Quantitative Trait Locus Analysis of Growth-Related Traits in a New Arabidopsis Recombinant Inbred Population[1]

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Arabidopsis natural variation was used to analyze the genetics of plant growth rate. Screening of 22 accessions revealed a large variation for seed weight, plant dry weight and relative growth rate but not for water content. A positive correlation was observed between seed weight and plant area 10 d after planting, suggesting that seed weight affects plant growth during early phases of development. During later stages of plant growth this correlation was not significant, indicating that other factors determine growth rate during this phase. Quantitative trait locus (QTL) analysis, using 114 (F9 generation) recombinant inbred lines derived from the cross between Landsberg *erecta* (L*er*, from Poland) and Shakdara (Sha, from Tadjikistan), revealed QTLs for seed weight, plant area, dry weight, relative growth rate, chlorophyll fluorescence, flowering time, and flowering-related traits. Growth traits (plant area, dry weight, and relative growth rate) colocated at five genomic regions. At the bottom of chromosome 5, colocation was found of QTLs for leaf area, leaf initiation speed, specific leaf area, and chlorophyll fluorescence but not for dry weight, indicating that this locus might be involved in leaf development. No consistent relation between growth traits and flowering time was observed despite some colocations. Some of the QTLs detected for flowering time overlapped with loci detected in other recombinant inbred line populations, but also new loci were identified. This study shows that Arabidopsis can successfully be used to study the genetic basis of complex traits like plant growth rate.

Analysis of plant growth is an essential step in the understanding of plant performance and productivity (Leister et al., 1999) and may reveal different strategies of plants to survive under limiting conditions.

Growth rate and, more specifically, relative growth rate (RGR) are comprehensive traits of plants, which characterize to a large extent plant performance and are also important components of fitness (McGraw and Garbutt, 1990). These parameters integrate morphological and physiological traits of plants. RGR is an inherent quantitative trait that may vary among plant species, occurring in a wide range of habitats. Plants in favorable environments often have an inherently high RGR, whereas those from less favorable habitats have an inherently low RGR, even when grown in the same favorable conditions (Grime and Hunt, 1975; Poorter and Remkes, 1990). In addition, plant growth rate is also affected by developmental changes such as the onset of flowering or the formation of storage organs.

Various parameters have been used to evaluate growth rate, including measurement of fresh or dry weight, root to shoot ratio, shoot number, or shoot length (Li et al., 1998; Leister et al., 1999). The measurement of fresh or dry weight is destructive and hence large numbers of plants are required to analyze growth in time. Although the analysis of growth by measuring the area covered by a plant instead of measuring its weight has been applied successfully (Smith and Spomer, 1987; Smith et al., 1989; Motooka et al., 1991), its use is hampered by complicated experimental designs. A nondestructive approach would be preferable, e.g. using image analysis. For Arabidopsis, which in its vegetative phase grows as a flat rosette with limited leaf overlap, Leister et al. (1999) showed that the use of digital video and image analysis was very effective in the determination of plant growth (rate) nondestructively, even during early developmental stages.

Growth rate can be seen as the integration of a wide range of processes, and thus genetic variation for such a complex trait may depend on many genes. Since also within species heritable differences in growth and morphology can be found (Maloof, 2003) these traits are amenable for genetic analysis. Complex polygenic traits can be studied genetically by quantitative trait loci (QTL) analysis. QTL analysis allows the dissection of quantitative genetic variation to the contribution of different loci. When mechanistically related traits map to similar map positions, this might suggest that variation for these traits at this locus is controlled by the same gene and in genetic terminology is pleiotropic. The extensive natural variation that occurs in

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Arabidopsis is being exploited increasingly as a source of genetic variation for the analysis of important adaptive traits, e.g. flowering time, plant and seed size, seed dormancy, pathogen resistance, and tolerance to abiotic stresses (for review, see Alonso-Blanco and Koornneef, 2000; Koornneef et al., 2004). Recombinant inbred lines (RILs) provide an immortal population, as each individual is practically homozygous, and large numbers of genetically identical individuals can be obtained, allowing repeated measurements of various traits in different conditions (Alonso-Blanco and Koornneef, 2000; Doerge, 2002).

We have used Arabidopsis natural variation to analyze growth rate by image analysis of plant leaf area, and by measuring a series of related parameters. From a greenhouse experiment involving approximately 130 Arabidopsis accessions, from a wide range of habitats, 22 accessions (Table I) were selected based on obvious differences in growth characteristics, carbohydrate content, and/or because they were used in generating Arabidopsis mapping populations (www.natural-eu.org). These accessions were studied to get insight in differences in various growth-related traits, which, when present, can be genetically analyzed further in segregating populations such as recombinant inbred lines (RILs). To investigate the genetic basis of differences in growth and growth-related traits and to see if relationships between traits in the selection of accessions might be due to a common genetic basis, we analyzed growth-related traits by QTL mapping. For this we used a newly developed RIL population derived from the cross between the laboratory accession Landsberg *erecta* (L*er*), originating from northern Europe (Rédei, 1992), and the accession Shakdara (Sha), originating from high altitudes in Tadjikistan (Khurmatov, 1982). These parental accessions were not the extremes in the accession screen, but they showed a considerable variation for various growth traits as was also observed in their progeny.

Since RGR depends on the gain of biomass via photosynthesis and on the starting mass of the plant, i.e. ultimately the seed from which it grows, we determined the seed weight and chlorophyll fluorescence as a nondestructive parameter for photosynthetic capacity. Allocation of biomass within the plant is expected to change upon flower induction and hence flowering time and related parameters were also analyzed in this study.

RESULTS

Variation among the Accessions

Screening the 22 accessions revealed a large variation for seed weight, growth rate, and plant fresh and dry weight but less for water content (Table I, Fig. 1). The seed weight of these accessions was not correlated with the latitude at which the accessions had originally been collected as suggested before by Li et al. (1998). Seed weight showed a positive correlation with plant area at 10 d after planting, suggesting that seed weight affected plant growth during early phases of development (Fig. 1A). During later stages of plant growth this correlation was not significant, indicating that other factors determined plant growth at that phase (Fig. 1B). Final plant dry weight correlated with RGR (based on area) especially during the last period (Fig. 1, C and D). A

Table I. *Names, stock numbers, origin, fresh and dry weight, water content and seed weight for 22 Arabidopsis accessions*

Name	Stock No	Country	Longitude	Latitude	Fresh Wt	Dry Wt	%WC	Seed Wt
					(gm)	*(gm)*		*(mg)*
Amel-1	CS 22526	Netherlands	E 5.6	N 53.4	1.43	0.19	87.0	0.026
Nes-1	CS 10041	Netherlands	E 5.8	N 53.3	0.87	0.14	83.9	0.030
Nes-3	CS 10042	Netherlands	E 5.8	N 53.2	0.83	0.11	86.4	0.028
Oerd-2	CS 10299	Netherlands	E 5.9	N 53.2	1.47	0.20	86.4	0.031
Oerd-4	CS 10040	Netherlands	E 5.9	N 53.2	1.03	0.12	88.4	0.030
Cerv-1	CS 22523	Italy	E 12.5	N 41.9	1.70	0.23	86.3	0.020
Rome-1	CS 22524	Italy	E 12.5	N 41.9	1.10	0.16	85.5	0.017
Ler	N 20	Poland	E 15.2	N 52.7	0.97	0.13	86.2	0.020
Col-2	CS 907	Poland	E 15.7	N 52.7	0.65	0.08	88.5	0.022
Cvi	N 8580	Cape Verde Islands	W 24.4	N 14.9	1.35	0.15	88.9	0.033
An-1	N 944	Belgium	E 4.4	N 51.2	0.37	0.06	84.5	0.021
Bla-10	JA 10185	Spain	E 2.8	N 41.7	0.93	0.12	86.8	0.022
Kond	CS 6175	Tadjikistan	E 68.5	N 38.5	1.50	0.20	86.9	0.019
Ely-1a	CS 6088	UK	W 0.3	N 52.4	0.53	0.07	87.5	0.020
Eri	CS 22548	Sweden	E 15	N 56.4	0.97	0.12	87.2	0.019
Hog	CS 6179	Tadjikistan	E 68.5	N 38.5	1.35	0.16	88.5	0.018
Kas-2	N 1264	India	E 71.8	N 34.3	0.17	0.03	84.0	0.028
Sid-1	CS 6077	UK	E 15.4	N 51.4	0.53	0.06	88.8	0.016
Sha	CS 929	Tadjikistan	E 71.3	N 37.3	0.90	0.10	88.9	0.019
Pak-3	JW 10214	Pakistan	E 73.4	N 33.9	0.15	0.01	93.3	0.032
Ik	JW 10223	Japan	E 135.1	N 35.5	1.30	0.19	85.4	0.030
Kyo-1	JW 10231	Japan	E 135.8	N 35.0	0.70	0.15	79.3	0.021

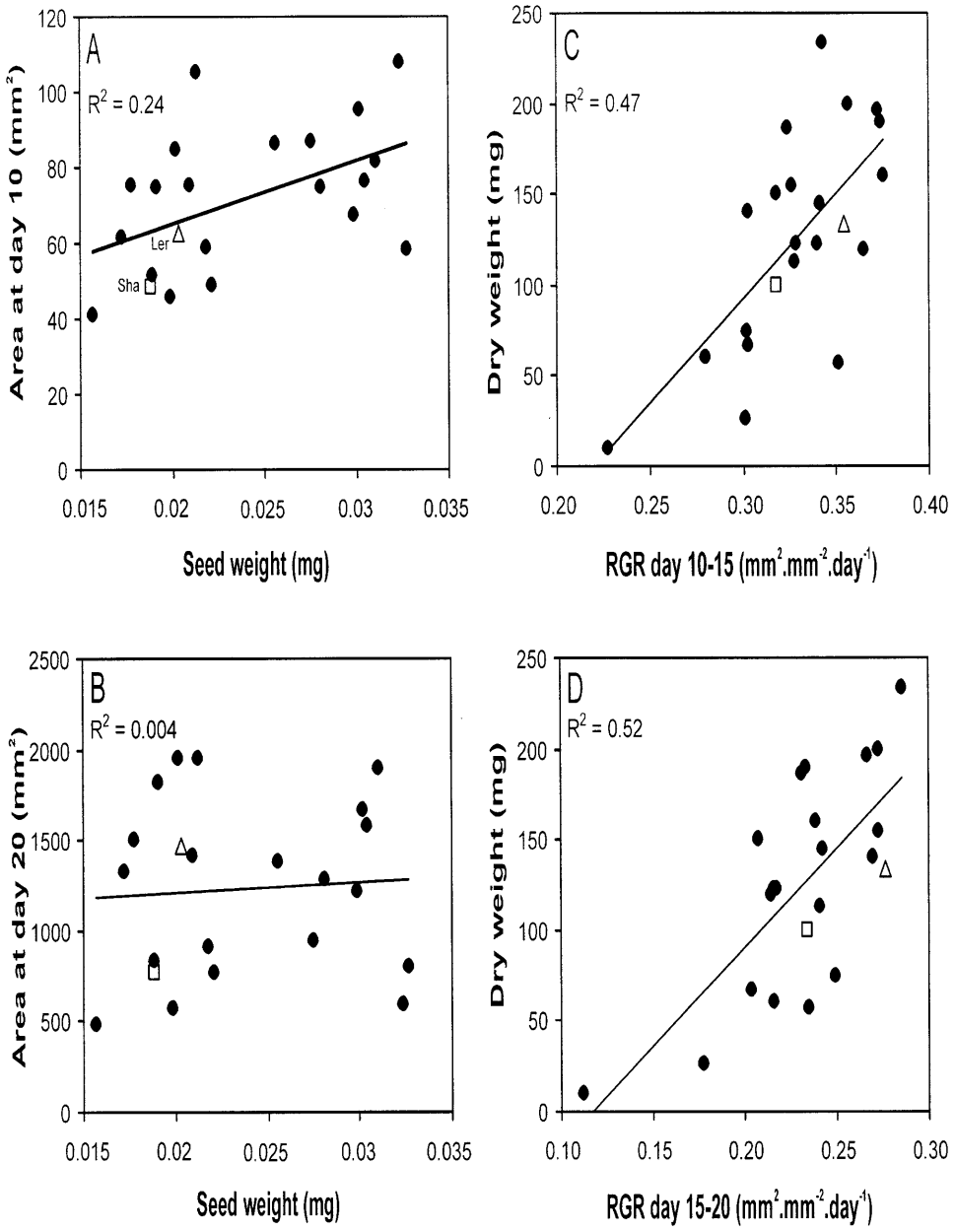

Figure 1. Correlation between seed weight and plant area at day 10 (A) and day 20 (B) for 22 Arabidopsis accessions. Correlation between dry weight at 35 d and relative growth rate (RGR) day 10–15 (C) and day 15–20 (D) on the basis of plant area. Δ and □ correspond to L*er* and Sha mean values, respectively.

positive correlation was observed between area at day 20 and the plant dry weight at day 35 (R^2 = 0.56, data not shown). We found a large variation between accessions for their growth rate as well as for their relative growth rate (on the basis of plant area) with Pak-3 having the lowest and Cerveteri-1, Kyoto-1, Oerd-2, and Kond the highest rates (Fig. 2, A and B). Ten days after planting, Pak-3 showed the largest area, but gradually its growth rate decreased due to early senescence, which was observed also for Kas-2 (Fig. 2A).

A principle component analysis indicated that the first three principle components (PCs) explained 97% of the variation for the six traits: fresh weight (FW), dry weight (DW), seed weight (SW), total leaf area 1 (TLA1), total leaf area 2 (TLA2), and total leaf area 3 (TLA3). PC1 showed a large variation between accessions and is mainly determined by growth related parameters (TLA3, TLA2, and DW). On the second function (PC2), TLA1, SW, and FW were the most important traits. On the third function (PC3), SW was the main variable discriminating between the accessions. Accessions with a large initial area (TLA1) and high seed weight (SW) were situated on the left side of the graph. The related accessions Oerd2, Oerd4, Nes1

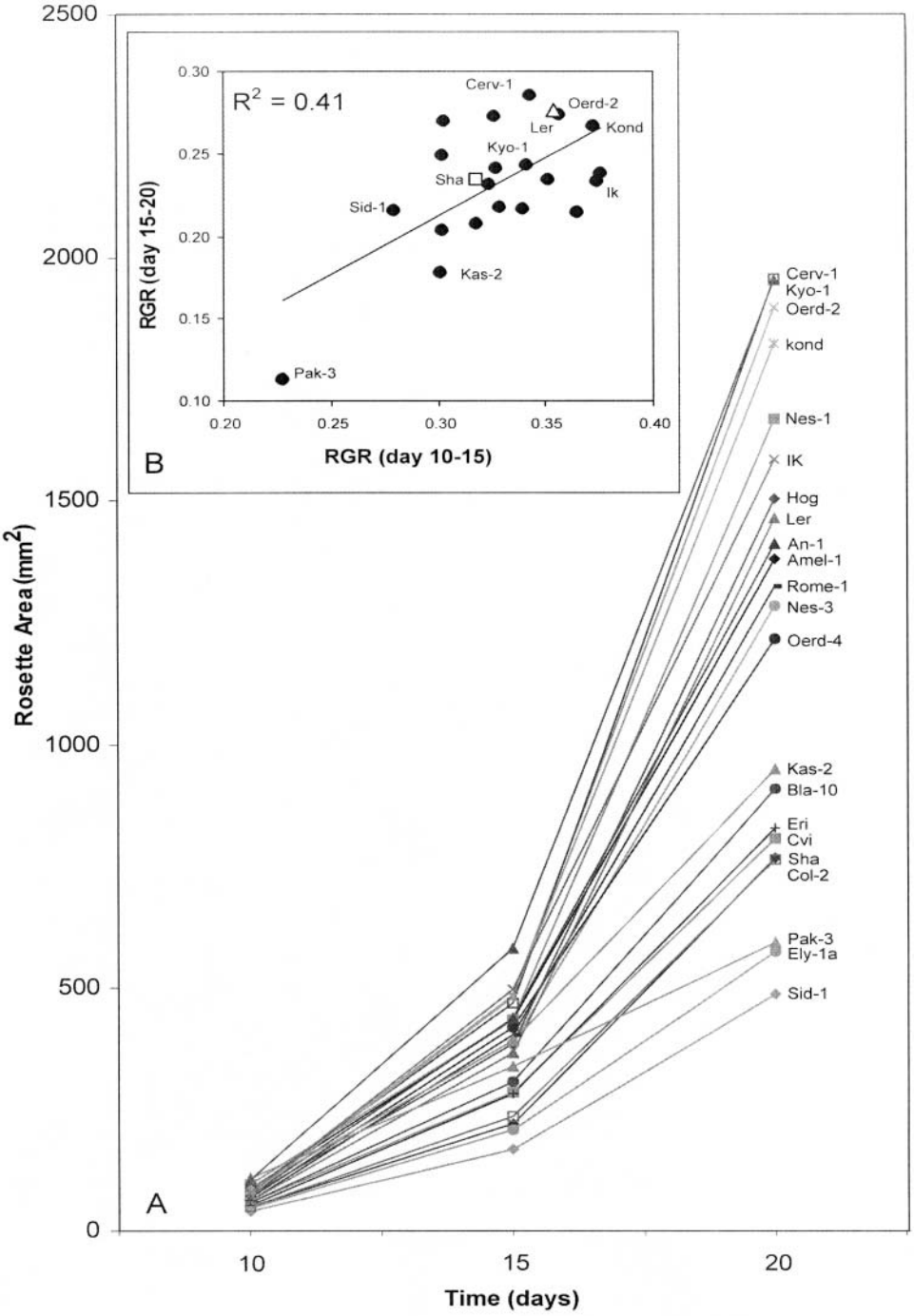

Figure 2. Growth rate curves for 22 Arabidopsis accessions determined by plant area (A). The correlation between relative growth rate (RGR) (day 10–15) and RGR (day 15–20) on the basis of plant area (B).

and Nes3, all collected in the dunes of the island Ameland in the north of The Netherlands, as well as IK, had high SW and moderate TLA1, were grouped in the middle (Fig. 3). PC1 discriminated between the smallest accession in final plant size (Sid-1) and the largest one (Cerveteri-1), while PC3, which was determined mainly by seed weight, gave the largest seeded accession Cvi a separate position that contrasted most with the low seed weight accession An-1.

Genetic Variation among the Ler × Sha RILs

For all traits analyzed significant variation was observed between RILs as indicated by the broad

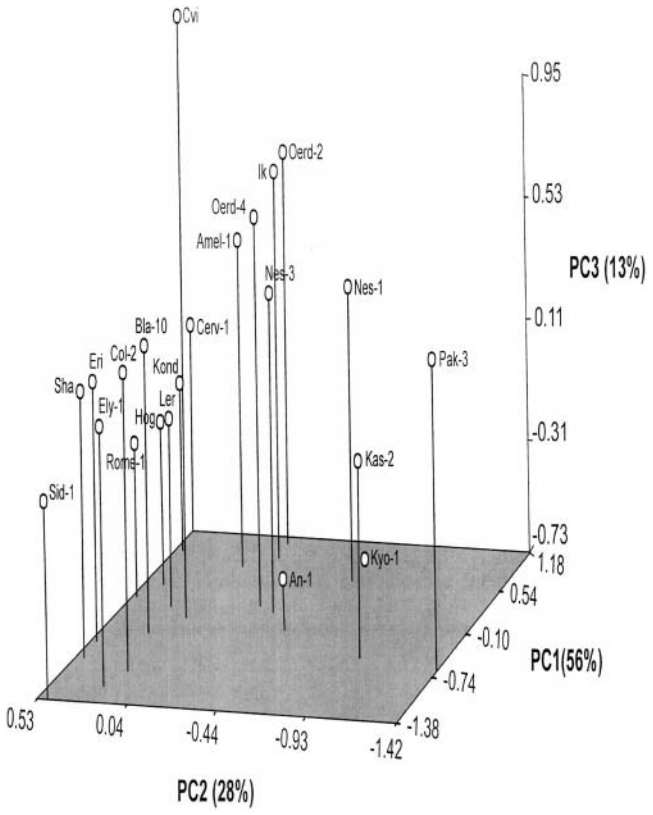

Figure 3. Principle component analysis of variables: fresh and dry weight, seed weight, total leaf area1, total leaf area2 and total leaf area3 for the 22 accessions. The first principle component (PC1) is determined by growth-related parameters; total leaf area3, total leaf area2, and dry weight. The second principle component (PC2) is determined mainly by total leaf area1, seed weight, and fresh weight. In the third principle component (PC3), seed weight was the most important variable discriminating between the accessions.

sense heritabilities ranging from 0.86 to 0.33 for flowering time traits and number of side branches, respectively (Table II and Fig. 4). Transgression beyond the parental values was observed for all traits including those for which parental values hardly differed, such as chlorophyll fluorescence. This amount of genetic variation indicated that QTL mapping was likely to reveal QTLs for most of the traits.

QTL Mapping

Seed Weight

Although the difference in seed weight between the L*er* and Sha parents was small (Fig. 4A and Table II), QTL mapping revealed one QTL on chromosome 5 (Fig. 5). This QTL, for which the Sha allele increased seed weight, explained 14.3% of the variance.

Plant Total Leaf Area and RGR

Figure 5 and Table III summarize the QTLs found in L*er* × Sha RILs for total leaf area (TLA) (four, five, and four QTLs for TLA1, TLA2, and TLA3, respectively). The detected QTLs showed a total explained phenotypic variance of 34%, 43%, and 37.5% for TLA1, TLA2, and TLA3, respectively (Table III). For TLA1, the L*er* alleles increased plant area at three QTLs (at msat1-10, nga692, and msat5-14), whereas at the CHIB locus the Sha allele increased the area. The Sha alleles at *ER*, CHIB, and nga129 increased the TLA2, whereas the L*er* alleles did so at msat1-10 and SO262. For TLA3, the Sha alleles at *ER*, CHIB, and MBK5 loci, and the L*er* allele at msat1-10 increased the area.

Four, three, and two QTLs were found for RGR2-1, RGR3-2, and RGR3-1, respectively. The detected QTLs showed a total explained phenotypic variance of 34.3%, 18.3%, and 16.7% for RGR2-1, RGR3-2, and RGR3-1, respectively (Table III). For RGR2-1, the Sha alleles at *ER*, CHIB, and MBK5 and the L*er* allele at msat1-10 increased plant growth rate. At the nga361, the Sha allele increased the RGR3-2 values, whereas the L*er* alleles did so at the msat1-10 and nga225 loci. For RGR3-1, at two QTLs (msat1-10 and nga225), the L*er* alleles increased growth rates.

At the top of chromosome 1 (msat1-10), colocation was found of the loci for TLA1, TLA2, TLA3 and for all three RGR parameters, as well as with flowering-related traits, i.e. flowering time (FT), total leaf number (TLN), cauline leaf number (CL), and plant length until first silique (PLTS). Colocation of QTLs for these different traits could also be observed at the bottom of chromosome 2 at the *ER* locus, at the CHIB marker near the top of chromosome 3 and at the top and bottom of chromosome 5 (Fig. 5). Colocation of these QTLs, at the top of chromosome 3, with a QTL for speed of germination (Clerkx et al., 2004) was observed, the Sha allele increasing the speed of germination.

The two detected QTLs for relative growth rate as based on dry weight (RGRdw) colocated with the QTLs for RGR calculated on the basis of plant area.

Plant Dry Weight and Relative Growth Rate

Despite the small differences in plant dry weight (DW) between L*er* and Sha, large variation was found between the RILs for dry weight of young (DW1) as well as older plants (DW2; Fig. 4, D and E). Three QTLs were detected for each parameter, explaining together 25.8% and 24.1% of the total variance, respectively (Fig. 5, Table III). With the exception of msat1-10, QTLs for DW1 and DW2 were at different positions. For CHIB and SO262 only DW1 QTLs were detected, whereas *ER* and nga225 revealed QTLs for DW2, indicating that growth in different phases of development may be controlled by different genes but also by the same genes. In agreement with the observed transgression QTLs in which either L*er* alleles increased growth (msat1-10, SO262, and nga225) and those for which the Sha alleles (CHIB and *ER*) lead to higher DW were found. Based on DW1 and DW2, the RGR for DW could be calculated, which revealed

Table II. *Parental values, ranges and heritabilities in the Ler × Sha RILs of all measured traits*

Trait	L*er* value	Sha value	Range	Mean	Heritability
Seed weight (mg)	0.018	0.017	0.014–0.023	0.018	nd
Total leaf area1 (mm^2)	130	153	48–365	161	0.65
Total leaf area2 (mm^2)	436	551	155–1319	555	0.71
Total leaf area3 (mm^2)	2191	2556	576–7418	2703	0.78
Relative growth rate 2-1(area)	0.3	0.32	0.22–0.36	0.31	nd
Relative growth rate 3-2(area)	0.27	0.26	0.08–0.32	0.26	nd
Relative growth rate 3-1(area)	0.28	0.28	0.13–0.33	0.28	nd
Dry weight young plant (mg)	2.5	3.5	1.4–8	3.5	0.68
Dry weight old plant (mg)	37	46	9–74	33	0.74
Relative growth rate (weight)	0.34	0.32	0.15–0.35	0.28	nd
Water content (%)	89	89.4	85.1–92.1	89.5	0.52
Specific leaf area ($mm^2.mg^{-1}$)	175	160	90–350	162	nd
Speed of leaf initiation	13	14	8–19	13.7	nd
Chlorophyll fluorescence	0.72	0.71	0.65–0.75	0.71	0.53
Flowering time SD (days)	33.2	32	21–48.7	35.3	0.86
Flowering time LD (days)	25.2	28.8	21.3–46.3	29.3	0.83
Total leaf number	7.9	9.5	6.6–31.1	10.6	0.86
Rosette leaf number	6	7.7	4.8–24.2	8.4	0.86
Cauline leaf number	1.9	1.8	1.3–6.9	2.3	0.59
Total plant length (cm)	17.5	37.4	11–50.4	26.7	0.85
Plant length till 1st silique (cm)	7.4	8.7	4–21.1	9.5	0.74
Inflorescence length (cm)	10.1	28.7	7–29.3	17.2	0.80
Number of side branches	1.9	2.4	0.4–3.9	1.83	0.33

nd, not determined because only one replica per line was analyzed.

QTLs at *ER* and nga225, which are the DW2 specific QTLs. In contrast the DW2 QTL at msat1-10 is due to the growth in the first phase, which is continued.

For RGWdw a significant interaction between msat1-10 and CHIB, which represented the DW1 QTL, was found explaining 5.5% of the phenotypic variance.

As might be expected plant total area and plant dry weight, were strongly correlated ($R^2 = 0.61$) at day 15.

Specific Leaf Area, Leaf Initiation Speed, and Chlorophyll Fluorescence

For specific leaf area (SLA) only one QTL, located near MBK5 and explaining 13.1% of the phenotypic variance, was detected (Fig. 5). This QTL, for which the Sha allele has a higher value, indicating thinner leaves, colocated with QTLs for leaf initiation speed (LIS), TLA, RGR, and FT traits.

Two QTLs controlling the speed of leaf initiation were found at the F8J2 and MBK5 (Fig. 5) explaining 7.8% and 12.8% of the phenotypic variance, respectively. For the detected QTLs, the L*er* alleles increased the rate of leaf development compared with the Sha alleles. Although the QTL for LIS at MBK5 colocated with one of the flowering time QTLs, this was not the case for the QTL at F8J2. No significant correlation ($R^2 = 0.15$) was observed between leaf number at day 24 and FT in short day (SD) indicating that a higher leaf initiation speed does not account for the major variation in flowering time.

One QTL for chlorophyll fluorescence (ChFl), also located near the MBK5 and explaining 21.4% of the phenotypic variance, was detected, which together with the interaction between MBK5 and msat4-14 (a minor QTL) explained 27.6% of the total variance (Fig. 5). For the detected QTL, the L*er* allele increased the photosynthetic capacity of the plant compared with the Sha allele.

Flowering Time and Flowering-Related Traits including Plant Length and Branching

In Arabidopsis, flowering time is often correlated with the number of leaves formed prior to flowering, and one might expect that the mass of the vegetative parts may influence the elongation of the inflorescence and, therefore, may affect total plant height. Furthermore, the number of leaves determines the number of axillary buds that might develop into secondary inflorescence, thus affecting branching. Because of the expected correlation the data obtained for these parameters are discussed together. The expected relationships were indeed observed in the present material as indicated by the strong correlation between flowering time and total leaf number (TLN, $R^2 = 0.86$), rosette leaf number (RL, $R^2 = 0.86$), and cauline leaf number (CL, $R^2 = 0.61$).

Flowering time differences between L*er* and Sha were relatively small and the relative order of both genotypes depended on the day-length condition, Sha being slighter earlier in short day (SD), but later

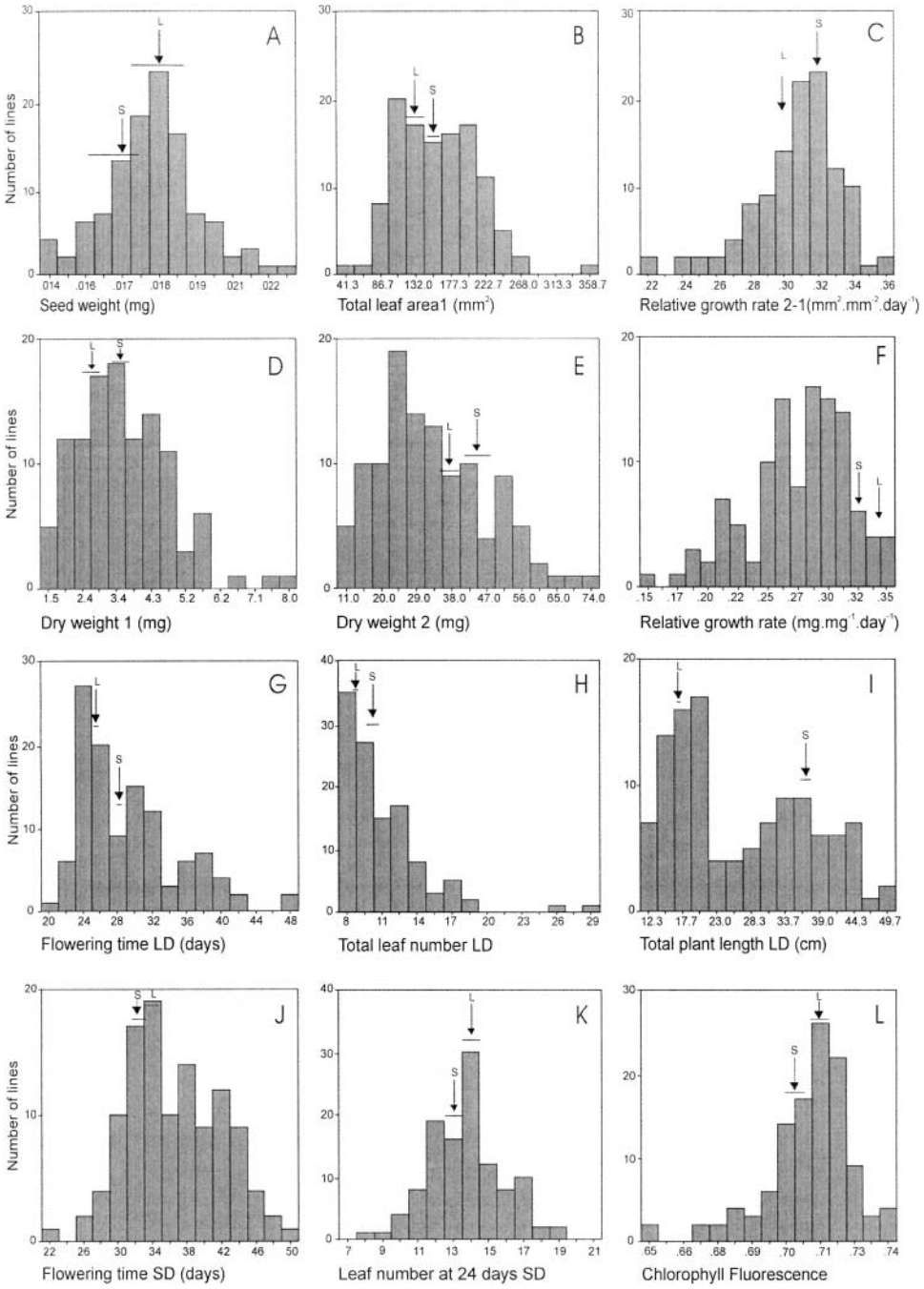

Figure 4. Frequency distribution of nonnormalized data of some traits in the L*er* × Sha RIL population. Growth traits: Seed weight (A), Plant area at 10 d (B), Relative growth rate calculated on the basis of plant area (C), Dry weight 1 at 15 d (D), Dry weight 2 at 25 d (E), Relative growth rate calculated on the basis of plant dry weight (F). Flowering traits: Flowering time scored in long day (LD) conditions (G), Total leaf number counted in LD (H), Total plant length measured in LD (I), Flowering time scored in Short Day (SD) conditions (J), Leaf number counted at 24 d in SD referring to speed of leaf initiation (K), Chlorophyll fluorescence measured in SD (L). The average parental value is indicated with an arrow for both parents, L for L*er* and S for Sha, and the horizontal bars represent the SE for these parental values.

in long day (LD) condition (Table II). However, variation between RILs was considerable and had the same magnitude in LD and SD conditions, with a highly significant correlation ($R^2 = 0.71$) (Fig. 4, G and J). Figure 5 and Table III summarize the QTLs found in L*er* × Sha RILs for flowering time in LD and SD conditions (seven and four QTLs, respectively). Detected QTLs showed a total explained phenotypic variance of 66.6% and 52.4%, respectively. In SD, at two of the four detected QTLs, viz,

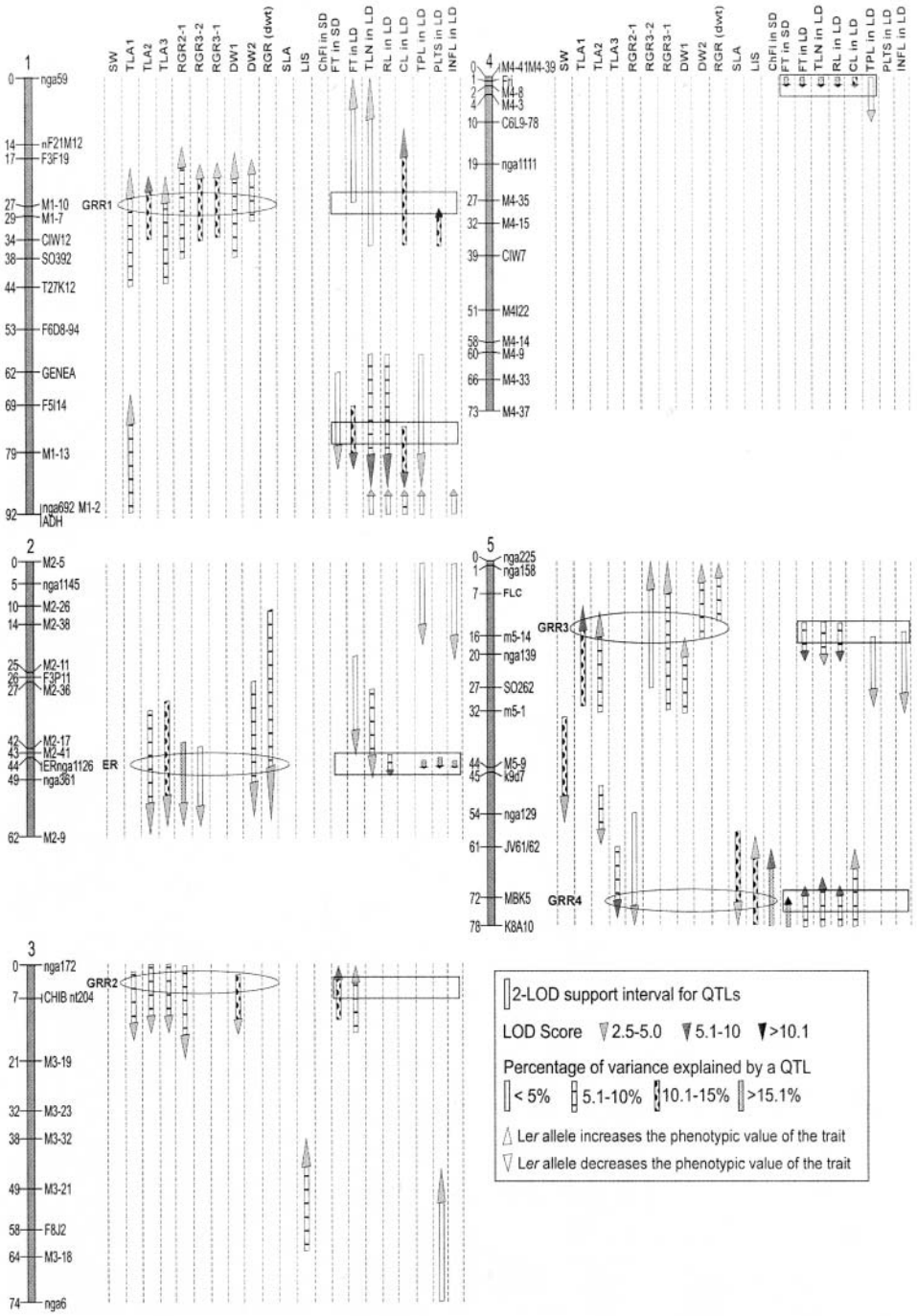

Figure 5. The L*er* × Sha linkage map showing the locations of QTLs for the traits analyzed. The lengths of the arrows indicate the 2-LOD support intervals. The direction of the arrow's head indicates the allelic effect: upward, L*er* increasing and Sha decreasing; downward, Sha increasing and L*er* decreasing. The gray scale of the arrow's head indicates the LOD score value. The filling pattern of the arrow's stem refers to the percentage of variance explained by each QTL. The ovals indicate QTLs of growth regions, *GRR1* until *GRR4* and the *ER* region, while the rectangles indicate the flowering time related QTLs.

at msat1-13 and *FRI*, Sha alleles delayed flowering, whereas Sha alleles at CHIB and K8A10 accelerated flowering. In LD these four QTLs were also detected and showed the same allelic effects. In addition two QTLs were detected in LD, at msat2-36 and msat5-14, where the Sha alleles delayed flowering and one QTL, at nga 59, where the Sha allele promoted flowering.

Seven QTLs were found for TLN, six of them colocating with FT QTLs in LD condition, with similar

Table III. *Characteristics of the detected QTLs explaining growth traits, flowering time and flowering related traits in Ler × Sha RIL population*

Trait	QTL	Map position	LOD score	% of variance	Additive allele effect
Seed weight (mg)					
	K9D7	5–45	3.6	14.3	−0.12
Toal leaf area1 (mm^2)				34	
	M1-10	1–26.7	3.08	6	13
	nga692	1–91.9	3.18	6.1	14
	CHIB	3–6.8	4.55	9	−17
	m5-14	5–16.3	6.13	12.9	19
Total leaf area2 (mm^2)				43	
	M1-10	1–26.7	5.53	11.1	68
	ER	2–43.7	3.66	7.1	−55
	CHIB	3–6.8	4.7	9.3	−64
	SO262	5–27.2	4.16	8.1	62
	nga 129	5–53.9	3.83	7.4	−65
Total leaf area3 (mm^2)				37.5	
	M1-10	1–26.7	3.57	8.1	346
	ER	2–43.7	4.37	10.1	−379
	CHIB	3–6.8	3	6.7	−331
	MBK5	5–72.4	5.36	12.6	−441
Relative growth rate 2-1 (area)				34.3	
	M1-10	1–26.7	2.89	7.4	0.07
	ER	2–43.7	3.51	17.4	−0.09
	CHIB	3–6.8	2.99	5.2	−0.06
	MBK5	5–72.4	2.75	4.3	−0.04
Relative growth rate 3-2 (area)				18.3	
	M1-10	1–26.7	3.25	10.2	0.11
	nga361	2–48.6	2.58	3.5	−0.09
	nga225	5–0	2.88	4.6	0.10
Relative growth rate 3-1 (area)				16.7	
	M1-10	1–26.7	3.45	10.4	0.12
	nga225	5–0	2.69	6.3	0.09
Dry weight 1 (mg)				25.8	
	M1-10	1–26.7	3.24	8.3	0.39
	CHIB	3–6.8	4.23	11.1	−0.44
	SO262	5–27.2	2.52	6.4	0.30
Dry weight 2 (mg)				24.1	
	M1-10	1–26.7	2.65	6.8	3.73
	ER	2–43.7	3.8	10	−4.58
	nga225	5–0	2.83	7.3	3.68
Relative growth rate (weight)				20.4	
	ER	2–43.7	2.75	6.7	−0.11
	nga225	5–0	3.33	8.2	0.12
	M1-10 × CHIB			5.5	
Specific leaf area ($mm^2.mg^{-1}$)					
	MBK5	5–72.4	3.41	13.1	−16
Leaf initiation speed				20.6	
	F8J2	3–58	2.56	7.8	0.59
	MBK5	5–72.4	4.06	12.8	0.78
Chlorophyll fluorescence (SD)				27.6	
	MBK5	5–72.4	6.28	21.4	0.008
	m4-14 × MBK5				6.2
Flowering time SD (days)				52.4	
	M1-13	1–78.7	3.77	4.8	−1.19
	CHIB	3–6.8	7.44	11	1.78
	FRI	4–0.8	11.48	18.7	−2.33
	K8A10	5–77.7	11.08	17.9	2.31
Flowering time LD (days)				66.6	
	nga59	1–0	3.38	3.6	1.05
	M1-13	1–78.7	8.92	11	−2.13
	M2-36	2–26.6	3.69	4	−1.05
	CHIB	3–6.8	4.83	5.5	1.15
	FRI	4–0.8	17.14	25.5	−2.52

(*Table continues on following page.*)

Table III. *(Continued from previous page.)*

Trait	QTL	Map position	LOD score	% of variance	Additive allele effect
	m5-14	5–16.3	5.84	7	−1.32
	K8A10	5–77.7	8.06	10	1.63
Total leaf number				63.4	
	nga59	1–0	2.51	3	0.71
	M1-13	1–78.7	7.24	9.7	−1.54
	ADH	1–92.3	3.63	4.7	1.07
	ER	2–43.7	4.84	6.1	−1.00
	FRI	4–0.8	15.47	25.2	−1.81
	m5-14	5–16.3	4.88	6.4	−0.98
	K8A10	5–77.7	6.13	8.3	1.07
Rosette leaf number				63.8	
	M1-13	1–78.7	6.69	8	−1.24
	ADH	1–92.3	3.79	4.5	0.91
	ER	2–43.7	7.45	9	−1.01
	FRI	4–0.8	17	25.9	−1.58
	m5-14	5–16.3	6.13	7.5	−0.88
	K8A10	5–77.7	7.07	8.9	0.93
Cauline leaf number				48.1	
	F3F19	1–17.2	5.61	11.4	0.22
	M1-13	1–78.7	6.69	13.8	−0.31
	ADH	1–92.3	2.73	5.2	0.20
	FRI	4–0.8	5.86	12	−0.24
	K8A10	5–77.7	2.97	5.7	0.17
Total plant length (cm)				81.7	
	F5I14	1–69.1	2.7	1.5	−1.51
	ADH	1–92.3	4.99	2.9	1.70
	M2-26	2–10	3.34	1.8	−1.49
	ER	2–43.7	46.26	70.1	−9.24
	FRI	4–0.8	4.47	2.5	−1.68
	SO262	5–27.2	4.98	2.9	−1.74
Plant length till 1st silique (cm)				60.6	
	CIW12	1–34.5	10.93	14.5	1.20
	ER	2–43.7	23.95	42.6	−2.10
	F8J2	3–58	3.14	3.5	0.65
Inflorescence length (cm)				79.9	
	nga692	1–91.9	4.14	2.7	1.52
	nga1145	2–5.3	2.61	1.7	−1.09
	ER	2–43.7	43.6	72.4	−7.19
	SO262	5–27.2	4.59	3.1	−1.49

contributions and allelic effect (Fig. 5, Table III). At CHIB a minor QTL (at the border of significance) could be detected.

Rosette leaf number and cauline leaf number, being the two components of TLN, showed six and five QTLs, explaining 63.8% and 48.1% of variance, respectively. One QTL, specific for cauline leaf number, colocating with FT (LD) but not with RL, was found on chromosome 1, near marker F3F19. For RL, two QTLs at chromosome 2 and top of chromosome 5 colocated with FT but not with CL QTLs, indicating that although flowering time is intimately linked with number of leaves initiated before the transition to flowering, the number of elongating internodes is under separate genetic control. The remaining QTLs for RL and CL colocated with each other and with QTLs for TLN and FT (Fig. 5).

For total plant length (TPL) and its two length components (length until the first silique and inflorescence length), the total explained variance was relatively high (81.7%, 60.6%, and 79.9%, respectively), which was largely due to the effect of the *ER* locus, explaining 70.1%, 42.6%, and 72.4% of the observed variation. The remaining five QTLs for TPL contribute little and for all loci, except *ADH*, the Sha alleles increased plant length (Table III).

For PLTS and inflorescence length (INFL) fewer QTLs were detected per trait (Table III). One locus at marker CIW12 might be specific for PLTS since it did not colocate with any other QTLs for TPL or INFL. For INFL, two loci at the bottom of chromosome 1 and near the middle of chromosome 5, colocated with TPL but not with PLTS (Fig. 5), suggesting that they might only be responsible for the increase in the internode length between the flowers.

No QTLs could be detected for the number of side branches derived either from the axillary buds of the rosette leaves or the cauline leaves, which may be due to the fact that many genes with small effects segregate in this population or due to the low heritability (0.33).

DISCUSSION

Variation specifically for growth of leaves among Arabidopsis accessions as such has not been studied, in contrast to hypocotyl growth (Maloof et al., 2001; Borevitz et al., 2002; Botto and Smith, 2002) and flowering time (for review, see Koornneef et al., 2004). A study by Li et al. (1998) compared growth for a number of accessions and tried to relate growth variation with other parameters such as seed weight and geographical distribution. In addition, growth was studied in genetic analyses of nitrogen-use efficiency (Rauh et al., 2002; Loudet et al., 2003). Ungerer et al. (2002) analyzed leaf size together with developmental traits dealing with flowering and plant architecture, while Pérez-Pérez et al. (2002) used Arabidopsis natural variation for genetic analysis of leaf morphology and leaf area.

Growth-Related Traits

In this article we provide a genetic analysis of traits related to plant growth. A comparison of the more extreme phenotypes among a collection of Arabidopsis accessions showed that large differences for growth rate exist, which may be different between accessions during consecutive phases of development. Differences in biomass may result from differences in seed mass, emergence time, or variation in RGR (Van Andel and Biere, 1990; Poorter and Navas, 2003). Differences in RGR can be explained by differences in leaf area per unit plant mass (LAR; leaf area ratio) or by differences in the rate of increase in plant mass per unit leaf area (ULR; unit leaf rate; Evans, 1972). In this study we found that RGR and final dry weight were not correlated with seed weight as was described by Li et al. (1998). However, early growth, determined as rosette area, was significantly correlated with seed weight. This correlation weakened during subsequent growth, implying that other factors started to dominate growth rate. A 2-fold difference was observed for seed weight; the low latitude accessions from the Cape Verde Islands and Pakistan but also accessions from the Dutch island of Ameland had heavy seeds, disproving the negative correlation between seed size and latitude suggested by Li et al. (1998). In the latter study this correlation was based almost exclusively on the Cvi accession.

The extensive heritable variation present in natural populations is shown in the analysis of a new RIL population derived from the cross L*er* × Sha, in which we studied a number of traits directly related to biomass production as well as to flowering. For most traits we detected heritable variation and QTLs could be mapped. The highest percentages of explained variation were obtained for flowering time and related traits, which have a high heritability. Less variation could be attributed to specific loci for growth-related traits and even less for parameters that were derived from two measured parameters, for which the variation of both measurements is added up. The usefulness of nondestructive growth measurements is clearly shown by the higher explained variance of leaf area than for dry weight, which is most likely due to the fact that more plants could be measured per genotype.

QTLs that were found for leaf area, dry weight, and RGR colocated in many cases, which is expected since they all measure different, but related, aspects of overall plant growth. However, in several cases no colocations were found for these growth-related traits. This indicates that some loci may have an overall effect on plant growth, whereas others specifically regulate certain processes that contribute more to some but less to other of the measured parameters, or act during a specific phase of growth. For example the QTLs on top of chromosome 3 were found mainly for the earlier phases, indicating that this QTL has a development-specific effect.

Colocation of QTLs for traits that are less obviously related might suggest pleiotropy. In case developmental changes such as flowering would be influenced by growth or vice versa, this would be reflected by colocation. Similarly, one could predict that larger late flowering plants would have longer stems. When traits have a causal relationship the allelic effects should also be in the same direction and a high overall correlation of these traits in the RIL population should be observed. Since only two out of the four FT QTLs found in SD, where growth analysis was performed, colocate with growth QTLs but have opposite allelic effects for the two traits and because the overall correlation between DW2 and FT was not significant ($R^2 = 0.03$), we conclude that both traits are genetically different. Although a flowering time QTL is found in the *ER* region, we do not consider this a pleiotropic effect because the line with the *ER* wild-type allele in L*er* background does not show this effect (data not shown).

For plant length the strongest effect is due to the *ER* locus, where the Sha allele promotes both growth and length. However, at the top part of chromosome 5 the QTLs for growth and total plant length colocate but the alleles act in opposite direction, which indicates that at this locus rosette growth might have a trade-off with total plant length. A weak, but significant, overall correlation was found between FT and length when the lines with mutant and wild-type *ER* alleles were treated separately. The highest correlation was between FT and length until the first silique, which was $R^2 = 0.49$ for *ER* plants and 0.19 for *er* plants. The relationship between both traits is also suggested by colocations at three positions with allelic effects in the same direction (Fig. 5).

For plant growth-related traits we found five regions with QTLs (Fig. 5). The effects of the loci were never more than 2-fold. The characteristic of the QTLs around msat1-10 near the top of chromosome 1, which is called *GRR1* (Growth Rate 1), is that it affects all parameters and therefore, growth as such during the vegetative phase of development. This locus might be the same as DM10.1 described by Loudet et al., (2003) in the Bay × Sha population, in which the Sha allele also has a negative effect. An interesting colocation found at this position, but not elsewhere, is between the number of cauline leaves and the length until the first silique. When the two traits are combined, this implies that the L*er* allele promotes formation of cauline leaves and stimulates elongation of internodes between leaves.

The second growth-related QTL region (*ER*) is around the *ERECTA* locus and very likely the *ERECTA* gene itself, since the analysis of a near-isogenic line, having the wild-type *ER* allele in a L*er* genetic background, showed similar differences with L*er* for the same traits (data not shown). Interestingly, the growth effects of this locus were not detected at the earlier phases of development. As shown before for both Col × L*er* and Cvi × L*er* RIL populations (Alonso-Blanco et al., 1999; Pérez-Pérez et al., 2002; Ungerer et al., 2002), this locus always makes a major contribution to plant length and leaf size.

Torii et al. (1996), Yokoyama et al. (1998), and Shpak et al. (2003) provided arguments that the *ER* gene plays a role in coordination of cell growth patterns within the organ primordia initiated from the shoot apical meristem. The gene is predominantly expressed in the shoot apical meristems and in organ primordia. The expression is weak during early plant development but increases with the transition from the vegetative to the reproductive growth phase, in agreement with the absence of effects during the early phase of growth. Douglas et al. (2002) also showed that the *ER* gene influences multiple processes during Arabidopsis development, including internode and pedicle elongation, leaf and silique morphogenesis, and thickness of stem tissue.

A third locus for growth on top of chromosome 3, named *GRR2*, mainly affected early growth. When comparing the accessions it was noted that early plant growth correlated positively with seed weight (Fig. 1A). However, in the L*er* × Sha RIL population, the *GRR2* locus affected early growth, but not seed weight. The finding of a QTL for speed of germination at that position (Clerkx et al., 2004) may suggest the cause of this early growth to be related to seed vigor, giving plants a faster start. Loudet et al. (2003) found a QTL for dry mass, which they named DM3.2 at the same position, for which the Sha allele also increases growth. This effect is rather small and was only observed when plants were grown at low nitrogen (3 mM) conditions (Loudet et al., 2003).

The locus near nga139 on top of chromosome 5 (*GRR3*) has not been described in other populations. It might actually consist of two loci that did not show up as significant in all analyses. A locus on top of this chromosome was described as DM10.7 by Loudet et al. (2003).

Probably the most interesting new QTL region is at the bottom of chromosome 5 (*GRR4*), where possibly two QTLs are located. Besides QTLs for growth rate and FT, also loci affecting LIS, SLA, and ChFl were found in this region, the latter two not being found in the other regions. Interestingly a higher rate of leaf initiation due to the L*er* allele coincided with smaller leaves and lower growth rate, suggesting that the leaves that are formed are smaller and also thinner as indicated by the reduction of SLA by the L*er* allele. The effect on chlorophyll fluorescence suggests that the physiology of these leaves is also different.

Flowering Time

In this study we have analyzed the flowering behavior of two early Arabidopsis accessions. They differed slightly in their flowering phenotype (measured as both FT and TLN) and in their response to photoperiod length. However, variation between segregating RILs derived from crosses between these two accessions showed a large variation as shown also in other crosses, viz, between L*er* and Cvi (Alonso-Blanco et al., 1998) and between Bay-0 and Sha (Loudet et al., 2002), and larger than that between L*er* and Col (Jansen et al., 1995). The significant correlation between flowering time in SD and in LD conditions (R^2 = 0.71) indicates that flowering time in both conditions is predominantly controlled by the same genetic factors in the L*er* × Sha RILs. The flowering behavior differences between the L*er* × Sha lines in both LD and SD conditions can be mainly attributed to QTLs located at msat1-13, CHIB, *FRI*, and K8A10. L*er* alleles at CHIB and K8A10 result in lateness, while at msat1-13 and *FRI* L*er* alleles are earlier, thus explaining the similar behavior of the parental lines and the transgression in the RILs. Three other loci that were found in LD only are located at nga59, msat2-36, and msat5-14, where L*er* alleles at the first locus give lateness while for the other two loci the L*er* alleles lead to earliness. Previously it was shown that the *FRI* and the *FLC* loci determine flowering time differences between very late, vernalization-responsive accessions and early ones (Johanson et al., 2000; Gazzani et al., 2003; Michaels et al., 2003). Sequence analysis has shown that Sha contains a wild-type *FRI* gene (Gazzani et al., 2003; Michaels et al., 2003) in contrast to L*er* (Johanson et al., 2000) making it most likely that the *FRI* locus is the gene for this QTL. This is further supported by the flowering time QTL, detected by Loudet et al. (2002) in the Sha × Bay-0 population. We could not find any QTL at the *FLC* locus, probably because both the Sha and L*er* parents carry weak alleles at this locus (Koornneef et al., 1994; Loudet et al., 2002; Gazzani et al., 2003; Michaels et al., 2003). The Sha accession was slightly less sensitive to changes in photoperiod

length compared to L*er*. Three of the detected QTLs might be specific to day length and, therefore, affect day length sensitivity, viz, at *ADH* (not significant for FT but detected for TLN) and at msat2-36, in LD condition only, and at CIW7 (minor QTL for FT) in SD condition. The first two QTLs might be the same found to be specific for photoperiod in the Bay-0 × Sha population in SD and LD, respectively (Loudet et al., 2002). Some of the flowering-related QTLs that we found in the L*er* × Sha population colocalized with previously published QTLs detected in other populations (Kowalski et al., 1994; Clarke et al., 1995; Jansen et al., 1995; Kuittinen et al., 1997; Alonso-Blanco et al., 1998; Loudet et al., 2002).

Concluding Remarks

Screening a number of Arabidopsis accessions revealed different patterns for growth. In this study we could identify a number of QTLs affecting plant growth. These loci appear to have different physiological functions, as concluded from colocations of QTLs for different traits. Especially the *GRR4* locus near marker MBK5 looks very interesting because it affects a plethora of physiological effects including speed of leaf initiation, specific leaf area, and chlorophyll fluorescence. However, it should be emphasized that due to the inaccuracy of QTL mapping in a population of this size, it cannot be excluded that independent but linked genes control these apparent pleiotropic effects. This should further be investigated by fine mapping, which is most effectively done when no other QTLs segregate, i.e. using near-isogenic lines (NILs, see Alonso-Blanco and Koornneef, 2000). In addition the loci *GRR1* and *3*, which might be related to nitrogen-use efficiency (Loudet et al., 2003) deserve further study. The *GRR3* QTL has the intriguing property that it affects early seedling growth. Because of the complexity of comprehensive traits like growth we cannot speculate on candidates for the QTLs, except for the *ERECTA* locus for which isogenic lines prove the involvement of the *ERECTA* gene. For flowering time several previously detected QTLs were found as well as a few new ones. Natural allelic variation for *FRI* and *FLC* is already studied at the molecular level (Johanson et al., 2000; Le Corre et al., 2002; Gazzani et al., 2003; Michaels et al., 2003). This may indicate the direction for future research, aiming to understand causes and consequences of natural genetic variation.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The seeds from different accessions were sown in petri dishes on water-saturated filter paper, followed by a 4-d cold treatment at 4°C, and then transferred to a climate room at 25°C and 16 h light for 2 d before planting in 7-cm pots with standard soil. In all descriptions of experiments, time is referred to as days after planting. Details of the selected 22 accessions are given in Table I. These accessions (24 plants/accession) were grown under controlled conditions in a growth cabinet, with 70% relative humidity, 22°C, 12-h day length and light intensity 25 Wm^{-2}, for a detailed growth analysis. Plants were placed on carts, and the carts were shuffled daily to avoid minor environmental differences within the growth cabinet.

F9 plants of a new set of 114 RILs, obtained by single-seed descent of F_2 plants derived from the cross L*er* × Sha, were analyzed for flowering time and growth-related traits in two different experiments. The first one was carried out in an air-conditioned green house supplemented with additional light (model SON-T plus 400W, Philips, Eindhoven, The Netherlands) providing a day length of at least 16 h light which is a long day (LD), and maintained at a temperature between 22°C and 25°C (day) and 18°C (night). The second one was carried out in a growth cabinet under 12 h light, which is a mild short day (SD) treatment for Arabidopsis. In the greenhouse experiment 12 plants/RIL were grown in the same conditions as mentioned before (LD), in a randomized two-block design to reduce environmental effects, while 10 plants/RIL were grown in the growth cabinet, in the same conditions as mentioned above, also in a randomized two-block design. A line with the *ERECTA* wild-type allele in the L*er* genetic background, the two reciprocal hybrids, and both parents were included in all experiments.

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) d after transferring the seedlings to the pots. 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), and analysis of the pictures using the computer program MetaMorph (version 4.01; Universal Imaging Corporation, West Chester, PA, www.image1.com). The mean TLA for each line of the 114 RILs was obtained by imaging five plants/line at day 10 (TLA1) and four plant/line at 15 (TLA2) and 20 (TLA3) d. The relative growth rate (RGR) was calculated according to the following equation: $(\ln A_x - \ln A_y)/dt_{(x-y)}$. 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 to 15, 15 to 20, and 10 to 20 d, respectively.

Weight, Water Content, and SLA Determinations

The mean seed weight (SW) for each accession was obtained by weighing two seed lots each of 100 seeds using a Perkin-Elmer microbalance (model AD-4 Autobalance, Boston). SW for each line of the 114 RILs was determined for one batch per line.

The mean fresh weight (FW) of the plants was determined at day 35 by harvesting and weighing the aboveground parts of two plants/accession. The mean FW for each RIL was determined at day 15 and 25, by harvesting and weighing two plants/line, one from each block. Dry weights (DW) were determined after drying the plants at 105°C for 48 h, and the water content (WC) was estimated as the relative ratio between water and dry weight using the formula [(FW-DW)/FW] × 100. The relative growth rate as based on dry weight (RGRdw) 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}). The relation between the 22 accessions based on seed weight, fresh and dry weights, and areas at 10, 15, and 20 d was described with principle component analysis using NTSYSpc version 2.10t. (Rohlf, 2001) with standardized data, which were converted in a correlation matrix from which three eigenvectors were extracted using the EIGEN function of the NTSYS-pc program.

Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence as a nondestructive means of photosynthetic capacity was measured 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 = Δ F/Fm′).

Measurement of Flowering Time and Related Traits in RILs

From the greenhouse experiment, in which 12 plants/RIL were grown in LD condition, FT for each plant was recorded as the number of days from

planting until the opening of the first flower. Flowering time was also scored by counting the TLN, i.e. RL plus CL, 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: TPL, PLTS, and total number of side shoots or inflorescences (TSSN) (number of branches in the main inflorescence plus the number of side shoots from the rosette). In the phytotron experiment (SD), flowering time was scored as described above, for six plants/line. In addition, the number of rosette leaves was counted at day 24 from planting (i.e. before flowering) which refers to the leaf initiation speed (LIS).

Genetic Mapping

The mapping of the segregating population was done by using 66 molecular markers, including the morphological marker *erecta*, located at a distance from 1 to 15 cM on the genetic map to obtain a regular distribution among the five chromosomes. These markers were used to generate the linkage map; details are published elsewhere (Clerkx et al., 2004). This map was used for QTL analysis of the various traits.

Statistical Analysis and QTL Mapping

For each RIL, the mean value of the traits under investigation was ($\log_{10}$) transformed to improve normality of the distribution, except for the relative growth rates, rosette areas, and the specific leaf area. Transformed data were used for QTL analysis. The software package MapQTL version 4.0 (van Ooijen, 2000) was used to identify and locate QTL on the linkage map by using interval mapping and multiple-QTL model (MQM) mapping methods as described in its reference manual (http://www.plant.wageningen-ur.nl/products/). In a first step, putative QTLs were identified using interval mapping. Thereafter, the closest marker at each putative QTL was selected as a cofactor, and the selected markers were used as genetic background controls in the approximate multiple QTL model of MapQTL. Log of the odds (LOD) threshold values applied to declare the presence of a QTLs were estimated by performing the permutation tests implemented in MapQTL version 4.0 using at least 1,000 permutations of the original data set for each trait, resulting in a 95% LOD threshold between 2.4 and 2.6.

Two-LOD support intervals were established as 95% QTL confidence interval (van Ooijen, 1992). 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 restricted MQM Mapping implemented with MapQTL.

Two-way interactions among the QTL identified for each trait were tested by ANOVA using the corresponding two markers as fixed factors and the trait as dependent variable, using the general linear model of the statistical package SPSS version 11.5.0. A Bonferroni correction to adjust the 0.05 threshold of significance was applied if multiple tests were performed on the same data set. Only those interactions that were significant after the Bonferroni correction are presented.

Heritabilities were calculated based on measurements on 6 to 12 plants.

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