a strong constitutive QTL of the number of rosette leaves has also been detected in this region, consistently with a QTL detected in the L*er* x An-1 population for flowering time in long-days (El-Lithy *et al.*, 2006). The effect of this region on leaf number being independent of day-length, the specific long days effect of EI-3 on leaf expansion does not add support for a single pleitropic locus.

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TABLES

Table I. Growth conditions in the four experiments. For each experiment, mean micrometeorological conditions from germination to end of rosette leaf area development are presented.

Exp.	Soil water content (g H_2O g^{-1} dry soil)	Day-length (h)	Mean air temperature (°C)	Daily incident light (mol m^{-2} d^{-1})	$VPD_{leaf-air}$ (kPa)
1	0.40	12	20.54	10.8	0.80
2	0.20	12	20.47	10.4	0.76
3	0.40	16	19.14	8.31	1.13
4	0.22	16	20.45	8.97	0.65

Table II. Pearson's correlations between leaf growth variables in the Ler x An-1 RIL population grown in long days (a) short days (b), well-watered (upper right), and water deficit (lower left) conditions

*,**, and ***, significant correlations with P values <0.05, <0.01, and <0.001 respectively. RA, rosette area; LN, number of rosette leaves; A_{L3}, leaf 3 area; A_{L6}, leaf 6 area.

(a)	RA	LN	A_{L3}	A_{L6}	(b)	RA	LN	A_{L3}	A_{L6}
RA		0.87***	0.16	0.23*	RA		0.88***	0.18	0.25**
LN	0.96***		-0.21*	-0.16	LN	0.93***		-0.22*	-0.16
A_{L3}	0.47***	0.39***		0.90***	A_{L3}	0.70***	0.53***		0.89***
A_{L6}	0.66***	0.58***	0.78***		A_{L6}	0.73***	0.56***	0.84***	

Table III. Broad sense heritabilities (h^2) measured in the L*er* x An-1 RIL population grown under short-day (SD) and long-day conditions (LD) in well-watered (WW) and water deficit (WD) treatments.

Leaf growth variables	Day-length	h^2	
		WW	WD
RA (cm^2)	SD	0.88	0.74
LN (leaf)	SD	0.94	0.94
EI (cm^2)	SD	0.68	0.26
RA (cm^2)	LD	0.88	0.82
LN (leaf)	LD	0.88	0.86
EI (cm^2)	LD	0.70	0.45

Supplementary Table I. Characteristics of the detected QTLs for leaf growth variables in the L*er* x An-1 population grown in short days (a, b, c) and long days (d, e, f). QTLs were detected for rosette area (RA, cm²), number of rosette leaves (LN, leaf) and the expansion index (EI, cm²), measured on plants grown in well watered conditions (a, d) and in water deficit conditions (b, e), and for the ratio between water deficit and well watered conditions (c, f).

(a)

Traits	r²G[a]	r²P[b]	Chromosome-Marker[c]	Position (cM)	F	p-value[d]	r²p[e]	2a[f]
RA	80.1	68.3	4-FRI	3.0	10.16	0.002	10.2	2.25
			4-SNP295	34.4	11.39	0.001	11.6	2.46
			5-SNP77	13.3	9.35	0.004	9.2	-2.45
			5-SNP304	79.7	18.55	<0.001	19.8	2.94
			3*5-nga172*SNP236	3.7*28.4	25.70	<0.001	47.0	-
LN	80.8	73.5	5-SNP77	13.3	8.15	<0.001	8.7	-1.32
			5-CIW10	81.3	34.25	<0.001	28.5	2.40
			2*4-SNP71*M4-41	25.7*0.0	7.66	<0.001	21.1	-
			3*5-nga72*SNP236	3.7*28.4	39.02	<0.001	57.6	-
EI	81.9	57.1	2-F3P11	24.6	27.27	<0.001	22.5	-0.30
			4-SNP295	34.4	84.70	<0.001	47.4	0.53
			5-SNP204	45.3	9.86	0.002	9.5	0.18

(b)

Traits	r²G[a]	r²P[b]	Chromosome-Marker[c]	Position (cM)	F	p-value[d]	r²p[e]	2a[f]
RA	95.3	70.5	3*5-SNP105*SNP236	0.0*28.4	17.91	<0.001	29.4	-
			4*5-FRI*SNP236	3.0*28.4	13.15	<0.001	23.4	-
			5*5-SNP77*SNP236	13.3*28.4	14.69	<0.001	25.5	-
			5*5-SNP236*nga129	28.4*63.2	9.76	<0.001	18.5	-
			5*5-SNP236*CIW10	28.4*81.3	20.81	<0.001	32.6	-
LN	76.5	71.9	3*5-SNP105*SNP236	0.0*28.4	19.76	<0.001	31.5	-
			4*5-FRI*SNP236	3.0*28.4	8.59	<0.001	16.7	-

			5*5-SNP77*SNP236	13.3*28.4	10.97	<0.001	20.3	-
			5*5-SNP236*nga129	28.4*63.2	12.13	<0.001	22.1	-
			5*5-SNP236*CIW10	28.4*81.3	26.61	<0.001	38.2	-
EI	-	-						

(c)

Traits	r²G[a]	r²P[b]	Chromosome-Marker[c]	Position (cM)	F	p-value[d]	r²p[e]	2a[f]
RA	-	35.5	1*5-SNP100*nga76	25.3*42.8	4.93	0.003	14.3	-
			4*5-SNP254*SNP358	8.7*18.6	8.36	<0.001	22.0	-
LN	-	73.5	2*5-msat2-5*nga129	0.0*63.2	5.60	0.001	15.4	-
			4*5-SNP254*SNP358	8.7*18.6	15.04	<0.001	32.9	-
EI	-	17.6	4-SNP295	34.4	21.59	<0.001	17.6	-0.23

(d)

Traits	r²G[a]	r²P[b]	Chromosome-Marker[c]	Position (cM)	F	p-value[d]	r²p[e]	2a[f]
RA	64.5	56.8	4-FRI	3.0	19.94	<0.001	16.6	2.00
			5-CIW10	81.3	22.28	<0.001	18.2	2.09
			3*5-nga172*SNP236	3.7*28.4	26.44	<0.001	44.2	-
LN	70.2	61.8	2-SNP391	43.8	8.95	0.004	8.7	0.63
			3-nga172	3.7	28.08	<0.001	23.0	1.09
			4-SNP232	55.2	9.55	0.003	9.2	-0.66
			5-SNP236	28.4	88.47	<0.001	48.5	-1.83
			5-MBK5	84.6	25.28	<0.001	21.2	1.02
EI	80.1	56.1	1-107	7.8	10.96	0.001	11.0	0.12
			4-FRI	3.0	19.29	<0.001	17.8	0.17
			5-SNP204	45.3	19.96	<0.001	18.3	0.16
			2*4-F3P11*SNP295	24.6*34.4	17.34	<0.001	36.9	-

(e)

Traits	r^2G[a]	r^2P[b]	Chromosome-Marker[c]	Position (cM)	F	p-value[d]	r^2p[e]	2a[f]
RA	64.5	56.8	4-M4-41	0.0	12.48	0.001	11.6	0.43
			4-F8D20	55.7	9.55	0.003	9.1	-0.38
			3*5-nga172*SNP236	3.7*28.4	39.15	<0.001	55.8	-
LN	80.8	73.5	3-nga172	3.7	10.25	0.002	9.9	0.52
			5-SNP236	28.4	68.87	<0.001	42.5	-1.31
			5-MBK5	84.6	19.15	<0.001	17.1	0.69
			4*4-M4-41*F8D20	0.0*55.7	7.11	<0.001	18.7	-
EI	97.0	44.0	2-Erecta	34.8	14.92	<0.001	14.4	-0.13
			3-SNP105	0.0	39.20	<0.001	30.6	0.22
			4-SNP254	8.7	8.02	0.006	8.3	0.18

(f)

Traits	r^2G[a]	r^2P[b]	Chromosome-Marker[c]	Position (cM)	F	p-value[d]	r^2p[e]	2a[f]
RA	-	19.1	4-SNP295	34.4	16.07	<0.001	14.1	-0.07
			5-SNP204	45.3	7.34	0.008	7.0	-0.04
LN	-	-						
EI	-	37.4	3-SNP105	0.0	12.77	<0.001	12.5	0.09
			1*4-nga128*SNP295	61.3*34.4	13.77	<0.001	31.7	-

[a] Percentage of genotypic variance explained by the QTL model

[b] Percentage of phenotypic variance explained by the QTL model

[c] Marker determined in cofactor selection (see Materials and methods)

[d] Significance of the term of the QTL model (see Materials and methods)

[f] Mean effect of the replacement of both An-1 alleles by L*er* alleles at the QTL

[e] Percentage of variance phenotypic explained by terms of the QTL model.

FIGURE LEGENDS

Figure 1. Bivariate relationships between the number of rosette leaves and leaf 3 area (open circles) and leaf 6 area (closed circles) in the L*er* x An-1 RIL population grown in four environmental conditions: under short days (left panels), well-watered (a) and water deficit treatments (b), under long days (right panels), well-watered (c) and water deficit treatments (d). Each point represents a single plant with four repeats of each RIL by environment. Solid and dotted lines show the loess estimate of the relationships between the number of rosette leaves and leaf 6 area and leaf 3 area respectively in the four experiments.

Figure 2. Leaf growth variables of Ler and An-1 grown under short-day (a, c, e, g, i) and long-day (b, d, f, h, j), well-watered (WW) and water deficit (WD) conditions. Profiles of final leaf area for Ler (closed symbols) and An-1 (open symbols) in well-watered (circles) and water deficit (triangles) treatments under short (a) and long days (b). Means and standard error are shown for duration of the vegetative phase (c, d), leaf emergence rate (e, f), and epidermal cell area (g, h) and number (i, j) in leaf 6 for Ler (black bars) and An-1 (white bars). Letters marks values that are not significantly different from each other (Tukey test, $P<0.05$).

Figure 3. Variation in rosette area (a), leaf number (b) and expansion index (c) measured in the two parental accessions and the Ler x An-1 RIL population grown under short-day (SD) and long-day (LD), well-watered (WW) and water deficit (WD) conditions. Grey bars represent the 95th percentile of RILs and the two black circles the extreme values. Solid and dashed vertical lines indicate mean value and median value, respectively. Black and white arrows indicate the Ler and An-1 values, respectively.

Figure 4. The Ler x An-1 linkage map showing QTLs for the rosette area (RA), number of rosette leaves (LN) and expansion index (EI) for well watered (WW) and water deficit (WD)

conditions under short-day (A) and long-day conditions (B). QTLs are represented by arrows centred on the marker determined in cofactor selection. The direction of arrows indicates the sign of the additive effect: arrows pointing upwards indicate that Ler alleles have a positive effect. The shape of the arrow indicates the nature of the QTL : main effects (headed arrows); in epistatic interactions (non headed arrows). The greyscale of the arrows indicates the percentage of phenotypic variance explained by the QTL : respectively 0-10%, 10-25%, 25-50% and 50-100% from the whitest to the darkest. Loci identified only in the well-watered treatment are shown with ellipse and loci identified only in the water deficit treatment are shown with rectangles. Loci identified in Figure 5 are indicated on the right margin of each chromosom.

Figure 5. The Ler x An-1 linkage map showing QTLs for the water deficit response of three leaf growth variables : rosette area (RA), number of rosette leaves (LN) and expansion index (EI) under short (SD) and long days (LD). For the complete set of detected QTLs, see Supplemental Table I. QTLs are represented by arrows centred on the marker determined in cofactor selection. The direction of arrows indicates the sign of the additive effect: arrows pointing upwards indicate that Ler alleles have a positive effect. The shape of the arrow indicates the nature of the QTL : main effects (headed arrows), in epistatic interactions (non headed arrows). The greyscale of the arrows indicates the percentage of phenotypic variance explained by the QTL. respectively 0-10%, 10-25%, 25-50% and 50-100% from the whitest to the darkest.

Figure 6. Relationships between rosette area in well-watered (WW) and water deficit (WD) conditions for RILs carrying the Ler allele at FRI marker and the An-1 alleles at SNP77 and SNP 236 markers (white circles, n=13). RILs with recombination events in one of the adjacent markers (grey triangles. n=10) and RILs with the other combinations of alleles (black

circles, n=85). Solid and dotted lines show the linear regressions for RILs with the different combinations of alleles. Data are presented for plants grown in short (a) and long days (b).

Figure 7. Response of underlying leaf growth variables to water deficit in RILs carrying the Ler allele at FRI marker and the An-1 alleles at SNP77 and SNP 236 markers (white bars, n=23) and RILs with the other combinations of these three alleles (black bars, n=85) grown under short (a, c) and long days (b, d). Leaf growth variables presented here are either related to leaf production (a, b): the number of rosette leaves (LN), the duration of the vegetative phase (DV), the leaf emergence rate (LER) or to leaf expansion: (c, d) leaf 6 area (AL6), epidermal cell area (CA) and epidermal cell number (CN).

Figure 8. Allelic values at the SNP295 marker (a, b) and the nga76 marker (c, d) for final individual leaf areas at six nodal positions. RILs were grown under short (a, c) and long days (b, d) in the well watered (circles) and water deficit (triangles) treatments. For both markers, closed and open symbols refer to RILs carrying a Ler or An-1 allele, respectively.

Figure 9. Allelic values at SNP105 marker for final individual leaf areas at six nodal positions (a, b) and the response of leaf 6 area (AL6), epidermal cell area (CA) and epidermal cell number (CN) to water deficit (c, d).

Profiles of final leaf area are presented for RILs carrying either a Ler (closed symbol) or an An-1 allele (open symbol) at SNP105 marker in well watered (circles) and water deficit (triangles) treatments both in short (a) and long days (b). The response of leaf 6 area (AL6), epidermal cell area (CA) and epidermal cell number (CN) to water deficit are presented for RILs carrying the Ler allele at SNP105 (white bars. n=38) and RILs carrying the An-1 allele at SNP105 (black bars. n=61) grown under short (c) and long days (d).

Supplementary figure 1. Relationships between the expansion index and the number of rosette leaves in the Ler x An-1 population grown in short (a, c) and long-days (b, d) under

well watered (a, b) and water deficit (c, d) conditions. None of the Pearson's correlations calculated in the fours conditions is significant (P value >0.05).

Supplementary figure 2. Frequency distributions of the three leaf growth variables in the Ler x An-1 population grown under short (a-c) and long day conditions (d-f) in the well-watered (black bars) and water deficit (white bars) conditions. Growth variables are : rosette area (a,d), number of rosette leaves (b,e) and expansion index (c,f).

Supplementary figure 3. Identification of markers at which the alleles affect the slope of the relationship between rosette area in well-watered conditions and rosette area in water deficit conditions in short (full line) and long days (dotted line). Values issued from the R script are expressed in –log (P-value) as a function of the positions on the five chromosomes in cM. The horizontal line is the threshold set at a P-value of 0.001.

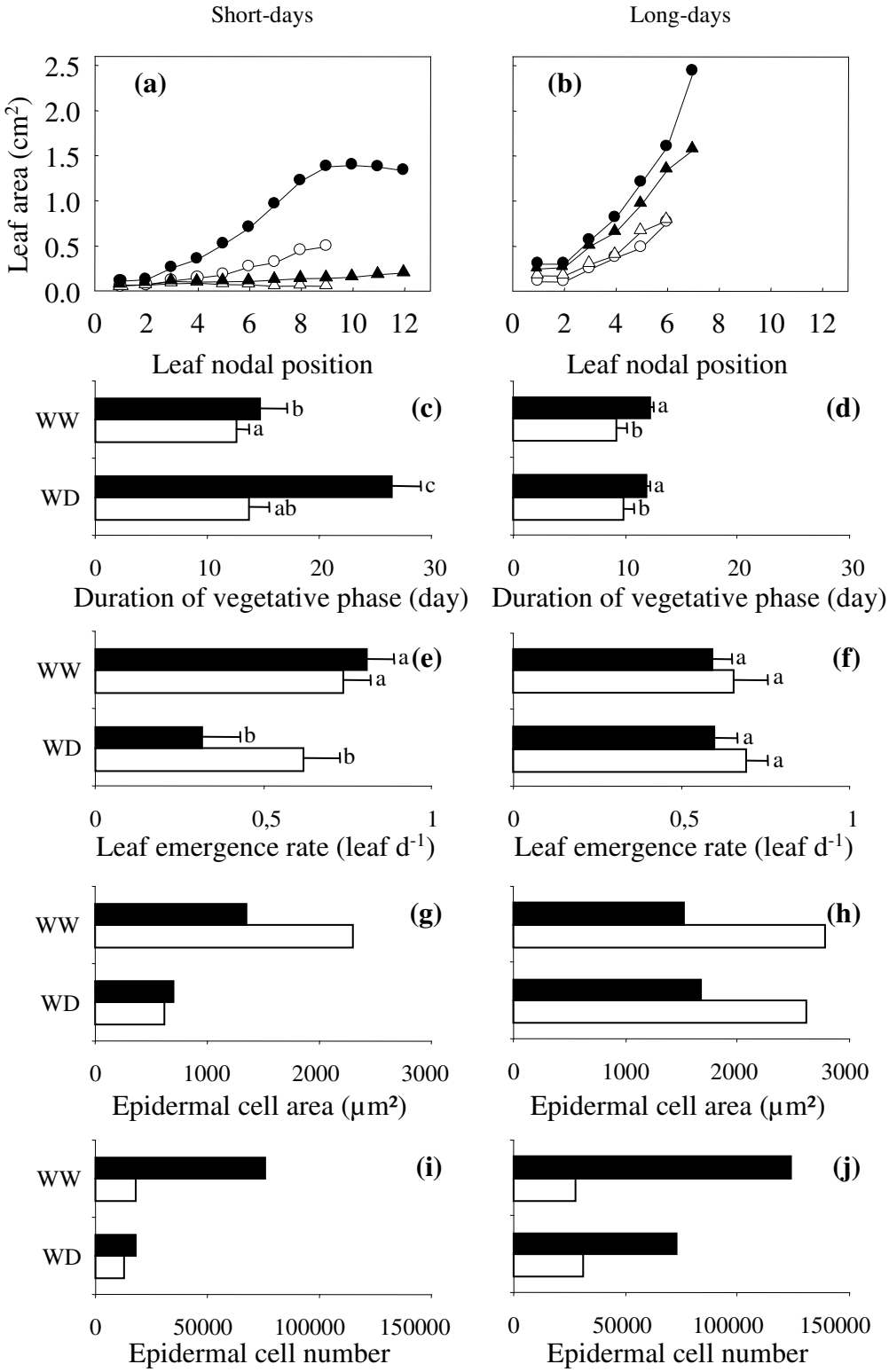


Figure 2. Leaf growth variables of Ler and An-1 grown under short-day (a, c, e, g, i) and long-day (b, d, f, h, j), well-watered (WW) and water deficit (WD) conditions. Profiles of final leaf area for Ler (closed symbols) and An-1 (open symbols) in well-watered (circles) and water deficit (triangles) treatments under short (a) and long days (b). Means and standard error are shown for duration of the vegetative phase (c, d), leaf emergence rate (e, f), and epidermal cell area (g, h) and number (i, j) in leaf 6 for Ler (black bars) and An-1 (white bars). Letters marks values that are not significantly different from each other (Tukey test, $P<0.05$).

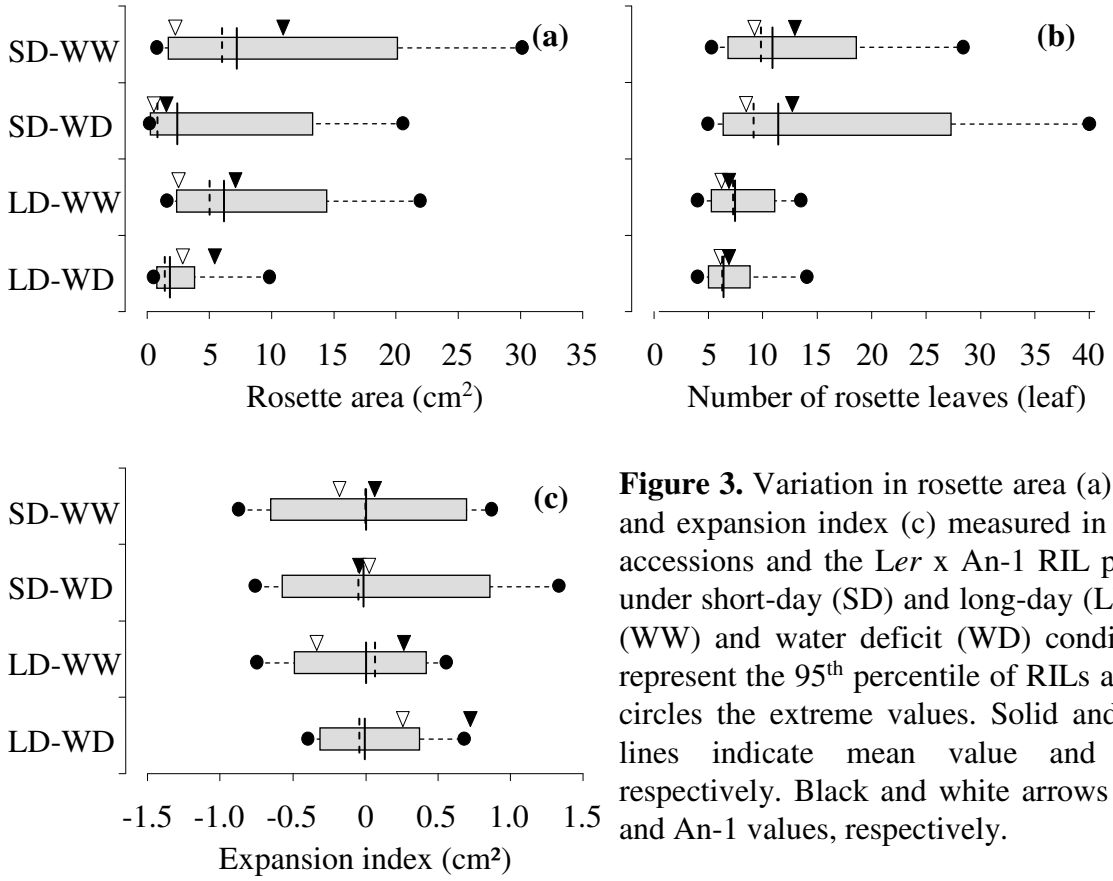
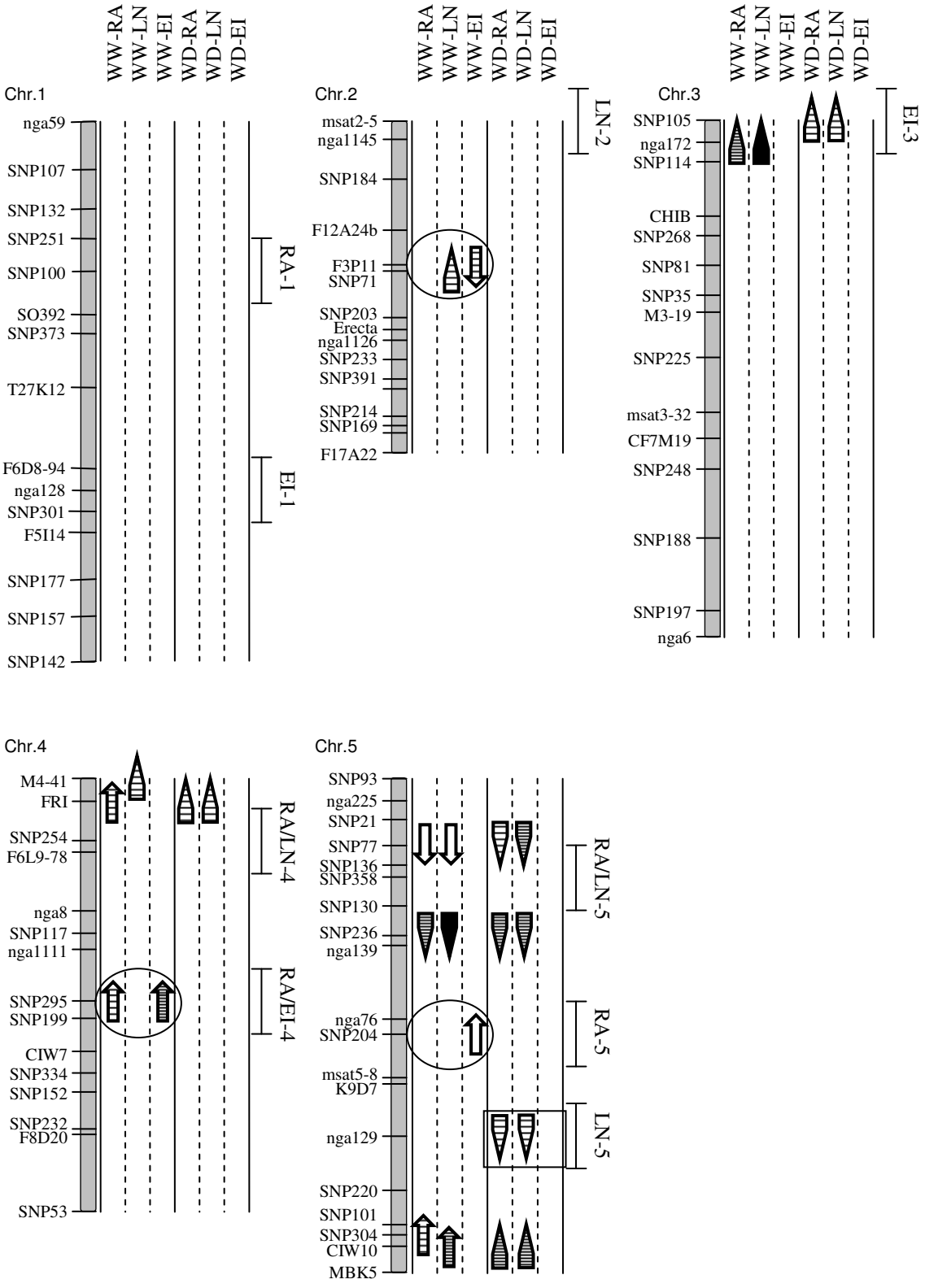


Figure 3. Variation in rosette area (a), leaf number (b) and expansion index (c) measured in the two parental accessions and the L*er* x An-1 RIL population grown under short-day (SD) and long-day (LD), well-watered (WW) and water deficit (WD) conditions. Grey bars represent the 95th percentile of RILs and the two black circles the extreme values. Solid and dashed vertical lines indicate mean value and median value, respectively. Black and white arrows indicate the L*er* and An-1 values, respectively.

(a) Short-day condition



(b) Long-day condition

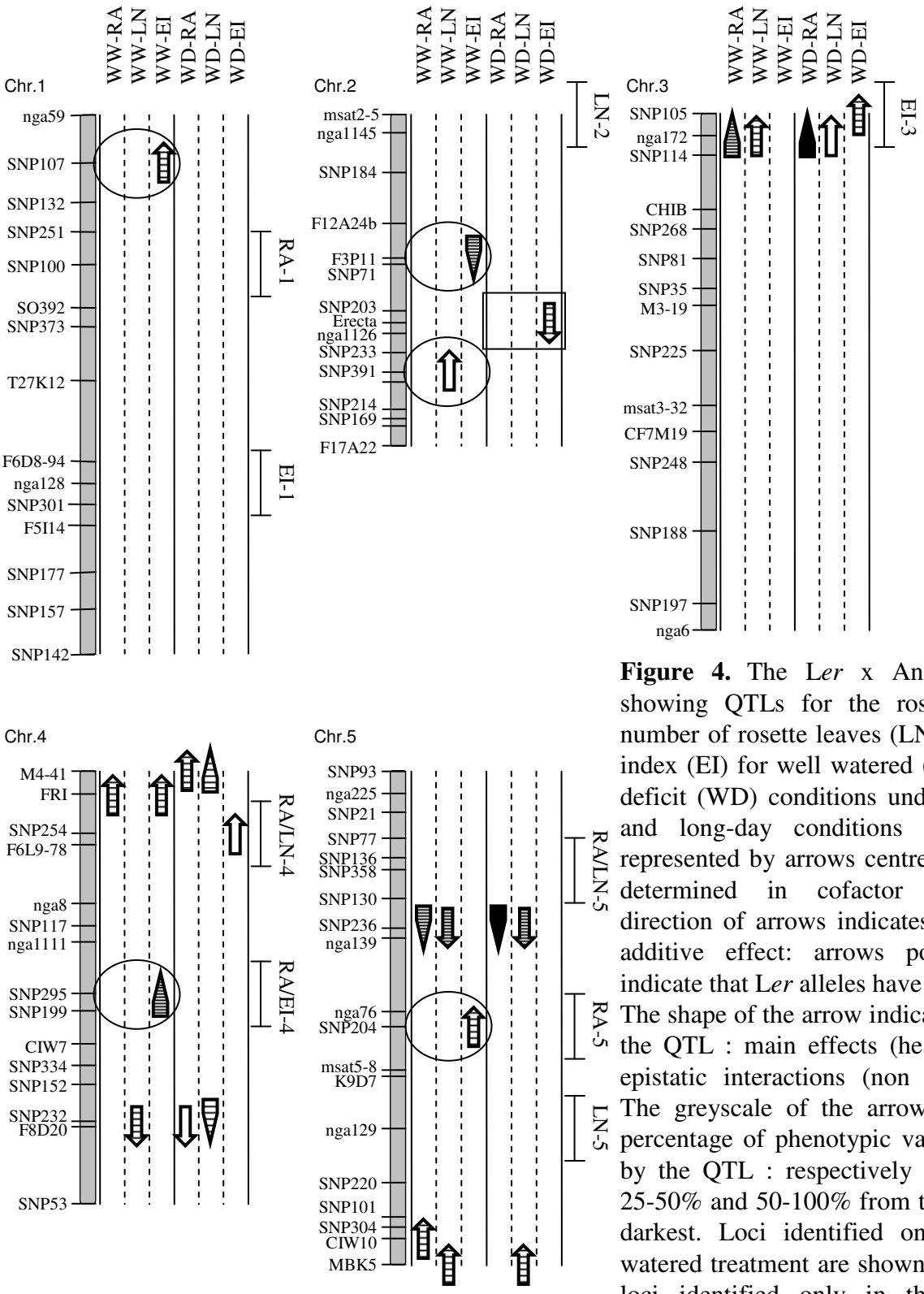


Figure 4. The L*er* x An-1 linkage map showing QTLs for the rosette area (RA), number of rosette leaves (LN) and expansion index (EI) for well watered (WW) and water deficit (WD) conditions under short-day (A) and long-day conditions (B). QTLs are represented by arrows centred on the marker determined in cofactor selection. The direction of arrows indicates the sign of the additive effect: arrows pointing upwards indicate that L*er* alleles have a positive effect. The shape of the arrow indicates the nature of the QTL : main effects (headed arrows); in epistatic interactions (non headed arrows). The greyscale of the arrows indicates the percentage of phenotypic variance explained by the QTL : respectively 0-10%, 10-25%, 25-50% and 50-100% from the whitest to the darkest. Loci identified only in the well-watered treatment are shown with ellipse and loci identified only in the water deficit treatment are shown with rectangles. Loci identified in Figure 5 are indicated on the right margin of each chromosom.

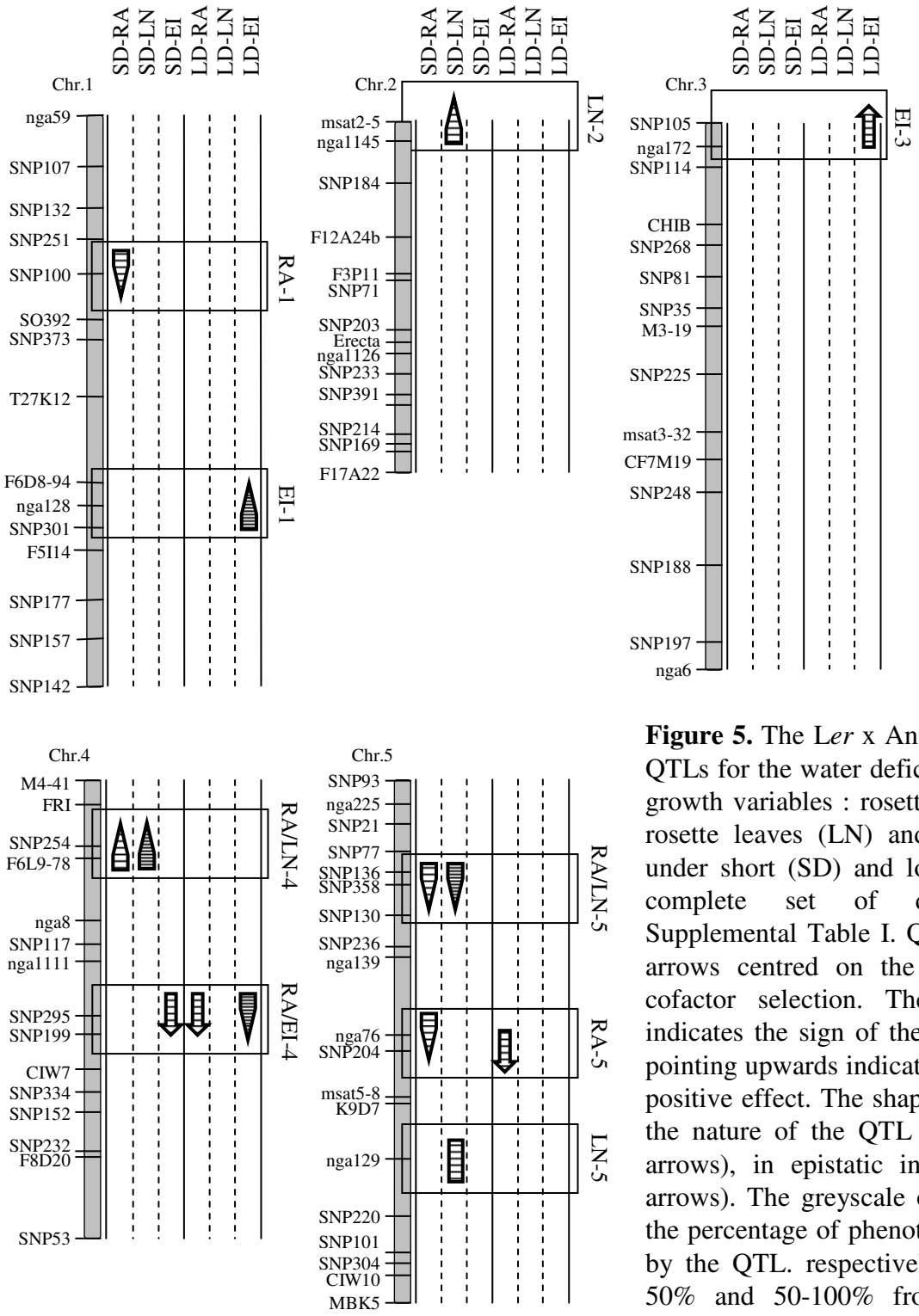


Figure 5. The L*er* x An-1 linkage map showing QTLs for the water deficit response of three leaf growth variables : rosette area (RA), number of rosette leaves (LN) and expansion index (EI) under short (SD) and long days (LD). For the complete set of detected QTLs, see Supplemental Table I. QTLs are represented by arrows centred on the marker determined in cofactor selection. The direction of arrows indicates the sign of the additive effect: arrows pointing upwards indicate that L*er* alleles have a positive effect. The shape of the arrow indicates the nature of the QTL : main effects (headed arrows), in epistatic interactions (non headed arrows). The greyscale of the arrows indicates the percentage of phenotypic variance explained by the QTL. respectively 0-10%, 10-25%, 25-50% and 50-100% from the whitest to the darkest.

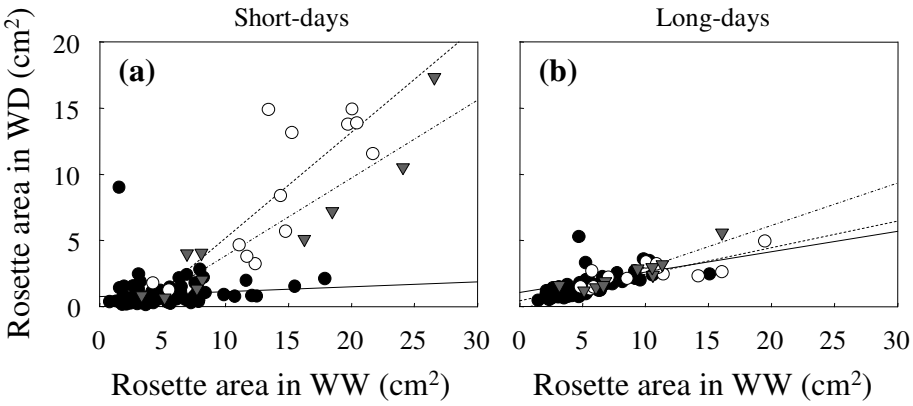


Figure 6. Relationships between rosette area in well-watered (WW) and water deficit (WD) conditions for RILs carrying the L*er* allele at FRI marker and the An-1 alleles at SNP77 and SNP 236 markers (white circles, n=13). RILs with recombination events in one of the adjacent markers (grey triangles. n=10) and RILs with the other combinations of alleles (black circles, n=85). Solid and dotted lines show the linear regressions for RILs with the different combinations of alleles. Data are presented for plants grown in short (a) and long days (b).

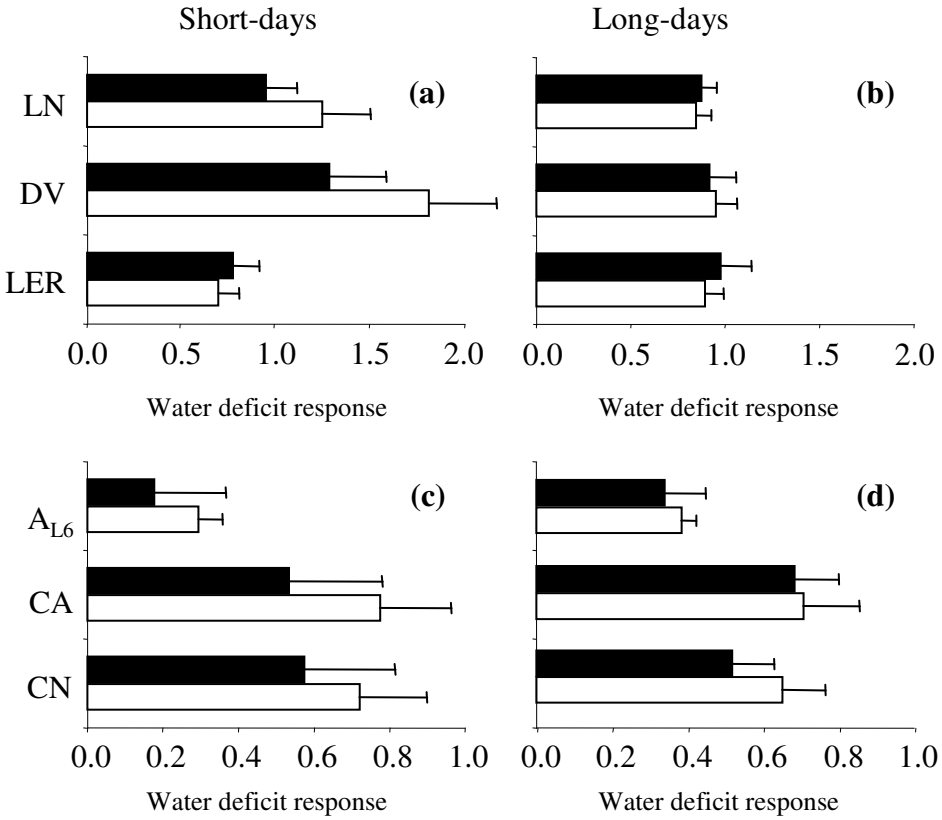


Figure 7. Response of underlying leaf growth variables to water deficit in RILs carrying the L*er* allele at FRI marker and the An-1 alleles at SNP77 and SNP 236 markers (white bars, n=23) and RILs with the other combinations of these three alleles (black bars, n=85) grown under short (a, c) and long days (b, d). Leaf growth variables presented here are either related to leaf production (a, b): the number of rosette leaves (LN), the duration of the vegetative phase (DV), the leaf emergence rate (LER) or to leaf expansion: (c, d) leaf 6 area (AL6), epidermal cell area (CA) and epidermal cell number (CN).

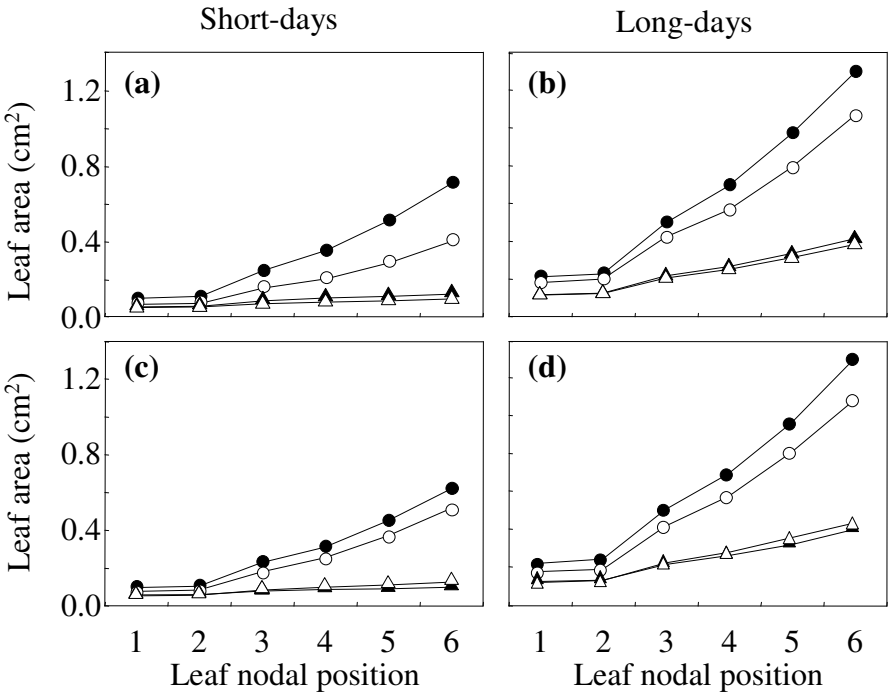


Figure 8. Allelic values at the SNP295 marker (a, b) and the nga76 marker (c, d) for final individual leaf areas at six nodal positions. RILs were grown under short (a, c) and long days (b, d) in the well watered (circles) and water deficit (triangles) treatments. For both markers, closed and open symbols refer to RILs carrying a L*er* or An-1 allele, respectively.

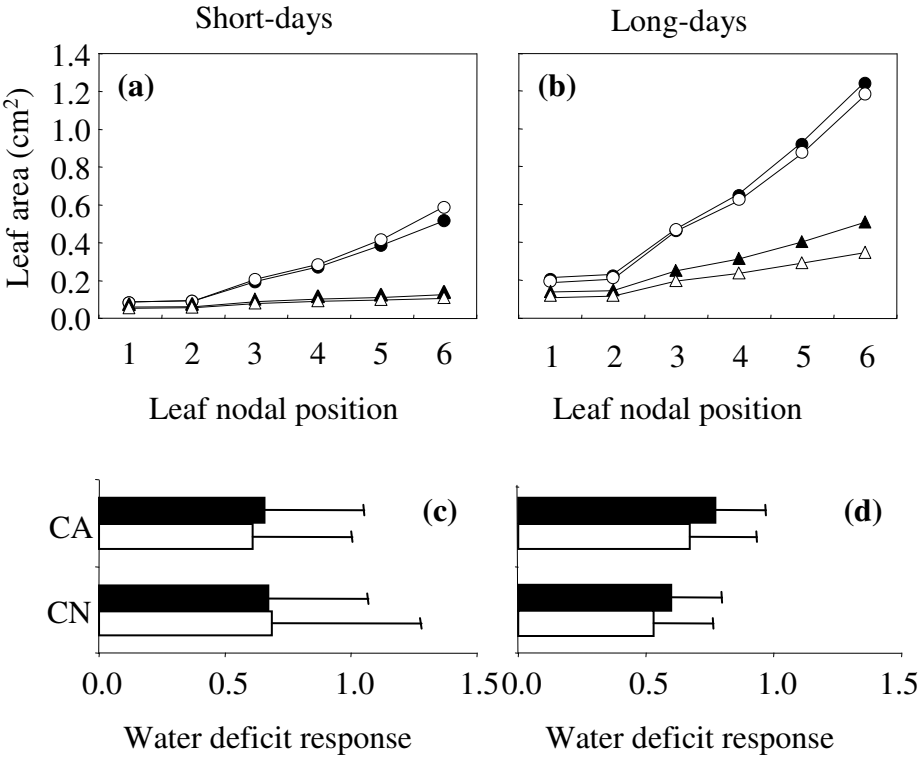
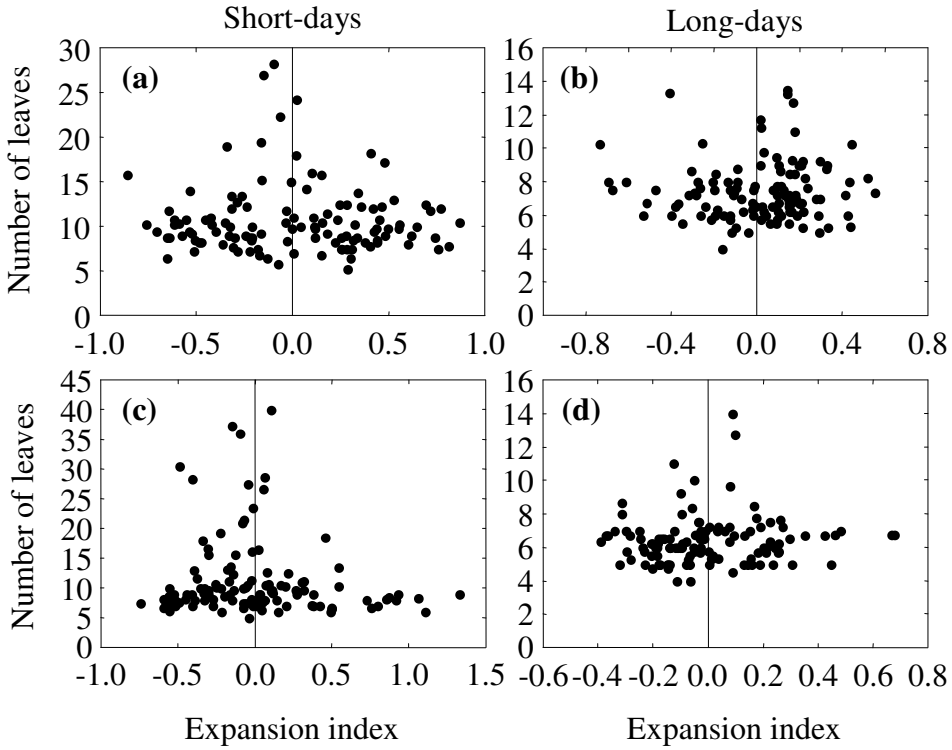
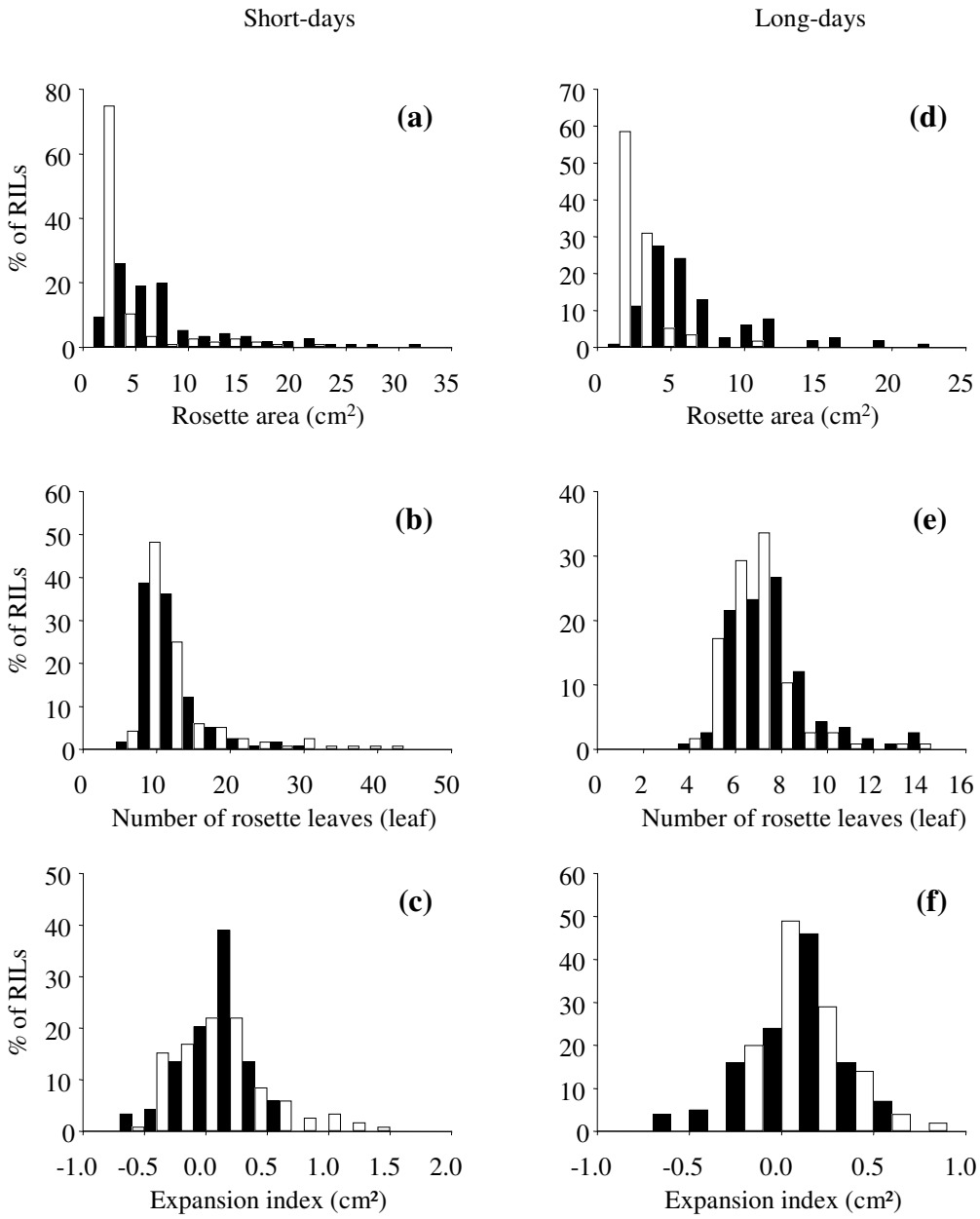


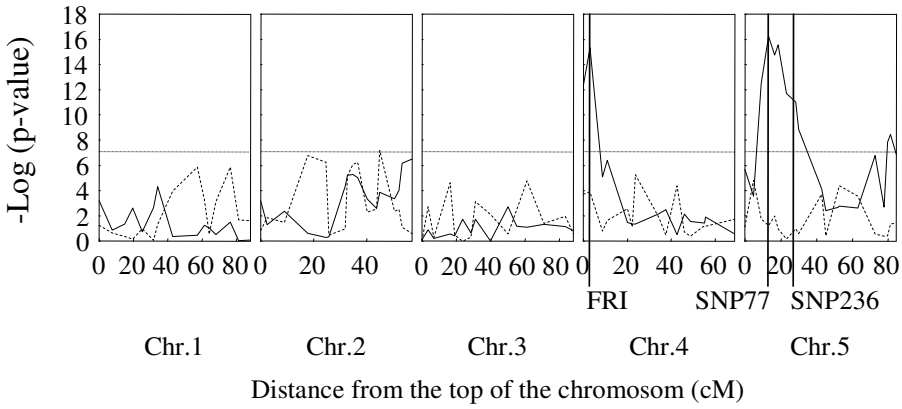
Fig. 9 Allelic values at SNP105 marker for final individual leaf areas at six nodal positions (a, b) and the response of leaf 6 area (A_{L6}), epidermal cell area (CA) and epidermal cell number (CN) to water deficit (c, d).
Profiles of final leaf area are presented for RILs carrying either a L*er* (closed symbol) or an An-1 allele (open symbol) at SNP105 marker in well watered (circles) and water deficit (triangles) treatments both in short (a) and long days (b). The response of leaf 6 area (A_{L6}), epidermal cell area (CA) and epidermal cell number (CN) to water deficit are presented for RILs carrying the L*er* allele at SNP105 (white bars. n=38) and RILs carrying the An-1 allele at SNP105 (black bars. n=61) grown under short (c) and long days (d).



Supplemental figure 1. Relationships between the expansion index and the number of rosette leaves in the L*er* x An-1 population grown in short (a, c) and long-days (b, d) under well watered (a, b) and water deficit (c, d) conditions. None of the Pearson's correlations calculated in the fours conditions is significant (P value >0.05).



Supplemental figure 2. Frequency distributions of the three leaf growth variables in the L*er* x An-1 population grown under short (a-c) and long day conditions (d-f) in the well-watered (black bars) and water deficit (white bars) conditions. Growth variables are : rosette area (a,d), number of rosette leaves (b,e) and expansion index (c,f).



Supplemental figure 3. Identification of markers at which the alleles affect the slope of the relationship between rosette area in well-watered conditions and rosette area in water deficit conditions in short (full line) and long days (dotted line). Values issued from the R script are expressed in –log (P-value) as a function of the positions on the five chromosomes in cM. The horizontal line is the threshold set at a P-value of 0.001.