The relation between carbohydrates, plant growth and flowering time in the Landsberg *erecta* x Kondara recombinant inbred line population

Running head: Natural allelic variation of carbohydrate traits in Arabidopsis.

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Abstract

Arabidopsis thaliana natural variation was used to study plant performance viewed as the accumulation of photo-assimilates, their allocation and storage, in relation to other growth-related features. A survey of Arabidopsis accessions revealed large variation for sugar and starch accumulation during the day.

Quantitative trait locus (QTL) analysis using recombinant inbred lines derived from the cross between Landsberg *erecta* and Kondara grown on hydroponics, revealed QTL for the different aspects of plant growth-related traits, sugar and starch contents and flowering-related traits. Co-locations of QTL for these different aspects were detected at different regions, mainly at the *ER* locus, the top of chromosome 3, 4 and 5 and the bottom of chromosome 5. In general, plant growth was more closely linked to leaf transitory starch levels than to the soluble sugar levels. From the significant correlation and the co-locations of the QTL for these aspects, we conclude that there is a complex relationship between plant growth-related traits, carbohydrate content and flowering-related traits.

Key words: Arabidopsis; dry weight; flowering time; area; QTL; RGR, sugars; starch.

Introduction

Growth of autotrophic plants depends on photosynthetic activity. Photo-assimilates can be either used directly for growth or respiration, or stored for a short period (e.g. in leaves, diurnal) or for a long period (e.g. in seeds or roots). The accumulation of carbohydrates in leaves has a role in regulating photosynthetic rate, as the level of these products is a function of the balance between photosynthesis and their use by growth processes of the plant.

Production of sugars through photosynthesis is an essential process, because sugar status modulates and coordinates internal regulators and environmental cues that control growth and development (Koch 1996; Sheen *et al*. 1999; Smeekens 2000). Sucrose is the major stable product of photosynthesis for most plants and it is also the form in which most carbon is transported in phloem vessels from leaves into sink organs such as roots, flowers, grains and tubers (Rolland *et al*. 2002).

Besides sucrose, starch serves as an important storage for carbohydrates. Many plants accumulate transitory starch in their leaves which serves as a short to medium term carbohydrate reserve. Transitory starch is degraded during the night, or when the rate of photosynthesis is low, to provide substrates for respiration and for the synthesis of sucrose and other translocated metabolites (Zeeman & Ap Rees 1999).

The extent to which starch accumulates in leaves differs between species. In *Arabidopsis thaliana* it is the major carbohydrate that accumulates and it is synthesized throughout the photoperiod (Caspar *et al.* 1985; Zeeman *et al*. 1998). The importance of storing carbohydrates as starch is reflected in the growth of plants that are unable to synthesize or to fully degrade transitory starch. In normal day/night conditions the starchless *Arabidopsis* lines *pgm-1* and *adg-1* (lacking plastidial phosphoglucomutase activity and ADP glucose pyrophosphorylase activity, respectively) grow more slowly than the wild type (Caspar *et al.* 1985; Lin *et al*. 1988). The growth rate of the *sex1-1* (starch excess) mutant, which has a reduced capacity to mobilize starch, is similarly affected (Caspar *et al.* 1991; Zeeman *et al*. 1998).

An alternative for laboratory induced-mutants, as source of genetic variation, is the use of genetic variation that can be found among naturally occurring populations of

Arabidopsis (Koornneef *et al*. 2004). The development of DNA markers has allowed studying naturally occurring allelic variation underlying complex traits by the association of trait values with specific alleles (Doerge 2002). Such analysis is often referred to as quantitative trait locus (QTL) analysis. The comparison of accessions allows genetically different parental lines to be selected for further studies. In addition, genetic variation undetectable by accession comparison might be revealed when analyzing segregating populations derived from crosses between accessions. This is the case when segregating individuals display phenotypes outside the range of variation of the parents (transgression) (Alonso-Blanco and Koornneef 2000).

Recently several studies have been published that describe the genetic variation for carbohydrate and growth traits in *Arabidopsis*. Cross *et al*. (2006) reported that extensive variation is present for a number of metabolic parameters and rosette weight among a diverse set of 24 Arabidopsis accessions. Significant correlations were detected between various traits. However, accessions were also found in which these correlations deviated from the general trends. Because correlations between traits might have non-genetic causes such as common selection or ancestry, genetic analysis should be performed to allow insight both the genetic basis of differences and the true pleiotropic effects due to common genetic regulation. Especially the question if and how differences in growth are due to genetic difference in metabolism is of importance for the understanding of physiology because it provides causal relationships instead of only correlative arguments. In *Arabidopsis* such analyses have been performed in the Bay-0 x Sha recombinant inbred line (RIL) population (Calenge *et al.* 2006) and in the Col x C24 population (Meyer *et al.* 2007 and Lisec *et al.* 2008) using rosette material and by Keurentjes *et al.* (2008) for 1 week old seedlings in the L*er* x Cvi RIL population. These studies identified a large number of QTLs, often with co-location of QTLs for traits that relate biochemically. However, they also indicate that the relationship between metabolites can be complex and that growth only correlates with levels of multiple metabolites (Meyer *et al*. 2007).

The present report describes the analysis of variation in carbohydrate content in a set of accessions and a QTL analysis of a RIL population derived from two parental lines

exhibiting contrasting levels of carbohydrates, viz., Landsberg *erecta* and Kondara (El-Lithy et al. 2006). Plants were grown on hydroponics, allowing the analysis of both rosettes and roots. Monitoring of flowering-related traits in the same material also allowed the analysis of the commitment to flowering in relation to growth and carbohydrate content. Furthermore, a QTL analyses were performed to the extracted values of the principal component analysis of the 45 studied traits of the L*er* x Kond RIL population.

Materials and Methods

Plant material and growth conditions

Arabidopsis thaliana accessions were obtained from the Arabidopsis stock centers ABRC, NASC and Sendai (www.arabidopsis.org), supplemented with accessions collected by members of the Laboratory of Genetics at Wageningen University and deposited at ABRC and NASC. Details of all accessions used in this study are given in supplementary Table 1. For the screening of the 123 accessions for their sugar and starch content at two time points, seeds were sown in Petri dishes on water-saturated filter paper followed by a 4-days cold treatment at 4 °C. They were then transferred to a climate room at 25°C and 16h light for two days before planting in 7-cm pots with standard soil. The plants (12 plants/accession) were grown in an air-conditioned greenhouse with 70 % relative humidity, supplemented with additional light (model SON-T plus 400W, Philips, Eindhoven, The Netherlands) providing a day-length of at least 16 h light (long day), with light intensity 125 µmol $m^{-2}s^{-1}$, and maintained at a temperature between 22-25 °C (day) and 18 °C (night).

To study the diurnal pattern of sugar and starch accumulation and for growth analyses, a number of 23 accessions and three mutants (24 plants/accession) were selected and grown under controlled conditions in a growth cabinet, with 70 % relative humidity, 22^{o}C, 12h day length and light intensity 25 Wm^{-2}. Plants were placed on carts and the carts were shuffled daily within the growth cabinet to avoid minor environmental differences within the cabinet. In all descriptions of experiments, time (days) is referred to as days after planting.

The RIL population was obtained from a cross between the accessions Landsberg *erecta* as a female (L*er*, N20) and Kondara (Kond, CS6175) as pollen parent.

For the analysis of sugars, starch, growth-related traits and flowering-related traits, the F10 seeds were transplanted in agar filled tubes and grown on hydroponic solution (Tocquin et al. 2003). The agar filled tubes were prepared by cutting a small part of the conical end of the 0.5 ml Eppen-dorf tubes (SARSTEDT, 72.698.200) then filled by 0.55% DAISHIN agar (DUCHEFA BIOCHEMIE, D1004.1000) and left to solidify. The

agar filled tubes were placed in holes prepared in black plastic sheets used as lids for the gray containers (46 x 31 x 8 cm, FAVORITE, 36.140) containing 9 liters of the hydroponic solution. The experiment was carried out in a randomized two-block design containing 18 plants per RIL of the F10 seeds. Seeds were sown in Petri dishes on water-saturated filter paper followed by a 4-days cold treatment at 4 °C, and then transferred to a climate room at 25°C and 16h light for one day before transplanting in the agar filled tubes. The experiment was also carried out in a growth cabinet under the same growth conditions mentioned before for growing the Arabidopsis accessions. A line with the *ERECTA* wild type allele in the L*er* genetic background and both parents were included in the experiment.

Measurement of sugars and starch

For analyses and quantification of soluble sugars (glucose, fructose and sucrose) and starch, for the screening of 123 Arabidopsis accessions or for determining the diurnal pattern of the accessions plus the three mutants, three leaves per plant were sampled together and immediately frozen in liquid nitrogen. This analysis was carried for three plants per accession at each time point. For the analysis of carbohydrate levels in the RILs of (L*er* x Kond), whole rosette leaves and roots were harvested separately for all the measurements, and then freeze-dried. The freeze-dried materials were ground, and samples between 5-10 mg were weighed. Two plants per block for each RIL were harvested for the analysis of sugars and starch.

Sugars were extracted by boiling in 80% methanol and quantified as described by Bentsink et al. (2000), with one exception, that the soluble sugars were separated by elution in an increasing linear gradient concentration of NaOH (20-150mM), with a flow rate of 1mL per minute. Starch was determined from the pellets of the sugar extractions, after extensive washing with water. Starch is determined as glucose, using a commercially available kit (EnzyPlus starch testkit, EZO 942).

Digital imaging, computer analysis and RGR determination

The mean total leaf area (TLA) of each accession was obtained by imaging 20 to 24 plants per accession at 10 (TLA1), 15 (TLA2) and 20 (TLA3) days after transferring the seedlings to the pots. The mean total leaf area (TLA) of each RIL was obtained by imaging 5 plants per RIL at the same time points after transferring the seedlings to the agar filled tubes. Leaf areas were determined with an image processing technique, using a Nikon digital camera (model COOLPIX 950) (Nikon Corporation Imaging Products Division, Shinagawa-Ku, Tokyo, Japan), and analysis of the pictures using the computer program MetaMorph (version 4.01) (Universal Imaging Corporation, West Chester, USA, www.imagel.com). The relative growth rate (RGR), on the basis of rosette areas, was calculated according to the following equation: $(\ln A_x - \ln A_y)/dt_{(x-y)}$, where "A" is the rosette area measured at "x" and "y" the second and the first time points, and "dt" is the time difference in days between these two points. RGR was calculated for each line based on the three measurements of rosette area, resulting in RGR2-1, RGR3-2 and RGR 3-1, referring to RGRs in the intervals 10 to15, 15 to 20, and 10 to 20 days, respectively.

Weight, water content, shoot/root ratio, RGR, SLA and root length determinations

The mean fresh weights (FW) of shoots for each RIL were determined at day 15 and 25, by harvesting and weighing two plants per line from each block. Dry weights (DW) of shoots (at day 15 and 25) and roots (only at day 25) were determined after drying the plants at 105 ^{o}C for 48 h. The water content (WC) of shoots was estimated as the relative ratio between water and dry weight using the formula [(FW-DW)/FW]*100. The shoot/root ratio (Sh/Ro) was calculated as shoot dry weight divided by root dry weight at day 25. The relative growth rate as based on dry weight of shoots (RGRsdw) was calculated in the same way as RGR based on leaf area. The specific leaf area (SLA) was calculated as area divided by weight ($mm^2.mg^{-1}$). Plant root length was measured at day 15 and 25 using a ruler for two plants for each RIL from each block.

Chlorophyll fluorescence, chlorosis and rosette leaf length measurements

Chlorophyll fluorescence as a non-destructive means of photosynthetic capacity was obtained for each RIL by measuring three different leaves per plant for three plants

for each RIL from each block. The measurements were done by using a MINI-PAM (S/N: 0133) (WALZ Mess- und Regeltechnik, Effeltrich, Germany), with the determination of the effective quantum yield of photosynthetic energy conversion (Yield = $\Delta F/F_{m'}$); where ΔF = variable fluorescence and $F_{m'}$ = fluorescence yield at zero photochemical and non-photochemical quenching for dark non-adapted leaves) (Van Kooten and Snel 1990). ChlF was measured at 125 µmol $m^{-2}s^{-1}$ light intensity, 70 % relative humidity and 22 °C.

Chlorosis was estimated at day 48, by scoring the color of the whole rosette leaves on a scale ranging from 1 (dark green) to 7 (yellow). Rosette leaf length was measured upon flowering using a ruler from the plant stem till the tip of the three largest leaves per plant for three plants for each RIL from each block.

Measurement of flowering time and related traits in RILs

From the hydroponics experiment, in which 18 plants/RIL were grown in SD condition, flowering time (FT) for three plants was recorded as the number of days from planting till the opening of the first flower. Flowering time was also scored by counting the total leaf number (TLN) i.e., rosette leaf number (RLN) plus cauline leaf number (CLN), excluding the cotyledons, since there is a close correlation between leaf number and flowering time (Koornneef et al. 1991). The following traits were also recorded: total plant length (TPL), plant length till first silique (PLTS), inflorescence length (INFL), number of rosette branches (RB), number of stem branches (SB) and total number of side shoots or inflorescences (TNB) (number of branches in the main inflorescence plus the number of side shoots from the rosette).

Genetic mapping

The mapping of the segregating population derived from the cross Landsberg *erecta* x Kondara was done by using 51 SNPWave, 23 SSLP markers and the morphological marker *erecta*, located at a distance from 0.5 –13 cM on the genetic map to obtain a regular distribution among the five chromosomes. These 75 markers were used to generate the linkage map as described in El-Lithy et al. (2006). To reduce the size of few gaps that still there and to have dense markers in certain regions, 24 new SSLP

markers were added (supplementary Table 2) and 2 SNPs were removed (SNP117 and SNP 292, chromosome 4) as they cause a disorder in relation to the physical map and therefore their presumed neighboring markers. These 97 markers were used to generate the new linkage map using JoinMap program (version 3.0, www.kyazma.nl), this new map was used for QTL analysis of the various traits used in this work.

QTL mapping and statistical analysis

The software package MapQTL® 5 was used to identify and locate QTLs on the linkage map by using interval mapping and multiple-QTL model (MQM) mapping methods as described in its reference manual (www.kyazma.nl). In a first step, putative QTLs were identified using interval mapping. Thereafter, the closest marker at each putative QTL was selected as a cofactor (van Ooijen and Maliepaard 1996; van Ooijen 2000) and the selected markers were used as genetic background controls in the approximate multiple QTL model of MapQTL. LOD threshold values applied to declare the presence of QTLs were estimated by performing the permutation tests implemented in MapQTL version 5.0 using at least 1000 permutations of the original data set, resulting in a 95% LOD threshold at 2.4. Two-LOD support intervals were established as 95% QTL confidence interval (van Ooijen 1999) using restricted MQM mapping implemented with MapQTL. The estimated additive genetic effect and the percentage of variance explained by each QTL and the total variance explained by all the QTLs affecting a trait were obtained using MQM mapping.

Heritability (broad sense) was estimated as the proportion of variance explained by between-line differences using the general linear model module of the statistical package of SPSS version 11.0.1 (SPSS Inc., Chicago, IL) based on measurements on 4-18 plants.

The relationship between the 23 Arabidopsis accessions with their carbohydrate measurements as variables was described with the principle component analysis (PCA) using NTSYSpc version 2.10t. (Rohlf 2001) with standardized data. For the PCA the standardized data were converted in a correlation matrix from which three eigenvectors were extracted using the EIGEN function of the NTSYS-pc program. The relations

between the 45 studied traits in the RILs of (L*er* x Kond) were performed by using the Pearson correlation coefficients and PCA taking the genotypes as variables of the statistical package of SPSS version 11.0.1 (SPSS Inc., Chicago, IL).

Results

Carbohydrate natural variation in Arabidopsis

To evaluate if there are differences in the diurnal pattern of sugar accumulation as well as starch turnover we screened 123 Arabidopsis accessions at two time points; two hours before the end of the light period and two hours before the end of the dark period. A large variation for the contents of sugars (glucose, fructose and sucrose) and starch was observed (El-Lithy 2005).

To study in more details the diurnal pattern of the carbohydrate content, 23 Arabidopsis accessions were selected on the basis of the previous screen and other growth-related traits. In addition three mutants were analyzed, known to have altered starch synthesis and degradation patterns, viz., the starchless lines *pgm* and *adg1* (lacking plastidial phosphoglucomutase activity and ADPglucose pyrophosphorylase activity, respectively) and the *sex1-1* (starch excess) mutant, which has a reduced capacity to mobilize starch. Carbohydrate analysis was carried out at six time points; the first was two hours before the end of the dark period and the last was two hours after the onset of the next dark period. The additional four determinations were carried out with material sampled during the light period. A principle component analysis (PCA) was performed to summarize the diurnal carbohydrates content of the different genotypes, where the first three principle components (PCs) explained 70 % of the total variation for carbohydrate content (Fig. 1). Based on their different levels of carbohydrate accumulation as revealed by the PCA analysis, the two accessions L*er* and Kond were chosen for a QTL analysis to map these and other traits. This population was grown on hydroponics, since it allows more uniform growth and the analysis of roots.

Genetic variation within the L*er* x Kond RIL population

In total 45 traits were studied in the RILs of (L*er* x Kond) which can be grouped in three main categories: vegetative growth traits both for shoots and roots, carbohydrate contents and flowering-related traits. Traits and abbreviations used are listed in Table 1, and the numbers 1 or 2 after the trait abbreviations refer to samples from 15 and 25 days

old plants, respectively (except only for TLA traits; 1 or 2 or 3 after the trait abbreviations refer to samples either at 10, 15 or 20 days old plants); L stands for light and D for dark. For all traits analyzed significant variation was observed between RILs. The broad sense heritabilities ranged from 0.30 for shoot Ra2L to 0.92 for TLN and RLN (Table 1). Transgression beyond the parental values was observed for all traits including those for which parental values hardly differed, such as RGR3-1, WC2 and St1L (see supplementary figure 1A, C and J). This amount of genetic variation indicated that QTL mapping was likely to reveal significant loci for most traits.

QTL mapping

The ultimate aim of this study is to unravel (genetic) relationships between carbohydrate-related traits and overall growth of plants including flowering time traits. Therefore, the growth QTLs were first described by investigating the genetics of size, weight and related parameters. The striking collocations between the different studied traits (carbohydrate-related traits, growth and flowering time traits) were explained in each section.

Vegetative growth

Shoot-related traits

At the *ERECTA* (*ER*) locus, co-location was found of QTLs for TLA1, TLA2, TLA3 and for RGR parameters on the basis of plant area and weight, as well as for shoot DW1 and DW2. Co-locations occurred also with SLA, RLL, all with the same allele effect; the Kond allele increasing the trait value (Fig. 2). The explained variance for each individual QTL ranged from 11.3% to 43.6% for SLA and TLA3, respectively (Suppl. Table 3). At the *ER* locus also QTLs for carbohydrate-related and flowering-related traits were detected, that will be described later in more detail. Also, at the top of chromosome 2 QTLs for TLA2, TLA3 and RGR3-1 co-located with L*er* allele increasing all trait values. In this chromosome region also QTLs for carbohydrate related traits and FT were detected.

At the top of chromosome 3, QTLs for TLA2 and TLA3 co-located with other QTLs for root weight and length, sugars and starch traits and FT and TPL, where the L*er* allele increases the trait values except for sucrose (Fig. 2).

On the top of chromosome 4, around *FRI*, there is a co-location for QTLs of WC1 and 2 with a QTL for RGR 3-1, with the L*er* allele increasing the trait values (Fig. 2). In the middle of chromosome 4 there is a co-location of QTLs for TLA3, RLL, Sh/Ro and ChlF and sugars with Kond alleles increasing the trait values. Here no FT QTLs were detected. QTLs for a limited number of traits or single growth related trait were found at the bottom of chromosome 4 (WC2 and RoL1, Fig. 2). QTLs for TLA1, TLA2 and DW1 were also detected at the top chromosome 5.

Root-related traits

The measurement of root dry weight of 15 days old plants was highly variable because of the very low weight of the roots, resulting in relatively large errors in the quantifications. Despite the small differences in root dry weight at day 25 (RoDW2) between L*er* and Kond, large variation was found between the RILs for this trait (Table 1). Around the *ER* locus, two QTLs were detected for RoDW2 and for Sh/Ro ratio on the basis of dry weight at day 25, each explaining 16.4% and 21.3% of the total variance (Fig. 2 and suppl. Table 3). These two QTLs co-located with the earlier mentioned shoot-related traits at this position.

At the top of chromosome 3, QTL for RoL1 co-located with a QTL for RoDW2 with the same allele effect but with an opposite allele effect for the Sh/Ro ratio in agreement with the observation that no QTL for shoot DW2 was detected at this position. However, 2 QTLs for TLA2 and TLA3 with similar allele effect as the QTL for RoL1 and RoDW2 were located at that position.

QTLs for RoD2, RoL1 and RoL2, with the L*er* allele increasing the trait values were found at the top of chromosome 4. These QTLs co-located with QTL for RGR3-1, WC1 and WC2 with a similar allele effect (Fig. 2).

Around marker SNP77 (top chromosome 5), QTLs for RoDW2 and RoL1, were detected for which the L*er* allele increased the trait values. These QTLs co-locate with

QTLs for TLA1, TLA2 and TLA3 and shoot DW1 with similar allele effects (Fig. 2) indicating that growth of the whole plant is affected at this locus. No QTLs for RoL1 and 2 were found at the *ER* locus.

Chlorophyll fluorescence (ChlF) and chlorosis (Chlo)

Three QTLs for ChlF were detected, located on chromosome 2, 4 and 5 at F3P11-6b, SNP408 and SNP236 and explaining 33.6% of the phenotypic variance (Fig. 2). For the first QTL, the L*er* allele increases the trait value whereas for the other two QTLs, the Kond allele increased the photosynthetic capacity of the plant compared with the L*er* allele. The ChlF QTLs at F3P11-6b at top of chromosome 2 and SNP408 at the middle of chromosome 4 co-located with sugar QTLs with similar allele effects.

For chlorosis five QTLs, explaining 51.7% of the phenotypic variance, were detected (Fig. 2). At three positions, the L*er* alleles accelerated leaf senescence. No co-location was detected with ChlF QTLs but four of the chlo QTLs co-located with FT QTLs at four different places, around the middle of chromosome 1, at the bottom of chromosome 2 and 5 and the top of chromosome 4 around *FRI* (Fig. 2) with opposite allele effect.

Carbohydrate-related traits

The analyses were carried out for shoots and roots, at two time points, at two hours before the end of the dark or light periods with 25 days old plants, while for 15 days old plants the analysis was done only at the end of the light period.

Carbohydrate content of shoots

Figure 2 and suppl. table 3 show the QTLs detected for sugars and starch. Around F3F19 on chromosome 1 a cluster of sugars QTLs (Gl1L, Gl2D, Fr2D, Ra2D, Gl2L and Su2L) has been detected with Kond allele increasing the trait values. Such QTLs did not co-locate with vegetative growth related traits. One other particular region to notice is located at the vicinity of the *ER* locus, where QTLs for Gl2D, Gl2L and St2D co-located with the same allele effect with other vegetative growth QTLs as well as with plant length

traits but with opposite allele effect with FT, CLN and SB QTLs. Also at the top of chromosome 3 carbohydrate QTLs for Su1L and Su2L co-located with a QTL for Sh/Ro ratio where Kond alleles increased the trait value. Three QTLs for St1L and St2L both in light and St2D at darkness co-located with QTLs for TLA2, TLA3, RoDW2 and RoL1 and with FT where L*er* allele increased the trait value.

At the top of chromosome 4, three QTLs for Gl2D, Fr2D in darkness and Gl2L in light co-located with vegetative growth-related traits with opposite allele effects and with other QTLs for flowering time-related traits with the same direction, Kond alleles increasing the trait values. Another interesting region is in the middle of chromosome 4 where QTLs for different sugar parameters co-located with QTLs for ChlF, Sh/Ro ratio, RLL and TLA3 with Kond alleles increasing the trait value. Around SNP152 on chromosome 4 QTLs for Fr2D, Ra2D, Fr2L and St2D at darkness co-located with QTLs for TLA3 and WC2, with opposite allele effects (Fig. 2).

Both at the middle and at the bottom of chromosome 5 several sugar QTLs co-located with other QTLs for growth-related and flowering-related traits. The explained variance for individual QTL detected for carbohydrates ranged from 5.2% to 30.3% of the total variance (Suppl. Table 3).

Carbohydrate content of roots

Growing this population on hydroponics allowed investigation of carbohydrates accumulation in roots, being an important sink in still vegetative Arabidopsis plants, together with growth-related traits of roots. For Ra2L three QTLs were detected at the bottom of chromosome 2 and 5 and at the top of chromosome 3 with explained variance of 9.6%, 7% and 24.1%, respectively. Two other QTLs for Fr2L and Su2L content in light were detected at the top and bottom of chromosome 1 (Fig. 2). The root Ra2L QTL at the top of chromosome 3 co-located with QTLs for Su1L and Su2L in the shoot with opposite allele effects, while it also co-located with QTLs for St1L, St2D, St2L and FT with the same allele effect (Fig. 2). No QTLs were found for starch content in roots at day 25 when measured before the end of the photoperiod. The levels of starch in roots were much lower than in leaves, ranging from 0.32 to 5.6 mg/g dry weight.

Flowering time and flowering-related traits including leaf numbers, plant length and branching

In Arabidopsis, a high correlation is usually observed between flowering time and total leaf number formed prior to flowering and one might expect to find correlations between rosette leaf number and rosette branches and between cauline leaf number and stem branches. Furthermore, plant height can be affected by the vegetative mass (rosette) and this is likely to have an effect on both stem leaves and on branches. Therefore, these traits are presented together.

The highly significant correlation between FT and TLN, RLN, CLN, RB and SB (R^2 = 0.85, 0.70, 0.88, -0.41 and 0.73 respectively) and between CLN and SB (R^2 = 0.67) confirm the expected relationships between these traits (Figure 3). A positive correlation between plant length traits (TPL, PLTS and INFL) and stem branching was observed, and a negative correlation was found with rosette branching.

On average L*er* flowered about 13 days earlier than Kond and transgression beyond the parental values was observed (Table 1, suppl. Fig. 1M). A large portion of the variation between lines (85.4%) could be explained by ten QTL, for six of which the L*er* allele promoted flowering (Fig. 2). In LD conditions (El-Lithy et al. 2006) eight out of the ten QTLs detected here in SD conditions were also observed in this L*er* x Kond population and showed the same allele effects. The two QTLs only detected in SD condition were both located on chromosome 2, viz., at *ER*, where a L*er* allele in this region delayed flowering, and at SNP166, where the L*er* allele promoted flowering.

Four QTLs were detected for TLN, which co-located with the FT QTL with the same allele effects (Fig. 2) explaining 71.4% of the total variance. Rosette leaf number (RLN) and cauline leaf number (CLN), being the two components of TLN, showed five QTLs each, explaining 75.3% and 55.3% of the variance, respectively. One QTL at SNP110 (chromosome 1), specific for RLN, did not co-locate either with FT, TLN or CLN QTLs, while for CLN a specific QTL was detected at SNP197, at the bottom of chromosome 3. The remaining QTLs for RLN and CLN either co-located with each other or with QTLs of TLN or FT (Fig. 2).

For total plant length and its two components (length till first silique and inflorescence length), the total explained variance was relatively high (86.4%, 79.7% and 81.8%, respectively), which was largely due to the effect of the *ER* locus, explaining 84.6%, 55.0% and 79.2% of the observed variation (Suppl. Table 3), where Kond alleles increased plant length. The frequency distribution of the RILs for INFL (Suppl. Fig. 1O) shows two classes, which could be explained by the detection of only two significant QTL, viz., at *ER* and SNP197 at chromosome 3.

For PLTS, five QTLs were found; two at chromosome 1 at SNP388 and F5I14, the last one being specific for PLTS with L*er* alleles increasing the length, suggesting that it might only be responsible for the increase in length of the stem internodes. The third QTL at the *ER* locus co-located with the other plant length traits, vegetative growth traits and sugars and starch QTLs with the same allele effect, Kond alleles increasing the trait values (Fig. 2). The other two QTLs located at SNP35 and SNP193 at chromosome 3 and 5, respectively.

No QTLs were found for the total number of branches, because all the detected QTL for rosette branches co-located either with significant or suggestive QTLs detected for stem branches but with opposite allele effects. Rosette and stem branches, being the two components of TNB, showed four and six QTLs, explaining 40.6% and 59.5% of the variance, respectively. Three QTLs, at SNP188, around *ER* and near *FRI* co-located for both traits with opposite allele effects (Fig. 2). The three remaining QTLs for SB located at F6D8-94 and SNP236 on chromosome 1 and 5, respectively, and at F5I14 on chromosome1.

Relationships between all analyzed traits in L*er* x Kond RILs and the power of remapping the combined traits

The 45 traits analyzed in this study represented growth-related traits, sugar and starch content and flowering-related traits that might be correlated because these traits affect each other in a direct or an indirect way. Figure 3 presents Pearson correlation coefficients between all studied traits in the L*er* x Kond RIL population: significant positive correlations are shown above the diagonal and the negative correlations below

the diagonal and the P values corrected for the multiple use of the same date set. The correlation co-efficient (R^2), ranging from 20% to 90% at –Log P values ranging from 1.6 to 87, are grouped in three categories represented by a gray scale in Figure 3.

Highly significant positive correlations between the different growth traits were found. There is also a positive correlation between plant biomass traits (areas, RGRs, WC, DW, SLA, RLL, RoDW and Sh/Ro) on the one hand and plant length traits (TPL, PLTS and INFL) and RB on the other. Sugars content correlated negatively with plant biomass traits, whereas starch content correlates positively, which fits with the detected QTLs (at *ER*, top chromosome 3, 4 and 5) that show co-location for such traits with opposite allele effects explaining these correlations (Fig. 2). However, in a limited number of cases positive correlations were observed between sugars content and few plant biomass traits (Fig. 3), that also can be seen from the co-location of QTLs at top of chromosome 2 and middle of 4 with the same allele effect (Fig. 2). FT, TLN, RLN, CLN and SB exhibited a negative correlation with plant biomass traits (Fig. 3), as can also be seen at *ER* and at top of chromosome 4 and 5 (Fig. 2).

A principal component analysis was carried out to further describe the predicted relationships between these traits. The first 13 components with Eigen values not less than one describe 80.8% of the total variance explained by these traits. In the first component, the plant areas, RGRs, DW2, RoDW2 and plant length traits were the most important traits determining the pattern of clustering. FT, TLN, CLN, Fr2D, Gl2D, Chlo and RLL mainly determine the second component. While, on the third component sugars content and DW1 were the main traits discriminating between the others (Suppl. Table 4, Fig 4). Starch clustered more with growth traits than with soluble sugars. Sugars traits clustered together with other traits determining their production and consumption or use as sink including TLN, RLN, CLN, SB and FT and with DW1 and ChlF as well (Fig. 4).

A QTL analysis was performed using the extracted factors of the PCA that sum up the variation which exist for the traits. Such PCA should identify QTLs for combined traits when the components are arranged according to the QTL effects. Mapping factors 1 and 2 identified the major growth and FT QTLs at the *ER* and *FRI* loci respectively and at the upper part of chromosome 5, whereas the major QTL for Factor 3 was at the top of

chromosome 3. For all factors several other QTLs were observed, some but not all, co-locating with loci affecting the traits making up these factors (Fig. 2 and Suppl. Table 3). This may identify some novel loci that only arise from combined effects e.g. for Fac 3 on the middle of chromosome 1. However they may, due to the complex correlation structure lead to an inability to detect specific QTLs. This might be the case for the relatively strong growth and sugar QTLs in the middle of chromosome 4.

Discussion

In this study plant performance was investigated by analyzing different aspects of plant growth-related traits, sugars and starch content and flowering-related traits, trying to explain the correlations that were found between these different aspects. Growing the RILs of the L*er* x Kond population on hydroponics allowed the study of the source-sink relationship including the roots.

The relationship between plant growth-related traits and carbohydrate content

Plant growth as well as carbohydrate content is complex polygenic traits; therefore, QTL analysis was used to clarify the genetic basis of the variation for both traits. The overall significant positive correlations between plant rosette areas and plant biomass are presented in Fig. 3. This shows that non-destructive measurements of plant area well represent plant biomass accumulation, at least in the early stages of development (Leister et al. 1999; El-Lithy et al. 2004). The co-location of QTL for plant areas, RGR(areas), DW1, DW2, RGR(DW), SLA, RLL, RoDW2 and Sh/Ro ratio with similar allele effects confirm that these parameters all represent (different) aspects of plant growth. SLA, considered as a key factor for plant growth (Poorter 2002), shows a significant positive correlation with RGR3-1(area) and RGR(DW) and a significant negative correlation with sugar content at 15 days, which might indicate that the plants invested more assimilates to build new tissues. The overall negative correlation between a series of growth traits (TLA2, TLA3, RGR3-1, SLA, RLL and RoDW2) and the sugar levels of 15 days old plants might suggest that there is an imbalance between the source and the sink (roots at this stage) at the whole plant level (Geiger et al. 2000; Paul and Foyer 2001)

Five regions were detected where QTL for various growth parameters co-located: the top of chromosome 2, the ER locus also on chromosome 2, the top parts of chromosomes 3 and 4 and the region around SNP77 on the upper half of chromosome 5.

Three out of the five growth regions (GRR) have also been identified in the L*er* x Sha RIL population (El-Lithy et al. 2004). The top parts of chromosomes 3 and 4 were also identified as biomass QTL in the Col x C24 RIL population (Lisec et al. 2008) and

for chromosome 3 in the the Bay-0 x Sha population, where also co-location with carbohydrate was found (Calenge et al. 2006). It is not surprising that QTL at different positions were identified in the other populations, because the genetic basis of such complex and integrative traits can differ between genotypes and since the experiments were not performed in identical conditions.

The *ER* locus harbors a major QTL for these traits as was also found by El-Lithy et al. (2004) in the the L*er* x Sha RIL population, The observation that a NIL containing the wild type *ER* allele exhibited the phenotype predicted by the QTL analysis indicates that *ER* is the likely candidate gene. Earlier studies showed that the *ER* gene is involved in a series of processes during Arabidopsis development (reviewed in van Zanten et al. 2009), like internode and pedicle elongation, leaf and silique morphogenesis and thickness of stem tissue (Douglas et al. 2002 and at the cellular level cell division and cell expansion (Tisne et al. 2008).

In addition to these five regions, the middle of chromosome 4 and the bottom of chromosome 4 and 5 contain QTL for only a limited number of shoot growth parameters.

The QTL on chromosomes 2, 3, 4 and 5 all contain QTL for carbohydrates, especially when determined at the end of the light period in 15 day old plants, in agreement with the negative correlation detected between growth and these sugar levels. Starch content had the same allele effects at the top of chromosome 3 and at ER but an opposite effect for the QTL at the top of chromosome 2. A positive correlation between starch and rosette dry weight was described by Calenge et al. (2006) but Cross et al. (2006) found a negative correlation in their survey of 24 accessions. The latter observation is in line with the recent findings of Sulpice et al. (2009) describing starch as a major integrator in the regulation of plant growth, biomass correlating negatively with starch content. These data together indicate that the link between carbohydrate content and growth is complex and probably depends strongly on the interaction of the genotype with the physiological conditions and stage of the plants.

Not all carbohydrate QTLs co-locate with growth QTLs. Examples are the top of chromosome 1 and the middle of chromosome 4 and 5. Some of these locations were also

described by Lisec et al. (2008) and Chalenge et al. (2006) e.g., the middle of chromosome 4, although details of the sugar QTL profile may differ.

It is of interest to note that the relationship between shoot and root growth depends on the QTL and is reflected by the Sh/Ro ratio QTLs. For *ER* the relative growth of the root, due to the Kondara allele, is larger than for the shoot, whereas for the top of chromosome 3 this is the opposite. For the QTLs on chromosomes 4 and 5 no ratio QTL were detected and therefore both organs are affected similarly.

In the Bay-0 x Sha RIL population a QTL for WC has been found at the top of chromosome 4 (Loudet et al. 2003) that co-located with the QTL detected for WC1 and WC2 at the same region in the present study. Water content at 15 and 25 days correlated negatively with sugars and starch contents, suggesting a link between carbohydrate content and the water availability in plant cells, although Poorter and Nagel (2000) stated that changes in carbon allocation are smaller in the case of limited water supply. The two QTL detected for WC1 and WC2 at top of chromosome four co-located with two QTL for sugars with opposite allele effect that might partly explain this negative correlation. The positive correlation between RoL1 and WC1 and between RoL2 and WC2 in combination with the co-location of the QTL for these two traits confirms the assumption that turgor pressure in root cells might lead to cell expansion thus increasing root length.

Chlorophyll fluorescence (ChlF) showed a positive correlation with Ra1L and Ra2L and one of the two detected QTL for ChlF in the middle of chromosome 4 co-located with several sugar QTL with similar direction, which confirms the relationship between the photosynthetic efficiency and sugar accumulation. Leaf chlorosis correlated also negatively with sugars content, which could suggest that a high level of sugars, mainly sucrose, induces leaf senescence (Yoshida 2003). The alternative hypothesis is that when the leaves start to senesce there is a remobilization of sugars from senescing leaves which are transported into young leaves (Himelblau and Amasino 2001).

Although ChlF was correlated with chlorosis, (Chlo), no co-locating QTL were found, which might be explained by co-locations with (minor) QTLs that were not detected for either one or for both traits (the unexplained variance). It is somewhat surprising that despite strong location of Chlo with FT indicating that early flowering

gives also earlier chlorosis no significant correlation was detected. None of the Chlo QTLs co-locate with those identified in the Bay-0 x Sha population (Diaz et al. 2006).

The relationship between flowering-related traits and carbohydrate content.

Earlier studies have shown that flower induction and opening might be due to a combination of sugar import and mobilization of various polysaccharides (Corbesier et al. 1998). In this study we indeed found a significant positive correlation between carbohydrate content and flowering-related traits, although it should be stressed that the carbohydrate measurements were done before flowering. Flowering time and/or flowering-related QTL co-located with sugars and/or starch QTL at the bottom of chromosomes 1, 2, and 3, around the *ER* locus and at the top of chromosomes 3, 4, and 5. However, not all QTL for these traits have similar allele effects, indicating that the correlations between carbohydrates and flowering time and flowering-related traits are complex.

The relationship between plant growth-related traits and flowering related traits.

Among the co-location between the QTL detected for growth-related traits and the ones for flowering-related traits, three main regions are of interest. The first one is around the *ER* locus, where several growth traits co-located with TPL, PLTS, INFL and RB with similar allele effects while they co-located with CLN and SB but with opposite allele effects. Similarly, in the L*er* x Sha RILs (El-Lithy et al. 2004) co-location between QTL for growth-related traits with QTL for TPL, PLTS, INFL, TLN and RLN were detected. From the previous findings we concluded that *ER* has an effect on plant growth and flowering-related traits but not on the flowering time itself. In the present experiment the *ER* Nil flowered later than the Ler line, although the Ler allele provided lateness. An explanation for this observation can be that at a linked locus Kond provides early alleles. This relationship between growth and FT can be seen clearly from the significant positive correlation between the majority of the growth-related traits with all plant length traits and with rosette branching (Fig. 3). This suggests that large plants allow a larger increase in length and in the number of rosette branches. The second and third regions are located

at the top of chromosomes 4 and 5 where the growth-related QTLs co-located with QTL detected for flowering-related traits, with opposite allele effect. This co-location of the QTL with opposite allele effect of these two sets of traits was supported also by the significant negative correlation found between FT, TLN, RLN, CLN and SB with the majority of growth traits (Fig. 3). Such correlation is understandable since early flowering plants have fewer leaves and fewer branches, which means less area and biomass as well. It is interesting that some FT QTL do not co-locate with growth (bottom of chromosomes 1, 2 and 5).

The co-location of QTL for different traits does not prove pleiotropy, because of the limited accuracy of QTL mapping. It could also be explained by the presence of a set of closely linked genes, each affecting different traits, which cannot be separated by QTL mapping. For confirmation of pleiotropic effects further fine mapping is required.

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Figure legends:

Figure 1. Principle component analysis of the 23 Arabidopsis accessions plus three mutants (*pgm-1*, *adg-1* and *sex1-1*), using the diurnal pattern of their carbohydrate content. The ovals indicate 3 different groups, as the accessions for each group had more or less a similar diurnal pattern. L*er* and Kond are indicated with closed circles.

Figure 2. The L*er* x Kond linkage map showing the locations of QTL for the traits analyzed. The lengths of the arrows indicate the 2-LOD support intervals. The direction of the arrow's head indicates the allelic effect: upward, L*er* increasing and Kond decreasing; downward, Kond increasing and L*er* decreasing. The ovals indicate QTL of growth and flowering regions, while the rectangles indicate the carbohydrate-related QTL. The first three panels from right showed the QTL detected for the extracted factors of the PCA.

Figure 3. Pearson correlation between the studied growth-related traits, carbohydrate content and flowering-related traits in the L*er* x Kond RIL population. Cells above the diagonal represent positive correlations, whereas the ones below the diagonal represent negative correlations.

Figure 4. Principle component analysis of the 45 studied traits in the RIL of the (L*er* x Kond) population, using the trait values of the RILs.

Tables:

Table I. Parental values, ranges, means and heritabilities in the L*er* x Kond RILs for all measured traits

Supplementary data:

Suppl. Figure 1. Frequency distribution of non-normalized data of selected traits in the L*er* x Kond RIL population. Vegetative traits: Shoot relative growth rate 3-1 calculated on the basis of plant area (A), Shoot dry weight 2 at 25 days at light (B), Water content 2 at 25 days (C), Shoot relative growth rate calculated on the basis of plant dry weight (D), Specific leaf area (E), Shoot-root ratio (F), Root length 2 at 25 days (G). Chlorophyll fluorescence measured as yield (H). Carbohydrate traits: Shoot sucrose content 2 at light at 25 days (I), Shoot starch content 1 at light at 15 days (J), Shoot starch content 2 at dark at 25 days (K), Shoot starch content 2 at light at 25 days (L). Generative traits: Flowering time (M), Total leaf number (N), Inflorescence length (O). The average parental value is indicated with an arrow for both parents, L for L*er* and K for Kond.

Suppl. Table I Names, stock numbers, origin, sugars and starch content for 123 Arabidopsis accessions.

Suppl. Table II. New markers used to genotype the L*er* x Kond population.

Suppl. Table III. Characteristics of the detected QTL explaining growth traits, carbohydrate levels, flowering time and flowering-related traits in L*er* x Kond RIL population.

Suppl. Table IV. Extracted values of the 45 traits using PCA method (13 components extracted).

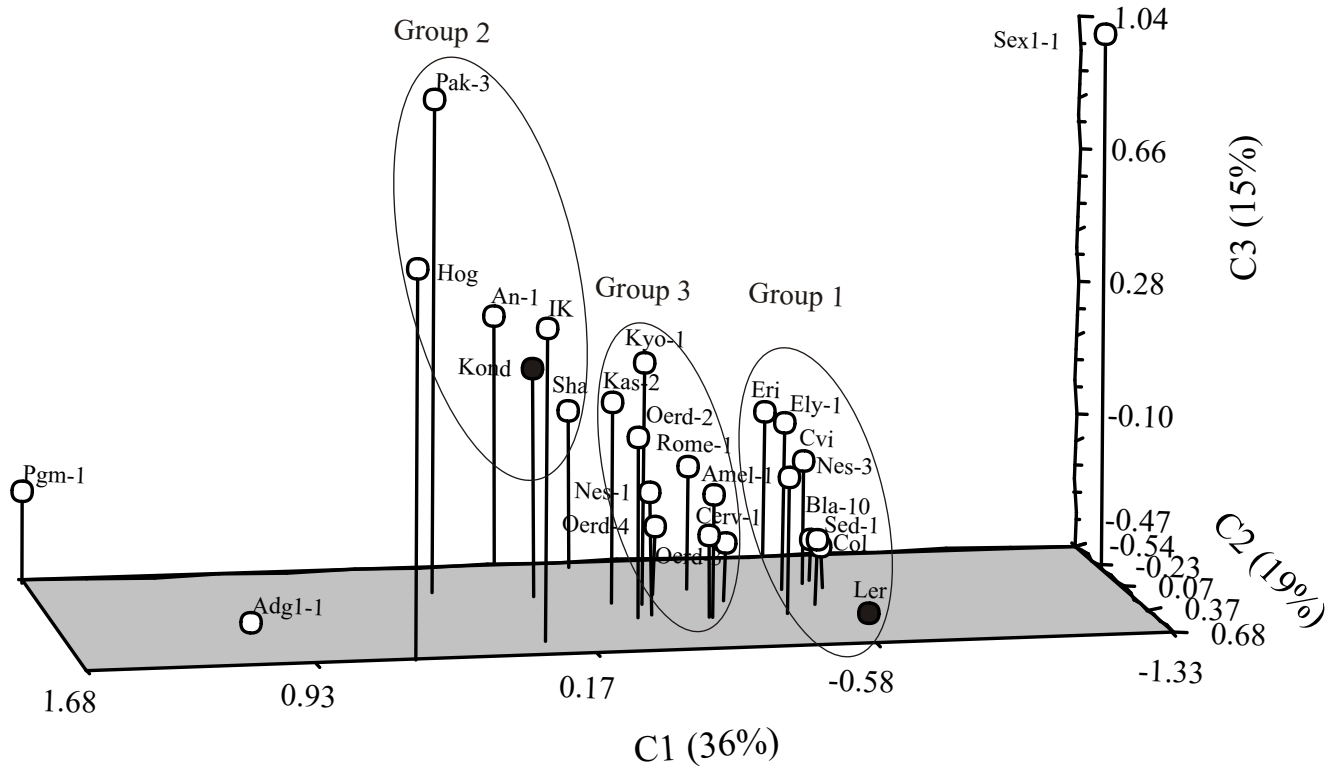
Table I. Parental values, ranges, means and heritabilities in the L*er* x Kond RILs for all measured traits

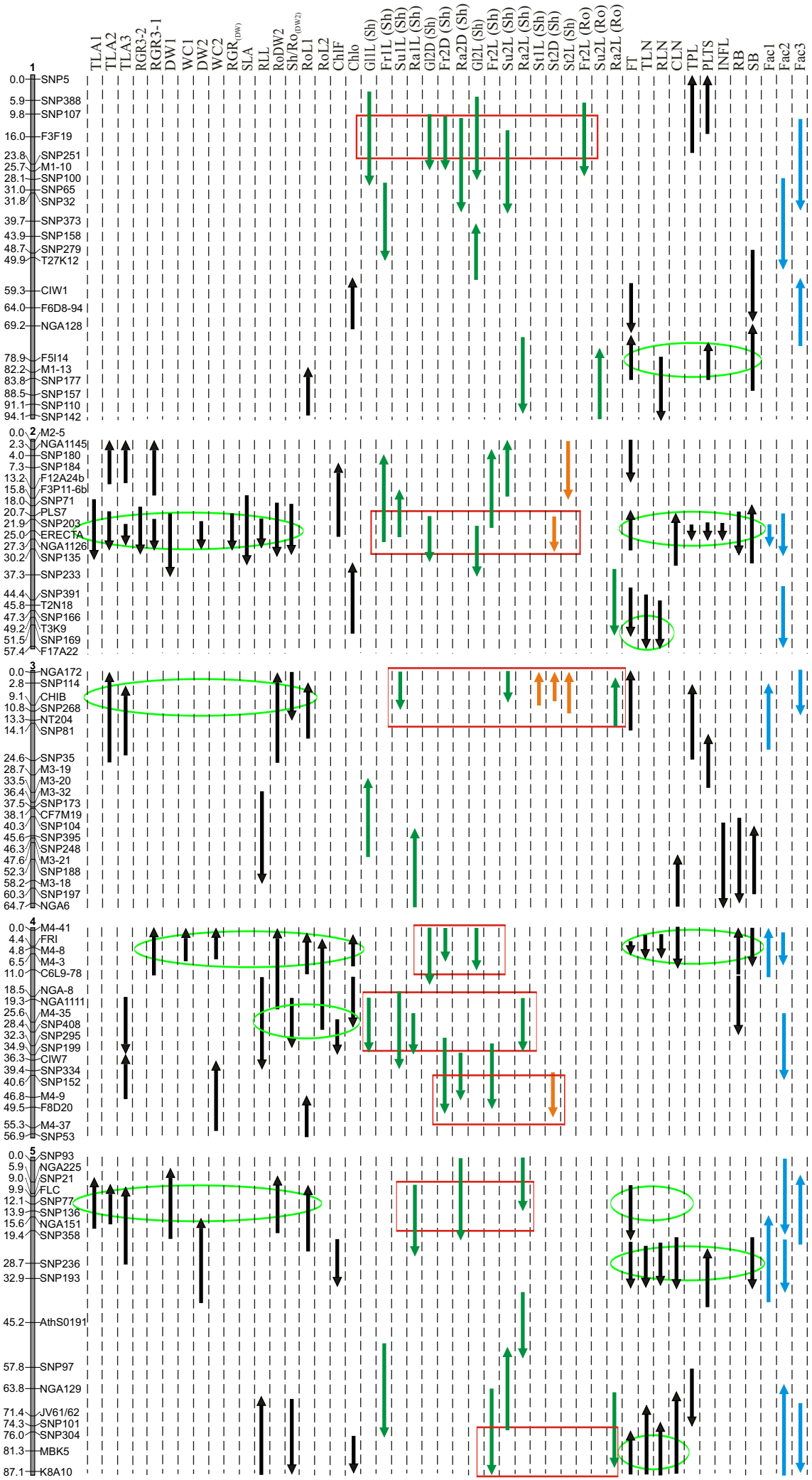
Trait	Ler value	Kond value	Range	Ler+	Mean	h^2
Shoot-related traits						
Total leaf area 1 (mm^2) (TLA1)	8.9	32.6	8.9 - 52.7	35.18	33.3	0.64
Total leaf area 2 (mm^2) (TLA2)	64.1	117.5	42.1 - 165.8	130.12	106	0.76
Total leaf area 3 (mm^2) (TLA3)	301.6	606.2	141.6 - 1007.9	578.5	502.5	0.80
Relative growth rate 3-2 (area) (RGR3-2)	0.31	0.33	0.21 - 0.37	0.30	0.31	nd
Relative growth rate 3-1 (area) (RGR3-1)	0.30	0.29	0.21 - 0.31	0.28	0.27	nd
Dry weight 1 (mg) (DW1)	2.0	2.3	1.08 - 3.2	2.5	2.1	0.62
Water content 1 (%) (WC1)	88.0	89.3	86.1 - 90.1	88.8	88.9	0.36
Dry weight 2 (mg) (DW2)	34.1	49.6	13.1 - 71.4	49.4	40.2	0.73
Water content 2 (%) (WC2)	89.8	89.6	88.0 - 91.2	89.6	89.6	0.48
Relative growth rate (DW)($RGR_{(DW)}$)	0.29	0.31	0.24 - 0.34	0.30	0.29	0.45
Specific leaf area ($mm^2.mg^{-1}$) (SLA)	32.5	50.5	31.2 - 75.9	52.6	51.7	nd
Rosette leaf length (cm) (RLL)	5.2	7.1	3.2 - 10.3	5.6	5.7	0.77
Root-related traits						
Root dry weight 2 (mg) (RoDW2)	7.4	8.3	3.1 - 14.7	10.3	8.1	0.58
Shoot/Root ratio (DW2) (Sh/Ro)	4.6	6.0	3.7 - 9.0	4.8	5.1	0.49
Root length 1 (cm) (RoL1)	14.9	10.4	4.0 - 16.2	13.9	11.7	0.56
Root length 2 (cm) (RoL2)	25.9	20.9	15.9 - 29.9	24.5	23.2	0.42
Chlorophyll fluorescence (ChlF)	0.743	0.758	0.614 - 0.772	0.736	0.728	0.39
Chlorosis (Chlo)	2.0	1.5	1.0 - 6.5	1.5	3.3	0.77
Shoot carbohydrate content						
Glucose 1 at light (mg/g DW) (Gl1L)	0.9	4.6	3.8 - 14.6	3.0	3.6	0.61
Fructose 1 at light (mg/g DW) (Fr1L)	6.6	8.5	0.8 - 21.8	7.9	6.9	0.68
Sucrose 1 at light (mg/g DW) (Su1L)	31.0	34.1	14.5 - 40.1	27.4	29.1	0.59
Raffinose 1 at light (mg/g DW) (Ra1L)	0.8	1.3	0.1 - 3.7	0.7	1.0	0.48
Glucose 2 at dark (mg/g DW) (Gl2D)	0.7	2.6	0.7 - 4.3	1.7	1.5	0.72
Fructose 2 at dark (mg/g DW) (Fr2D)	1.3	3.1	1.1 - 5.8	3.1	2.2	0.66
Sucrose 2 at dark (mg/g DW) (Su2D)	4.0	5.6	4.0 - 10.0	5.8	5.7	0.64
Raffinose 2 at dark (mg/g DW) (Ra2D)	0.2	0.3	0.1 - 0.7	0.3	0.2	0.63
Glucose 2 at light (mg/g DW) (Gl2L)	0.8	4.6	0.7 - 5.1	1.1	1.9	0.41
Fructose 2 at light (mg/g DW) (Fr2L)	1.3	2.9	0.4 - 7.1	1.9	1.9	0.59
Sucrose 2 at light (mg/g DW) (Su2L)	6.9	8.5	5.3 - 9.9	8.2	7.8	0.66
Raffinose 2 at light (mg/g DW) (Ra2L)	0.5	1.3	0.4 - 2.0	0.6	0.8	0.30
Starch 1 at light (mg/g DW) (St1L)	186.1	189.0	102.8 - 285.1	186.5	197.7	0.68

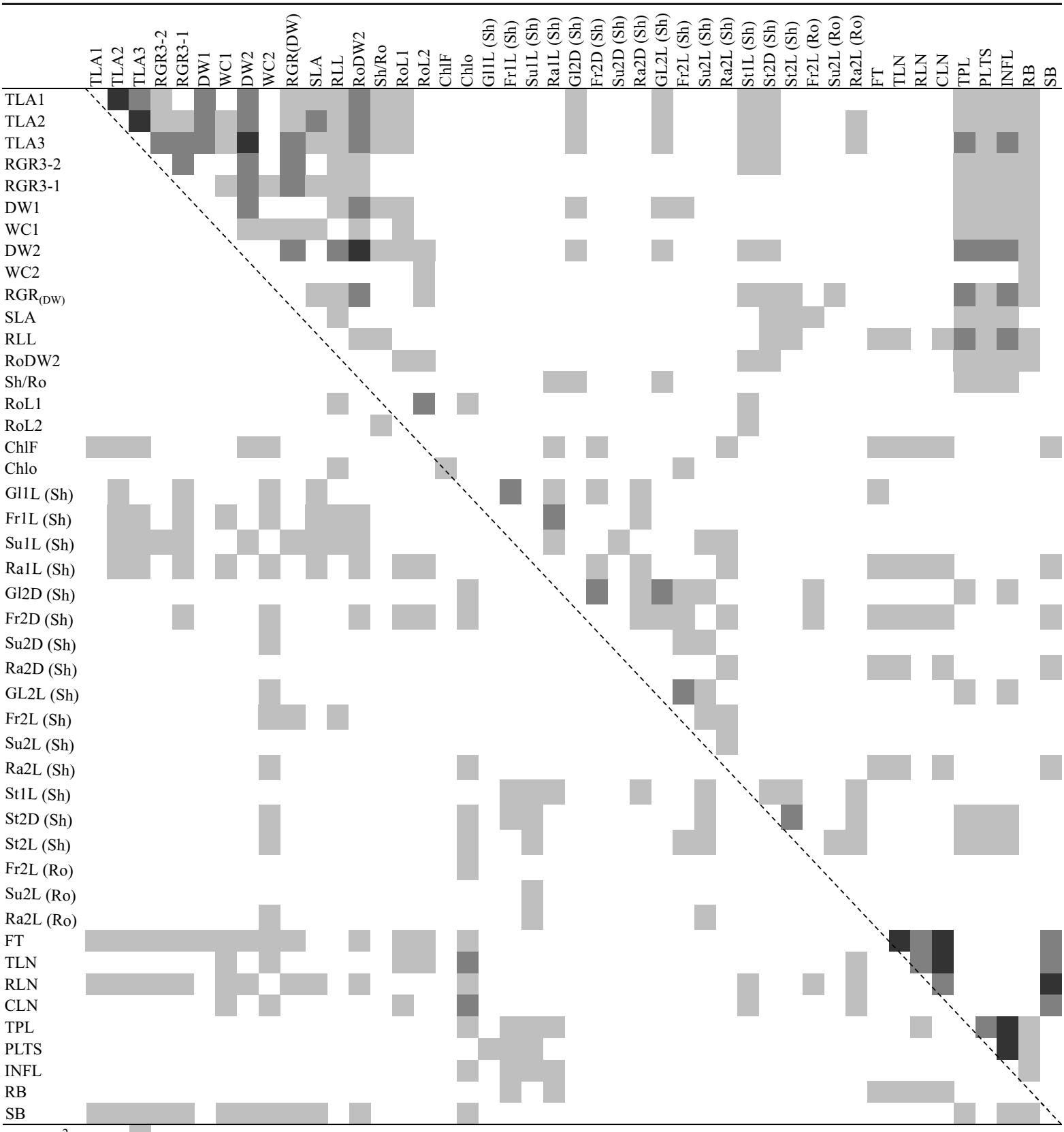
Starch 2 at dark (mg/g DW) (St2D)	44.0	58.9	8.6 - 106.0	32.4	51.1	0.52
Starch 2 at light (mg/g DW) (St2L)	102.5	124.1	63.0 - 140.2	74.0	100.7	0.45
Root carbohydrate content						
Fructose 2 at light (mg/g DW) (Fr2L)	2.7	2.6	1.0 - 9.5	4.5	3.1	0.43
Sucrose 2 at light (mg/g DW) (Su2L)	10.7	22.6	1.0 - 33.2	20.4	18.7	0.43
Raffinose 2 at light (mg/g DW) (Ra2L)	3.3	1.7	0.4 - 5.3	2.4	2.4	0.52
Flowering-related traits						
Flowering time (days) (FT)	35.5	48.3	24.7 - 71.5	42.3	42.0	0.89
Total leaf number (TLN)	17.5	41.0	8.5 - 64.3	30.0	24.8	0.92
Rosette leaf number (RLN)	13.0	37.0	6.5 - 53.3	22.0	20.4	0.92
Cauline leaf number (CLN)	4.5	4.0	1.5 - 9.9	8.0	4.6	0.81
Total plant length (cm) (TPL)	33.6	46.2	11.8 - 80.4	62.6	45.1	0.90
Plant length till 1st silique (cm) (PLTS)	13.0	10.4	4.4 - 25.8	22.6	13.8	0.85
Inflorescence length (cm) (INFL)	20.6	35.8	5.9 - 59.9	40.1	31.3	0.87
Rosette branches (RB)	6.5	3.3	0.0 - 13.4	1.8	5.1	0.59
Stem branches (SB)	5.2	4.0	1.7 - 11.0	8.3	5.1	0.78

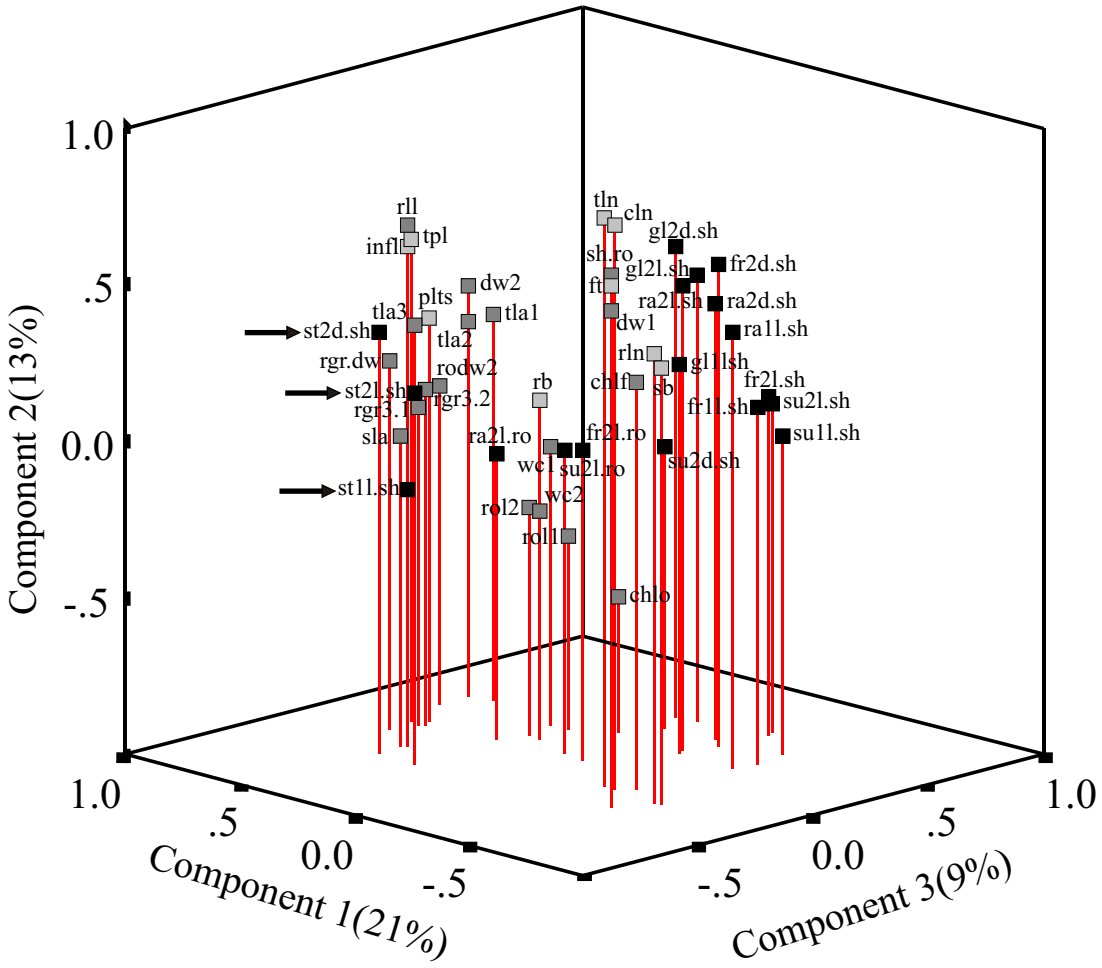
nd, not determined because these traits are calculated only on the basis of averages.

Ler+ is *ERECTA* wild type allele in the L*er* genetic background.


Group 2
Pak-3
Hog
An-1
IK
Kond
Sha
Group 3
Kyo-1
Kas-2
Oerd-2
Rome-1
Amel-1
Nes-1
Oerd-4
Cerv-1
Oerd-1
Group 1
Eri
Ely-1
Cvi
Nes-3
Bla-10
Sed-1
Col
Ler
Pgm-1
Adg1-1
Sex1-1
1.04
0.66
0.28
-0.10
-0.47
-0.54
-0.23
0.07
0.37
0.68
-1.33
C3 (15%)
C2 (19%)
1.68
0.93
0.17
-0.58
C1 (36%)


TLA1
TLA2
TLA3
RGR3-2
RGR3-1
DW1
WC1
DW2
WC2
RGR(DW)
SLA
RLL
RoDW2
Sh/Ro(DW2)
RoL1
RoL2
ChlF
Chlo
Gl1L (Sh)
Fr1L (Sh)
Su1L (Sh)
Ra1L (Sh)
Gl2D (Sh)
Fr2D (Sh)
Ra2D (Sh)
Gl2L (Sh)
Fr2L (Sh)
Su2L (Sh)
Ra2L (Sh)
St1L (Sh)
St2D (Sh)
St2L (Sh)
Fr2L (Ro)
Su2L (Ro)
Ra2L (Ro)
FT
TLN
RLN
CLN
TPL
PLTS
INFL
RB
SB
Fac1
Fac2
Fac3
1
0.0 SNP5
5.9 SNP388
9.8 SNP107
16.0 F3F19
23.8 SNP251
25.7 M1-10
28.1 SNP100
31.0 SNP65
31.8 SNP32
39.7 SNP373
43.9 SNP158
48.7 SNP279
49.9 T27K12
59.3 CIW1
64.0 F6D8-94
69.2 NGA128
78.9 F5I14
82.2 M1-13
83.8 SNP177
88.5 SNP157
91.1 SNP110
94.1 SNP142
2
0.0 M2-5
2.3 NGA1145
4.0 SNP180
7.3 SNP184
13.2 F12A24b
15.8 F3P11-6b
18.0 SNP71
20.7 PLS7
21.9 SNP203
25.0 ERECTA
27.3 NGA1126
30.2 SNP135
37.3 SNP233
44.4 SNP391
45.8 T2N18
47.3 SNP166
49.2 T3K9
51.5 SNP169
57.4 F17A22
3
0.0 NGA172
2.8 SNP114
9.1 CHIB
10.8 SNP268
13.3 NT204
14.1 SNP81
24.6 SNP35
28.7 M3-19
33.5 M3-20
36.4 M3-32
37.5 SNP173
38.1 CF7M19
40.3 SNP104
45.6 SNP395
46.3 SNP248
47.6 M3-21
52.3 SNP188
58.2 M3-18
60.3 SNP197
64.7 NGA6
4
0.0 M4-41
4.4 FRI
4.8 M4-8
6.5 M4-3
11.0 C6L9-78
18.5 NGA-8
19.3 NGA1111
25.6 M4-35
28.4 SNP408
32.3 SNP295
34.9 SNP199
36.3 CIW7
39.4 SNP334
40.6 SNP152
46.8 M4-9
49.5 F8D20
55.3 M4-37
56.9 SNP53
5
0.0 SNP93
5.9 NGA225
9.0 SNP21
9.9 FLC
12.1 SNP77
13.9 SNP136
15.6 NGA151
19.4 SNP358
28.7 SNP236
32.9 SNP193
45.2 AthS0191
57.8 SNP97
63.8 NGA129
71.4 JV61/62
74.3 SNP101
76.0 SNP304
81.3 MBK5
87.1 K8A10


TLA1
TLA2
TLA3
RGR3-2
RGR3-1
DW1
WC1
DW2
WC2
RGR(DW)
SLA
RLL
RoDW2
Sh/Ro
RoL1
RoL2
ChlF
Chlo
Gl1L (Sh)
Fr1L (Sh)
Su1L (Sh)
Ra1L (Sh)
Gl2D (Sh)
Fr2D (Sh)
Su2D (Sh)
Ra2D (Sh)
GL2L (Sh)
Fr2L (Sh)
Su2L (Sh)
Ra2L (Sh)
St1L (Sh)
St2D (Sh)
St2L (Sh)
Fr2L (Ro)
Su2L (Ro)
Ra2L (Ro)
FT
TLN
RLN
CLN
TPL
PLTS
INFL
RB
SB
RGR(DW)
0.2 < R2 < 0.5
0.5 < R2 < 0.8
0.8 < R2 < 0.9
1.6 < -LOG P value < 7.2
6.7 < -LOG P value < 20.8
26.5 < -LOG P value < 87


Component 2(13%)
1.0
.5
0.0
-.5
rll
tpl
infl
dw2
plts
tla3
tla1
tla2
st2d.sh
rgr.dw
rodw2
st2l.sh
rgr3.1
rgr3.2
sla
st1l.sh
rb
ra2l.ro
wc1
su2l.ro
wc2
rol2
rol1
tln
cln
gl2d.sh
sh.ro
gl2l.sh
fr2d.sh
ft
ra2l.sh
ra2d.sh
dw1
ra1l.sh
rln
gl1l.sh
chlf
sb
fr2l.sh
fr1l.sh
su2l.sh
su1l.sh
fr2l.ro
su2d.sh
chlo
1.0
.5
0.0
-.5
Component 1(21%)
Component 3(9%)
1.0
.5
0.0
-.5