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RESEARCH PAPER

Quantitative trait loci analysis of leaf and plant longevity in *Arabidopsis thaliana*

Virginia M. C. Luquez[1,*], Yamila Sasal[1], Micaela Medrano[1], María I. Martín[1], Mercedes Mujica[2] and Juan J. Guiamét[1,†]

[1] *Instituto de Fisiología Vegetal (INFIVE), Universidad Nacional de La Plata, CC 327, 1900 La Plata, Argentina*

[2] *Cátedra de Mejoramiento Genético, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, CC 31, 1900 La Plata, Argentina*

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Abstract

The natural variation in leaf and plant longevity in *Arabidopsis thaliana* was analysed in a set of 45 ecotypes and 155 recombinant inbred lines derived from a Cape Verde Islands (Cvi)×Landsberg *erecta* (L*er*) cross. Post-bolting longevity was inversely related to time to flowering and rosette leaf number in the set of 45 ecotypes, with Cvi having the longest and L*er* the shortest post-bolting longevity. The recombinant inbred line population was tested under low or high soil nutrient levels (LN or HN, respectively). Three quantitative trait loci (QTL), one in chromosome 3 and two in chromosomes 1 and 5, were associated with longevity of the 6th rosette leaf under LN and HN, respectively. Four QTL for post-bolting longevity were found in chromosomes 1, 3, 4, and 5, and two in chromosomes 1 and 5 under LN and HN, respectively. An epistatic interaction affecting post-bolting longevity under LN, but not HN, was detected. L*er* and Cvi carry a mix of increasing and decreasing alleles for the QTL affecting longevity of the 6th leaf and post-bolting longevity. Longevity of the 6th rosette leaf was associated with different QTL than post-bolting longevity, and it was affected by different QTL depending on nutrient availability. By contrast, the major QTL affecting post-bolting longevity exerted significant effects irrespective of soil nutrient availability.

Key words: *Arabidopsis thaliana*, leaf longevity, nutrient levels, quantitative trait loci.

Introduction

Leaf and plant longevity are strongly affected by the time of onset and the rate of progress of senescence processes, with earlier onset or faster rate of senescence significantly shortening longevity. Senescence is an orderly, genetically controlled type of programmed cell death (Gan and Amasino, 1997; Noodén *et al.*, 1997; Lim *et al.*, 2003), which is characterized by breakdown of macromolecules and redistribution of the released nutrients (particularly nitrogen) to younger leaves or to developing seeds, tubers, etc. During the vegetative growth phase, senescence of leaves progresses from the base upwards ('sequential' senescence). On the other hand, all leaves and other vegetative tissues die more or less simultaneously during the final stages of fruit development in monocarpic plants, i.e. those that flower and fruit once in their life cycle (Noodén *et al.*, 2004).

Senescence and, therefore, longevity are under the influence of environmental conditions, e.g. irradiance and spectral composition of light, photoperiod, water availability, etc. (Guiamét *et al.*, 1989; Noodén *et al.*, 1996; Rousseaux *et al.*, 1996; Pic *et al.*, 2002). In natural and agricultural environments nutrient supply is an important factor modulating leaf area duration, with nutrient shortage significantly accelerating senescence and reducing leaf longevity (Martignone *et al.*, 1987; Thomas and De Villiers, 1996). Quite often fertilization contributes to an increase in grain yield partly by delaying senescence of the whole canopy and, thereby, extending plant longevity and the grain-filling period (Bänziger *et al.*, 2002). The

* Present address: Umeå Plant Science Centre, SE-901 87 Umeå, Sweden.
† To whom correspondence should be addressed. E-mail: jguiamet@fcnym.unlp.edu.ar

Abbreviations: QTL, quantitative trait loci; LN, low nutrient level; HN, high nutrient level; Cvi, Cape Verde Islands; L*er*, Landsberg *erecta*; RIL, recombinant inbred line.

identification of genes regulating the response of leaf longevity to nutrient levels might help in the design of plants which are tolerant of moderate nutrient shortages.

The expression of many genes increases during senescence, and these genes include transcription factors and putative signalling components (Gepstein *et al.*, 2003; Lin and Wu, 2004). Only in a few cases has the function of these genes been examined with loss-of-function mutants (He and Gan, 2002). Several spontaneous mutations that affect senescence cause 'stay green' phenotypes where chlorophyll loss is delayed or blocked (Thomas and Howarth, 2000). The molecular basis of these 'stay green' variants is known for only a few mutations (Woo *et al.*, 2001; Yoshida *et al.*, 2002). A useful approach to uncover genes important in the regulation of leaf life-span is the identification of quantitative trait loci (QTL) responsible for variation in leaf and plant longevity in lines derived from crosses between contrasting genotypes. This approach allows the identification of chromosomal regions where allelic variation causes significant effects on a given trait. In sorghum, where the 'stay green' trait is associated with increased tolerance to post-anthesis drought (Borrell *et al.*, 2000), five to eight QTL account for a substantial proportion of the variation in the rates of leaf senescence under field conditions (Crasta *et al.*, 1999; Kebede *et al.*, 2001; Haussmann *et al.*, 2002). Some of these QTL are consistently detected in studies involving recombinant inbred lines (RILs) derived from crosses with different sources of the 'stay green' trait, and two of the sorghum QTL correspond to syntenic genomic regions of maize harbouring 'stay green' QTL (Kebede *et al.*, 2001). This implies that the genetic regulation of senescence might be relatively conserved among related species, and that the identification of QTL in one species might be useful in breeding programmes of related crops.

Arabidopsis thaliana is widely used as a model plant for genetic and physiological studies (Alonso-Blanco and Koornneef, 2000). In recent years, there has been a significant number of studies of leaf senescence and longevity in *Arabidopsis*, but these analyses were mostly limited to the laboratory strains Columbia and Landsberg, and mutants in those genetic backgrounds (Hensel *et al.*, 1993; Lohman *et al.*, 1994; Noodén *et al.*, 1996; Noodén and Penney, 2001; Woo *et al.*, 2001; Buchanan-Wollaston *et al.*, 2003; Guo *et al.*, 2004; Lin and Wu, 2004). Only in one paper was leaf senescence studied in other *Arabidopsis* accessions (Levey and Wingler, 2005). *Arabidopsis* has a widespread natural distribution, spanning a wide latitudinal and altitudinal range, and many different habitats (e.g. agricultural fields, prairies, forest floors, swamps, etc.). There is a large natural variation between accessions collected in different locations (Alonso-Blanco and Koornneef, 2000) and, when different accessions are grown under a similar environment, their phenotypic variation reflects their underlying genetic differences. The genetic analysis of crosses between *Arabidopsis* accessions has proved to be a fruitful strategy to find new genes involved in the regulation of flowering (Alonso-Blanco *et al.*, 1998*b*), in responses to light quality and hormones (Borevitz *et al.*, 2002) and in nitrogen-use efficiency (Rauh *et al.*, 2002; Loudet *et al.*, 2003), among other traits. As far as is known, the naturally occurring variation of *Arabidopsis* has not been used to analyse QTL associated with leaf and plant longevity.

The aims of this work were (i) to analyse differences in post-flowering longevity in a large set of *Arabidopsis* ecotypes collected at different latitudes and environments, and (ii) to detect QTL for leaf and plant longevity in a set of recombinant inbred lines (Alonso-Blanco *et al.*, 1998*a*) growing under two contrasting nutrient supply levels.

Materials and methods

Plant material and growing conditions

Ecotypes of *Arabidopsis thaliana* and RILs were obtained from the Arabidopsis Biological Resources Centre, Ohio, USA. The names and geographic origin of the ecotypes used are listed in Table 1.

Forty-five ecotypes were grown in 1999, and two [Landsberg *erecta* (L*er*) and Cape Verde Islands (Cvi)] were selected for further analysis in 2002. Seeds were planted on 14 May 1999 (45 ecotypes; Table 1) and 14 July 2002 (L*er* and Cvi). Seeds were sown in 200 ml plastic pots filled with a 1:1 (v/v) mixture of sterile soil and perlite, and kept for 4 d at 4 °C to overcome seed dormancy and ensure uniform germination. Thereafter, pots were arranged in a completely randomized design and grown in a greenhouse under natural irradiance and photoperiod. Plants were sub-irrigated with distilled water. When the first two true leaves appeared, plants were thinned to one plant per pot. Pots were watered weekly with 10 ml of complete nutrient solution from emergence to bolting of the earlier-flowering ecotype. The experiments ended in September 1999 and October 2002.

RILs from a cross between Cvi and L*er* (Alonso-Blanco *et al.*, 1998*a*) were grown in 2003 to identify QTL. RILs (155 lines) were planted on 15 May 2003 in 25×21 cm sheet pots, each pot 4 cm in diameter and 9 cm deep (total volume 90 ml), filled with a 1:1 (v/v) mixture of sterile soil and perlite. Pots were sub-irrigated with distilled water. Sixteen plants of each RIL were grown in four completely randomized blocks, each block with four plants of each RIL. After sowing, sheet pots were kept at 4 °C for 4 d to ensure uniform germination, and then placed randomly in a greenhouse. Starting 30 d after sowing, RILs were subjected to two nutrient availability regimes. Two blocks (eight plants per RIL) were fertilized weekly by adding 5 ml of complete nutrient solution per plant [fertilized treatment, i.e. high nutrient level (HN)], while the other two blocks (eight plants per RIL) received the same amount of distilled water [non-fertilized treatment, i.e. low nutrient level (LN)]. Fertilization was discontinued when the first rosettes dried out. Since the RIL×block interaction within each treatment was not significant, all the data from the same treatment were pooled and the mean used to calculate the QTL.

Typical average greenhouse temperatures during the day were around 19, 17, 16, 16, and 19 °C for May, June, July, August, and September, respectively. For all experiments, night temperatures in the greenhouse were below 10 °C during June, July, and August, so the plants were vernalized. The maximum irradiance was 1000 μmol m^{-2} s^{-1} on sunny days, and the minimum was 80 μmol m^{-2} s^{-1} on

Table 1. *Geographic origin, latitude, days to bolting, rosette leaf number at the start of fruit growth, post-bolting rosette longevity (PBL), and broad-sense heritability for all traits in a set of 45 ecotypes grown during 1999*

Values are mean ±standard error. Data for Cvi and L*er* are shown in boldface.

Ecotype	Geographic origin	Latitude	Bolting (d)	Leaf number	PBL (d)
Aa-0	Aua/Rhon, Germany	51	100.4±1.9	57.2±2.3	31.4±1.3
Ag-0	Argentat, France	45	98.8±1.2	52.0±2.5	33.6±0.9
Ak-1	Achkarren, Germany	48	82.2±0.4	25.4±0.6	39.6±1.5
Bay-0	Bayreuth, Germany	49	89.0±0.6	33.0±0.5	37.4±1.7
Bl-1	Bologna, Italy	44	88.4±1.3	40.0±1.1	33.4±2.5
Bla-3	Blanes, Spain	41	80.8±0.6	28.4±1.3	35.0±0.5
Br-0	Brno, Czech Republic	49	92.0±0.6	45.4±1.7	37.8±2.1
Bs-2	Basel, Switzerland	47	83.8±0.3	36.0±1.3	32.0±2.0
Bur-0	Burren, Ireland	53	104.6±0.4	48.0±1.3	31.6±0.8
Can-0	Canary Islands, Spain	28	92.0±0.6	36.6±1.1	35.2±1.4
Cnt-1	Canterbury, UK	51	90.4±0.2	40.8±1.2	34.0±1.5
Co-1	Coimbra, Portugal	40	65.2±0.9	21.0±1.0	39.8±0.9
Col-0	Columbia, MO, USA	38	95.8±95.8	38.8±1.0	28.8±1.7
Cvi-0	**Cape Verde Islands**	**16**	**60.0±1.6**	**11.6±1.0**	**45.0±1.6**
Di-2	Dijon, France	47	91.6±1.2	33.8±0.7	31.4±1.8
Edi-0	Edinburgh, UK	56	94.6±0.7	47.4±2.4	32.2±1.1
En-1	Enkheim, Germany	50	83.0±0.9	31.8±0.7	34.6±0.5
Es-0	Espoo, Finland	60	102.4±1.1	45.4±2.5	29.6±0.5
Est-1	Estland, Russia	58	95.2±1.3	37.4±1.7	30.8±1.2
Gre-0	Greenville, MI, USA	43	100.4±0.7	43.8±3.7	30.6±3.4
Hl-0	Holtensen, Germany	51	89.4±0.7	34.0±1.6	28.4±1.2
Kas-1	Kashmir, India	34	88.6±1.2	31.8±1.0	34.2±1.0
Ka-0	Carintia, Austria	46	82.4±1.8	27.4±1.3	42.6±2.1
Kil-0	Killian, UK	56	82.4±1.2	30.0±1.9	34.8±1.7
Lc-0	Loch Ness, UK	57	89.2±0.7	38.2±1.6	27.6±1.0
L*er*-0	**Landsberg *erecta*, Germany**	**51**	**87.0±1.3**	**24±1.0**	**23.8±0.9**
Li-8	Linburg, Germany	50	91.4±0.2	41.6±1.5	33.2±1.3
Lm-2	Le Mans, France	48	83.4±2.4	30.6±2.1	31.2±0.9
Lu-1	Lund, Sweden	55	92.8±0.6	40.4±1.6	35.2±0.8
Mh-0	Muhlen, Poland	53	99.2±0.5	42.8±2.4	32.8±0.5
Ms-0	Moscow, Russia	56	87.2±1.9	24.4±1.4	37.0±2.3
Mr-0	Montessoro, Italy	44	91.6±91.6	33.6±1.5	31.6±1.9
Nok-0	Noordwijk, Netherlands	52	98.0±1.5	41.4±1.9	31.6±3.4
Ost-0	Osthammar, Sweden	60	107.0±1.5	51.8 ±1.8	30.2±0.7
Pa-1	Palermo, Italy	38	74.4±1.0	25.4±0.9	33.2±2.0
Pog-0	Point Grey, BC, Canada	49	91.4±1.0	37.8±1.7	36.2±1.0
Rsch-4	Staraia Roscha, Russia	54	93.2±1.2	46.0±3.1	32.6±0.9
Shah	Pamiro-Alay, Tadjikistan	39	79.6±1.6	26.4±0.8	37.4±1.6
Sorbo	Sorbo, Tadjikistan	38	91.2±1.0	35.2±0.9	32.0±2.6
Su-0	Southport, UK	53	96.8±1.1	48.8±1.2	29.2±1.0
Te-0	Tenela, Finland	60	101.8±0.9	44.6±1.2	24.2±2.4
Ts-1	Tossa de Mar, Spain	41	72.4±1.0	20.4±0.7	37.6±1.6
Tsu-0	Tsu, Japan	34	84.2±0.5	40.4±0.2	38.0±0.8
Ws-0	Wassilewskija, Russia	53	89.0±0.7	45.2±3.0	36.0±1.2
Yo-0	Yosemite, USA	37	107.0±1.0	63.2±1.6	23.8±2.5
Average			89.8	37.3	33.3
Heritability (broad sense)			0.99	0.98	0.89

cloudy days. Day length was between a minimum of 9 h 49 min in June and 11 h 52 min in September.

Longevity of the 6th leaf

Twenty-nine days after sowing, when the sixth rosette leaf was 2 mm long, it was tipped with a small drop of white correction fluid. These leaves were considered to be of the same age, and were treated as a cohort (Noodén and Penney, 2001). Survival/death was scored every 2 d, and a leaf was recorded as dead when it was totally yellow. Leaf longevity was calculated as the number of days between emergence (i.e. the leaf 2 mm long) and death.

Phenology and post-bolting longevity

The dates of bolting (defined as the first inflorescence stalk 1 cm long) and death of the rosette foliage [last rosette leaf dead, as in Noodén and Penney (2001)] were recorded every 2 d. Post-bolting rosette longevity was calculated as the number of days between bolting and death of the rosette. Post-bolting rosette longevity is an estimate of the time when reproductive-driven senescence is complete, relative to the start of the reproductive period. Rosette leaf number was determined by counting all rosette leaves (dead or alive, including cotyledons) at the onset of fruit development (siliques 1 cm long).

Above-ground dry weight

When plants were recorded as dead, the above-ground parts of the plant were cut and dried at 80 °C for 48 h to determine dry weight.

QTL analysis

QTL were identified by marker regression analysis (Kearsey and Hyne, 1994) using the software available at the QTLCafé website

(www.bham.ac.uk/g.g.seaton/). The original set of RILs derived from Cvi and L*er* is completely mapped with AFLP markers (Alonso-Blanco *et al.*, 1998*a*). The map and the information about markers were obtained from www.arabidopsis.org. To avoid type 1 errors, only QTL with $P < 0.01$ were considered (Kearsey and Pooni, 1996). Markers with a high proportion of missing genotypic values or markers mapping at the same distance as other markers were discarded. In total, 95 markers covering 475 cM, with marker distances between 3 cM and 13 cM were used. The data were transformed to $\log_{10}$ to improve normality, but the results (i.e. QTL position) were similar to the original or log-transformed data. Tables and figures show the data in their original (non-transformed) values.

Epistasis

Conditional epistatic interactions affecting the phenotypic value of the QTL were found by using 'Epistat' software (Lark *et al.*, 1995). To detect epistatic interactions, a pairwise comparison of the phenotypic distribution of the four possible genotypic combinations of each QTL was run with each marker (other than the QTL), and the significance of the interactions tested by running Montecarlo simulations. Only interactions with a P value <0.001 were considered significant.

Heritability and statistical analysis

Broad-sense heritability (i.e. the ratio between genotypic and phenotypic variance) was calculated according to Lynch and Walsh (1998). The contribution of each QTL to the phenotypic variance was estimated by analysis of variance components using the General Linear Model and the REML procedure. For each trait, the genotype of the closest marker to the detected QTL was included as a random factor in the model, and the marker×marker interactions when significant. Heterozygous markers were excluded from the analysis.

Results

Post-bolting rosette longevity variation in a large set of Arabidopsis *ecotypes*

The ecotypes included in the 1999 experiment originated in different locations spanning a 44° latitudinal range (Table 1). These ecotypes were also collected in different environments, i.e. agricultural fields, forest floors, swamps, etc, and were therefore likely to be adapted to quite different conditions. As expected, they showed a large variation in their development, e.g. in the date of bolting, number of rosette leaves, and post-bolting rosette longevity (Table 1). For example, time to bolting ranged from 60 d to 107 d, the number of rosette leaves varied between 11.6 and 63.2, and post-bolting rosette longevity was between 23 d and 45 d. Post-bolting rosette longevity, i.e. the number of days from bolting to death of all rosette leaves, was inversely related to time to bolting ($r=-0.56$, $P < 0.001$) and rosette leaf number ($r=-0.42$, $P < 0.001$). Ecotypes that flowered later generally had more rosette leaves and shorter post-flowering longevities.

Broad-sense heritability estimates the degree to which the individual phenotypes are determined by the genotype (Falconer and Mackay, 1996). Broad-sense heritability for post-bolting longevity was 0.89 (Table 1), which is lower than the heritability values for days to bolting (0.99) and leaf number (0.98).

Landsberg *erecta* (L*er*) and Cape Verde Islands (Cvi) showed the shortest and longest post-bolting rosette longevities of the 45 ecotypes tested, with 23.8 d and 45 d, respectively (Table 1). Therefore, they were planted again in 2002 to test if they also differed in longevity of an early rosette leaf. As in the 1999 experiment, Cvi flowered about 20 d earlier and produced fewer rosette leaves than L*er* (Table 2). In addition to a longer post-flowering longevity; the 6th rosette leaf also had longer longevity in Cvi than in L*er* (Table 2).

*Longevity in the Cvi–*Ler *RIL set*

Since Cvi and L*er* differ in terms of post-bolting and leaf longevity, a set of mapped RILs derived from a cross between these two ecotypes (Alonso-Blanco *et al.*, 1998*a*) was used to identify QTL associated with longevity of an early leaf and post-bolting longevity of the rosette. The RILs were tested under two nutrient availability regimes that were expected to alter the progression of senescence and, therefore, affect longevity: LN (only nutrients available in the soil) and HN (watered weekly with complete nutrient solution). There were significant effects of nutrient levels on longevity of the 6th leaf and post-bolting rosette longevity (Table 3). Genotype×environment interactions were significant for time to flowering and leaf and rosette longevity (data not shown), indicating that lines responded differentially to the different nutrient supply regimes.

Nutrient levels affected above-ground dry weight accumulation, which was 30–50% higher in HN than in LN in L*er*, Cvi, and the RIL set (Table 3). The fertilization treatment did not affect phenological development (i.e. days to flowering and rosette leaf number). Longevity of the 6th leaf was extended by only 2 d in fertilized plants of Cvi and the RIL set, but it did not change in L*er* (Table 3). Post-bolting rosette longevity was not affected by nutrient levels in the parental lines, but it was extended by an average of 3 d in the RIL set. Broad-sense heritability for these traits was calculated for the RIL set. In all cases a high proportion of the total variance was determined by the genotype (Table 3).

The frequency distribution of both leaf and post-bolting longevity showed transgressive variation (i.e. lines with

Table 2. *Days to bolting, rosette leaf number, longevity of the 6th leaf, and post-bolting rosette longevity in Landsberg* erecta *(*L*er) and Cape Verde Islands (Cvi)*

Values are means ±standard error.

Ecotype	Days to bolting	Rosette leaf number	6th leaf longevity (d)	Post-bolting rosette longevity (d)
L*er*	63.3±0.8	22.8±0.4	31.8±0.1	23.3±0.9
Cvi	44.0±0.0	10.8±0.3	38.5±1.0	42.6±1.1

Table 3. *Phenological development, longevity, and dry weight accumulation in Cvi, Ler, and the average of the 155 RILs growing under two fertilization treatments (fertilized, HN; non-fertilized, LN)*

Broad-sense heritability was calculated for the RILs in each treatment. Traits are defined in Materials and methods. Values are means ±standard error.

Trait	Treatment	*Ler*	Cvi	RILs	Heritability (broad sense)
Above-ground dry weight (mg)	LN	184±13	271±24	154±24	0.92
	HN	249±33	350±9	231±3	0.93
Days to bolting	LN	74.7±1.1	56.9±1.2	57.1±0.5	0.99
	HN	76.5±0.6	56.7±0.9	57.1±0.5	0.99
Rosette leaf number	LN	19.4±0.6	13.1±0.4	13.5±0.2	0.99
	HN	20.6±0.5	12.2±0.4	13.6±0.2	0.99
6th leaf longevity	LN	42.2±1.2	47.0±1.2	42.9±0.2	0.85
	HN	42.7±0.9	49.9±1.2	44.4±0.2	0.91
Post-bolting rosette longevity	LN	31.1±0.8	40.3±1.0	33.5±0.2	0.94
	HN	29.8±0.7	40.3±1.0	36.2±0.2	0.97

higher or lower values for the traits than the parental lines; Figs 1, 2) indicating the presence of a mixture of alleles that increase or decrease the trait in each parent. The frequency distribution of 6th leaf longevity in both nutrient-level treatments in the RIL set is shown in Fig. 1. Clearly, the frequency distribution for the RILs was different in both treatments, with the distribution slightly skewed towards longer longevities and a slight decrease in the frequency of lines showing intermediate longevities in HN. The distribution of post-bolting rosette longevity was also different in both treatments, and, again, in HN the frequency of lines with longer longevities was higher than in LN, with an increase in the frequency of lines with post-bolting rosette longevity higher than 46 d (Fig. 2). There was no correlation between longevity of the 6th leaf under LN and HN (Fig. 3), implying that the genetic combinations that caused extended longevity under LN did not affect longevity in the same direction under HN. By contrast, post-bolting rosette longevity under LN and HN correlated very closely (R^2=0.63). Longevity of the 6th leaf and post-bolting longevity correlated quite well (R^2=0.39) under HN, but there was no correlation between these variables in plants growing with low nutrient supply (Fig. 4).

As in the original set of 45 ecotypes tested, post-bolting rosette longevity correlated negatively with days to bolting and rosette leaf number in both LN and HN (Table 4). Longevity of the 6th leaf did not correlate with days to bolting and rosette leaf number in low nutrient supply, but it showed a moderate correlation under high nutrient conditions (Table 4).

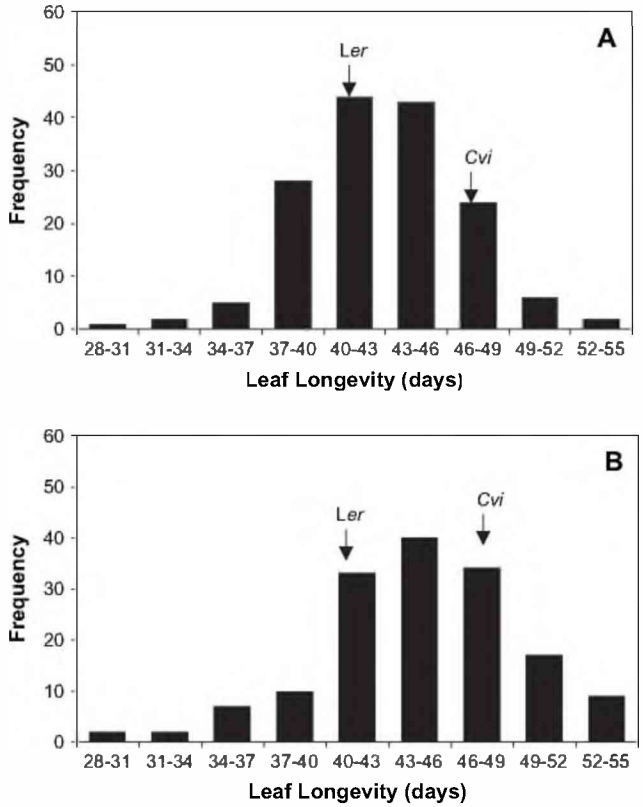

Fig. 1. Frequency distribution of longevity of the 6th leaf in the RIL set in each nutrient-supply treatment (A, without fertilization; B, watered with nutrient solution). Means for *Ler* and Cvi are indicated with arrows.

QTL affecting 6th leaf longevity

The significance levels and residuals for marker regression analysis are shown in Table 5. None of the residuals was significant, implying that there was only one QTL per linkage group. Different QTL had significant effects on 6th leaf longevity depending on the environment, i.e. the soil nutrient level. In the LN environment, one significant QTL in chromosome 3 at 60 cM (6ll-3LN), with an additive effect of 1.31 (Table 5), explained 8.1% of total variance in longevity of the 6th leaf. Under HN, a QTL in chromosome 1 at 18 cM (6ll-1HN) and another one in chromosome 5 at 32 cM (6ll-5HN) together accounted for 18% of the variance. It is interesting to note that each parental line carries alleles with opposite effects under high nutrient conditions; the Cvi allele at 6ll-1HN decreases and the *Ler* allele increases longevity, while, for 6ll-5HN, Cvi carries the increasing allele and *Ler* the decreasing one (Table 5).

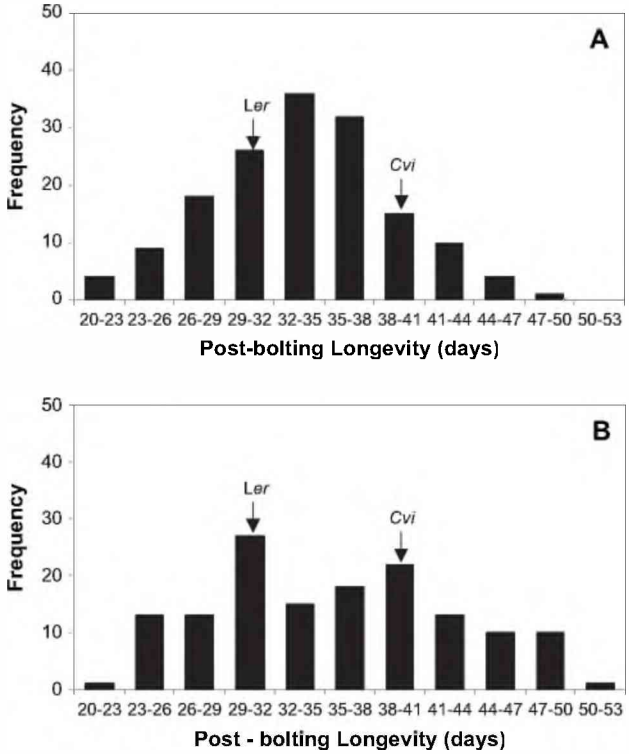

Fig. 2. Post-bolting rosette longevity frequency distribution in the RIL set in both environments (A, without fertilization; B, watered with nutrient solution). Means for L*er* and Cvi are indicated with arrows.

Analysis of QTL involved in post-bolting rosette longevity

Four QTL with significant effects on post-flowering rosette longevity explaining 41.4% of variance were detected under LN, whereas two QTL explained 48.8% of variance under HN (Table 5). The confidence intervals for the map position of two of the QTL detected under LN (i.e. pbl-1LN and pbl-5LN) overlapped with the two QTL with significant effects under HN (pbl-1HN and pbl-5HN), and they may represent the same genes with significant effects in both environments. However, pbl-3LN and pbl-4LN had significant effects only under LN conditions. As with longevity of an early leaf, each parental line carries a mix of increasing and decreasing alleles. For example, Cvi carries the decreasing alleles of pbl-1LN, pbl-1HN, and pbl-4LN, and the increasing alleles of pbl-3LN, pbl-5LN, and pbl-5HN.

Epistatic interactions for post-flowering longevity under nutrient-limiting conditions

Epistat software (Lark *et al.*, 1995) was used to search for conditional epistatic interactions affecting the phenotypic value of the QTL involved in post-bolting longevity. A pairwise comparison of the phenotypic distribution of the four possible genotypic combinations of each QTL with each marker (other than the QTL) detected only an interaction between pbl-1LN and the BF.134C-Col marker (chromosome 3; Table 6). BF.134C-Col had no effect on post-bolting longevity of plants carrying the Cvi allele of pbl-1LN. The L*er* allele of pbl-1LN reduces post-flowering longevity by 3.5 d in the presence of the L*er* allele of BF.134C-Col but, in lines carrying the Cvi allele of BF.134C-Col, the L*er* allele of pbl-1LN causes a longevity decrease of 10 d. No interaction between pbl-1HN and BF.134C-Col was detected in fertilized plants.

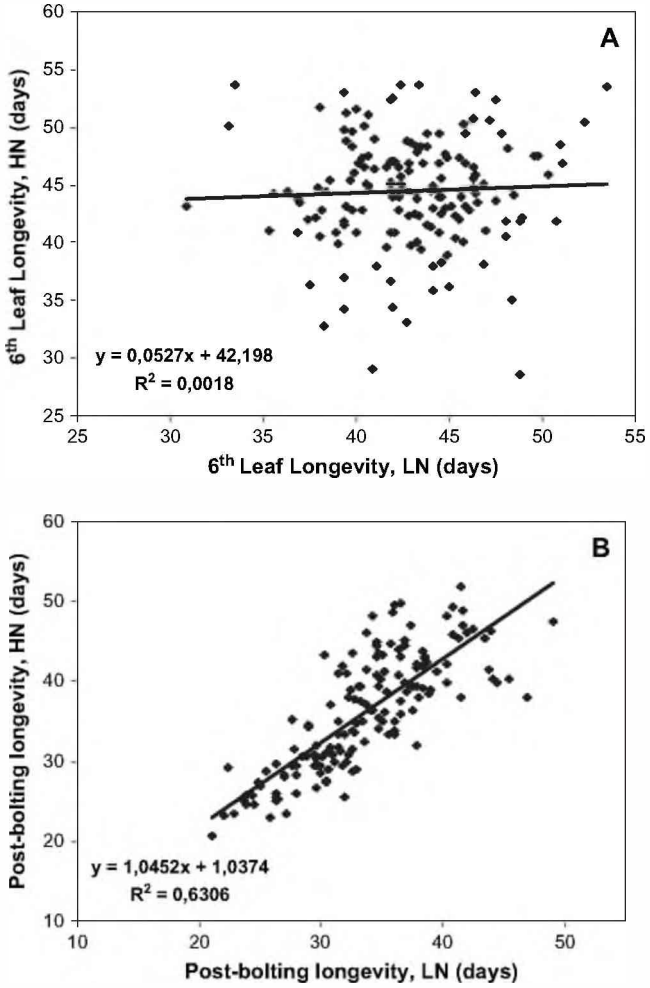

Fig. 3. Relationship between longevity in plants of the RIL set growing with low (LN) or high (HN) nutrient availability. (A) Longevity of the 6th leaf of plants supplied with nutrient solution (HN) versus distilled water (LN). (B) Post-bolting longevity in LN versus HN. The insets show R^2 and the equation for the linear model.

Discussion

QTL involved in leaf and plant longevity

In annual herbaceous plants, leaf senescence follows two well-defined patterns (Leopold, 1961; Noodén *et al.*, 2004). During the vegetative growth phase leaves senesce in a chronological sequence from the base upwards (sequential senescence), with older leaves dying at the

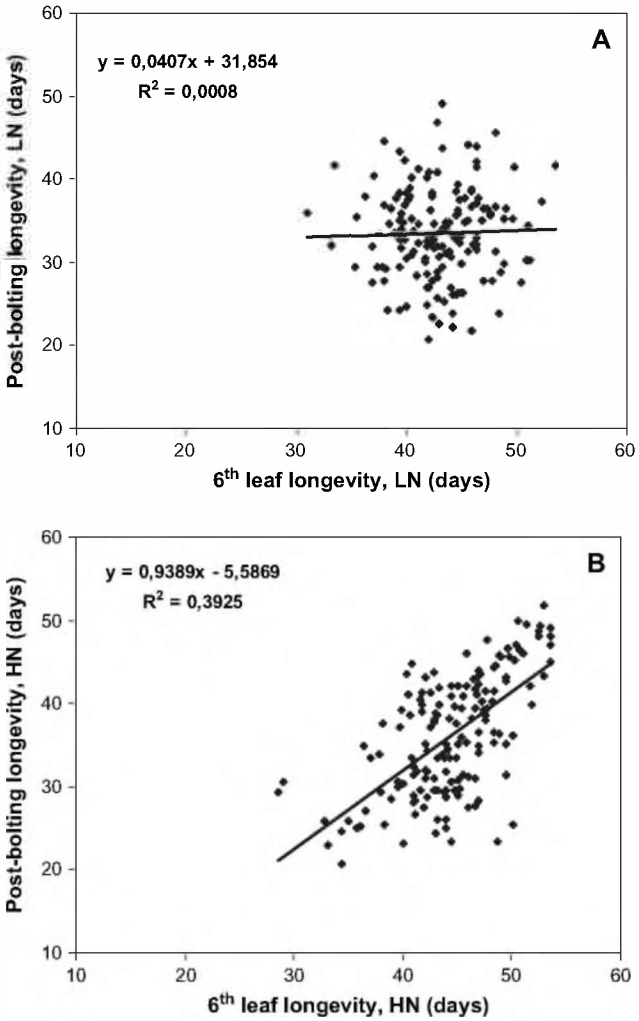

Fig. 4. Relationship between longevity of the 6th leaf and post-bolting longevity in the RIL set. (A) Low nutrient availability (LN); (B) high nutrient availability (HN). The insets show R^2 and the equation for the linear model.

Table 4. *Pearson correlation coefficient (r) between leaf longevity or post-bolting rosette longevity and other developmental parameters for the 155 RILs, with (HN) or without (LN) fertilization*

Some plants or leaves were missing, so the *N* value is indicated for each pair of variables.

	Treatment	6th leaf longevity[a]	Post-bolting rosette longevity
Days to bolting	LN	0.06^{NS} (*N*=1169)	−0.57* (*N*=1225)
	HN	−0.25* (*N*=1153)	−0.73* (*N*=1216)
Rosette leaf number	LN	0.01^{NS} (*N*=1169)	−0.47* (*N*=1223)
	HN	−0.22* (*N*=1155)	−0.57* (*N*=1214)

[a] * Significant at $P < 0.001$; ^{NS} non-significant.

base while new leaves develop on top of the plant. Longevity of the 6th rosette leaf was probably influenced by this sequential senescence pattern. Once plants flower and set fruit, this pattern ceases with the arrest of new leaf production. All the remaining leaves and other vegetative tissues die more or less synchronously at the end of the plant life cycle as fruits mature. These two patterns of senescence differ in terms of their controlling factors. Removal of the terminal bud shows that sequential senescence is controlled by the growing apex (Noodén, 1988), although the decrease in irradiance and the lower red:far red ratio of light at the bottom of the plant also play important roles accelerating sequential senescence (Guiamét *et al.*, 1989; Rousseaux *et al.*, 1996). On the other hand, the final demise of the plant is regulated by maturing fruits in many species, including *Arabidopsis* (Bleecker and Patterson, 1997; Noodén and Penney, 2001). Thus, longevity of the 6th leaf and post-flowering longevity of the rosette might be expected to be associated with different QTL. However, compared with L*er*, Cvi shows greater longevity both of the 6th leaf and of the rosette during the reproductive period. Longevity of the 6th leaf and post-bolting longevity correlate well in the set of 155 RILs grown with abundant nutrient availability. Likewise, a region mapping at 28–32 cM in chromosome 5 is involved in the regulation of 6th leaf longevity under high nutrient conditions, and of post-bolting rosette longevity in both environments. Cvi carries the increasing allele for the QTL mapping in this region (6ll-5HN, pbl-5LN, and pbl-5HN), lending support to the idea that these QTL may represent a single gene affecting longevity of an early leaf under HN, and post-bolting longevity irrespective of nutrient supply. Similarly, under low nutrient conditions, longevity of the 6th leaf and post-bolting duration of the rosette were associated with QTL mapping at 53–59 cM on chromosome 3. Again, Cvi carries the increasing alleles. This suggests that the same genes may be involved in determining longevity of an early developed leaf and post-flowering longevity of the rosette, although the QTL analysis shown here lacks the power to conclude unambiguously that these QTL represent the same genes.

QTL associated with leaf and post-bolting rosette longevity at different nutrient levels

Nutrient availability had a large effect on above-ground biomass accumulation, but a comparatively minor influence on longevity. None of the QTL affecting longevity overlapped with QTL involved in growth responses to nutrient supply levels (data not shown), suggesting that none of these QTL affects longevity through effects on nutrient assimilation. The lack of correlation between 6th leaf longevity under HN and LN (Fig. 3) seems to indicate that different genes are involved in longevity responses of an early leaf to different nutrient availability levels. This is substantiated by the finding that different QTL were associated with 6th leaf longevity in HN and LN. Similarly, in another set of RILs of *Arabidopsis*, dry matter accumulation and nitrogen content 35 d after planting were affected by different QTL in plants receiving 3 mM or 10 mM

Table 5. *Quantitative trait loci affecting longevity of the 6th leaf and post-bolting rosette longevity*

Only QTL with significant effects at $P < 0.01$ are indicated. LN, Low nutrient level (without fertilization); HN, high nutrient level (watered weekly with nutrient solution). Positive additive effects indicate that Cvi carries the increasing allele; negative additive effects indicate that the increasing allele is carried by L*er*.

		Name and chromosome	*P* marker regression[a]	*P* residual	Additive effect	Position (cM)	Nearest marker (position)	Simulated interval (95%)	Variance explained (R^2)
6th leaf longevity	LN	6ll-3LN (3)	0.002*	0.13	1.31	60	GH.172C (60)	59.0±8.0	*8.1*
	HN	6ll-1HN (1)	0.001*	0.06	−1.60	18	EC.480C (15)	19.7±10.9	5.8
	HN	6ll-5HN (5)	0.002*	0.69	2.24	32	DF.184L-Col (30)	32.1±4.3	12.2
Post-bolting rosette longevity	LN	pbl-1LN (1)	0.001*	0.06	−2.51	8	AXR-1 (7)	9.0±7.8	16.8
	LN	pbl-3LN (3)	0.004*	0.49	1.71	54	GD.296C-Col (57)	53.1±8.9	8.0
	LN	pbl-4LN (4)	0.001*	0.49	−1.93	64	HH.159C-Col (65)	64.3±5.9	7.3
	LN	pbl-5LN (5)	0.003*	0.17	2.48	28	DF.184L-Col (30)	28.1±5.5	9.3
	HN	pbl-1HN (1)	0.001*	0.54	−4.18	6	AXR-1 (7)	6.4±2.1	31.3
	HN	pbl-5HN (5)	0.002*	0.06	4.62	32	DF.184L-Col (30)	31.8±3.3	17.5

[a] * Significant at $P < 0.001$.

Table 6. *Effect of interacting QTL on post-bolting rosette longevity (d) of non-fertilized plants*

The phenotypic effect of the L*er* allele at the pbl-1LN QTL is significantly affected ($P < 0.01$) by the presence of the Cvi allele at the BF.134C-Col marker locus.

		BF 134C-Col	
		Ler	Cvi
pbl-1LN	*Ler*	31.9	25.2
	Cvi	35.5	35.2

nitrate (Loudet *et al.*, 2003). Growth was associated with treatment-specific QTL in another set of RILs grown with different N sources (Rauh *et al.*, 2002).

By contrast, post-bolting rosette longevity under HN and LN correlated closely, suggesting that the same QTL influenced longevity regardless of nutrient availability. In fact, two pairs of QTL (pbl-1LN and pbl1-HN, pbl-5LN and pbl-5HN) accounted for a large part of the phenotypic variation in post-bolting longevity in both nutrient-supply treatments. Both QTL in each pair map close together, with L*er* carrying the increasing alleles for pbl-1LN and pbl1-HN and the decreasing alleles for pbl-5LN and pbl-5HN. This suggests that these may represent two genes in chromosomes 1 and 5, respectively, affecting plant longevity irrespective of the nutritional status of the plant. The fact that the same genomic regions had a large influence on post-bolting rosette longevity under both nutrient availability regimes explains the close correlation between post-bolting rosette longevity at LN and HN. Lines with a greater post-bolting longevity under HN also had extended longevity under LN. Apparently, the internal factors that control post-reproductive longevity override the effects of nutrient availability. Likewise, 'stay green' lines of maize characterized by delayed senescence of the canopy during the grain-filling period also remain 'stay green' when they are grown under nitrogen-limiting conditions (Bänziger *et al.*, 1999). Therefore, it might be possible to breed for genotypes that do not accelerate reproduction-associated senescence in response to nutrient shortages and, thereby, maintain a reasonably extended grain-filling period.

Relationship between flowering time and post-bolting longevity

There was a close inverse correlation between post-bolting-rosette longevity and variables related to reproductive development (e.g. days to bolting and rosette leaf number at the start of fruit development), in the set of 45 ecotypes and in both nutrient-availability treatments (HN and LN) with the RILs derived from a Cvi×L*er* cross (Table 4). A relationship between flower or fruit development and post-bolting longevity is expected, because the development of reproductive organs triggers senescence and death of the whole plant in many monocarpic species (Noodén, 1988; Bleecker and Patterson, 1997; Noodén and Penney, 2001; Noodén *et al.*, 2004). In fact, rosette senescence starts later in later-flowering ecotypes grown under a constant photoperiod, temperature, and irradiance (Levey and Wringler, 2005). Therefore, it might be expected that rosettes of earlier-flowering lines would senesce earlier than those of later-flowering ecotypes, but earlier-flowering lines exhibited longer post-bolting duration of the rosette, compared with later-flowering ecotypes. QTL were also analysed for days to bolting in the present experiment. Two QTL were found in both nutrient-availability treatments: one in chromosome 1 at 6 cM and another one in chromosome 5 at 32 cM (data not shown). These QTL mapped in the same chromosome region as QTL for post-bolting longevity: pbl-1LN, pbl-5LN, pbl-1HN, and pbl-5HN (Table 5). Two QTL for post-bolting rosette longevity (pbl-1LN and pbl-1HN) mapped close to EDI, a major time-of-flower QTL in *Arabidopsis* identified as an allele of CRY2 (Alonso-Blanco *et al.*, 1998*b*; El-Assal *et al.*, 2001). This is consistent with an indirect effect of delayed flowering on longevity, for example, later-flowering

lines might have shorter post-bolting longevity because of longer photoperiods, slightly higher temperatures, etc., during their reproductive growth under the greenhouse conditions of the present experiments. However, a direct effect (independent of flowering time) of *CRY2*, or other genes in the same region, cannot be ruled out. It was found that the BF.134C-Col marker locus exerts an epistatic effect over pbl-1LN on post-bolting rosette longevity of non-fertilized plants, but there was no epistatic effect under high nutrient conditions. Moreover, no epistatic interaction of these two regions affecting time to flowering was detected (data not shown). This suggests that the effects of pbl-1LN on longevity might be independent of time of flowering.

Irrespective of the mechanistic basis for the inverse relationship between time to flowering and post-bolting rosette longevity, this suggests that earlier-flowering lines supported reproductive development mostly with currently fixed photosynthates, whereas later-flowering lines might have relied heavily on C and nutrient accumulation during a protracted vegetative period, and then redistribution from vegetative structures to developing siliques. This is similar to the behaviour of other Brassicaceae species where pod growth and development depend strongly on photosynthates redistributed from the senescing rosette to inflorescences (Biswas and Mandal, 1987).

A complex network

The analysis of RILs derived from a cross between the *Arabidopsis* ecotypes L*er* and Cvi reveal a number of QTL with significant effects on leaf longevity, and epistatic interactions between QTL that affected longevity in one environment. Likewise, the analysis of QTL involved in the expression of the 'stay green' trait in sorghum and wheat (Crasta *et al.*, 1999; Kebede *et al.*, 2001; Haussman *et al.*, 2002; Sanchez *et al.*, 2002; Verma *et al.*, 2004) uncovered several genomic regions with significant effects extending leaf area duration. It is assumed that the QTL identified in this paper represent a small number of the genes whose allelic variation may affect leaf longevity, and that examination of RILs derived from crosses between other ecotypes of *Arabidopsis* will help to pinpoint other genomic regions with significant effects on longevity. It is particularly interesting that even contrasting ecotypes, such as L*er* and Cvi used in this work, contain seemingly balanced sets of alleles extending or reducing longevity. If this is representative of natural populations of other species, there may be a large unexploited natural genetic variation that might be used to extend leaf area duration in crops if the appropriate alleles from different sources are combined together in improved varieties.

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