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RESEARCH PAPER

QTLs for shelf life in lettuce co-locate with those for leaf biophysical properties but not with those for leaf developmental traits

Fang Z. Zhang [1,*], Carol Wagstaff [1,†], Anne M. Rae [1,‡], Arinder K. Sihota [1], C. William Keevil [1], Steve D. Rothwell [2], Graham J. J. Clarkson [2], Richard W. Michelmore [3], María José Truco [3], Mark S. Dixon [1] and Gail Taylor [1,§]

[1] School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton S016 7PX, UK
[2] Vitacress Salads Ltd, Lower Link Farm, St Mary Bourne, Andover SP11 6DB, UK
[3] Genome Center and Plant Sciences Department, University of California, Davis, CA 95616, USA

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Abstract

Developmental and biophysical leaf characteristics that influence post-harvest shelf life in lettuce, an important leafy crop, have been examined. The traits were studied using 60 informative F_9 recombinant inbred lines (RILs) derived from a cross between cultivated lettuce (***Lactuca sativa*** cv. Salinas) and wild lettuce (***L. serriola*** acc. UC96US23). Quantitative trait loci (QTLs) for shelf life co-located most closely with those for leaf biophysical properties such as plasticity, elasticity, and breakstrength, suggesting that these are appropriate targets for molecular breeding for improved shelf life. Significant correlations were found between shelf life and leaf size, leaf weight, leaf chlorophyll content, leaf stomatal index, and epidermal cell number per leaf, indicating that these pre-harvest leaf development traits confer post-harvest properties. By studying the population in two contrasting environments in northern and southern Europe, the genotype by environment interaction effects of the QTLs relevant to leaf development and shelf life were assessed. In total, 107 QTLs, distributed on all nine linkage groups, were detected from the 29 traits. Only five QTLs were common in both environments. Several areas where many QTLs co-located (hotspots) on the genome were identified, with relatively little overlap between developmental hotspots and those relating to shelf life. However, QTLs for leaf biophysical properties (breakstrength, plasticity, and elasticity) and cell area correlated well with shelf life, confirming that the ideal ideotype lettuce should have small cells with strong cell walls. The identification of QTLs for leaf development, strength, and longevity will lead to a better understanding of processability at a genetic and cellular level, and allow the improvement of salad leaf quality through marker-assisted breeding.

Key words: Biophysical, biomechanical properties, leaf development, lettuce, microbiology, post-harvest, QTLs, shelf life.

Introduction

Pre-packed baby salads, consisting of lettuce, beets, herbs, and spinach, have become a popular and profitable product due to public demand for healthy and convenient food, with a US annual market value in excess of US$2 billion, but the commercial value of these crops is affected

* Present address: Centre for Advanced Studies, Cardiff University, 43…45 Park Place, Cardiff CF10 3BB, UK.
† Present address: School of Food Biosciences, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK.
‡ Present address: School of Biosciences, University of Nottingham, Sutton Bonnington Campus, Loughborough LE12 5RD, UK.
§ To whom correspondence should be addressed. E-mail: g.taylor@soton.ac.uk

Abbreviations: AGR, absolute growth rate; CHL, total chlorophyll; DW, dry weight; DWP, percentage dry weight; E, elasticity; ECA, epidermal cell area; ECN, epidermal cell number; EST, expressed sequence tag; FW, fresh weight; LA, leaf area; LG, linkage group (chromosome); LOD, logarithm of odds; ML, maximum load; OSM, osmolality; P, plasticity; P+E, total deformation potential; QTL, quantitative trait locus; RIL, recombinant inbred line; RGR, relative growth rate; SLA, specific leaf area; SD, stomatal density; SI, stomatal index; SL_H, shelf life from day of harvest; XTH, xyloglucan endotransglycosylase/hydrolase.

by a relatively short shelf life. There is a need to extend shelf life, but understanding the genetic basis of post-harvest shelf life in leafy crops is limited, although likely to be linked to pre-harvest leaf traits. Recent research has suggested that the 'ideal' leaf for processability and shelf life is likely to be characterized by small cells, with favourable water relations (high solute potential) and limited cell wall extensibility and loosening. Other favourable traits include increased leaf thickness and a waxy cuticle (Clarkson *et al*., 2003). Understanding of the genetic determinants of such traits in a leafy crop such as lettuce is extremely limited, despite the economic value of this and other leafy salads. However, many of these characteristics are tractable in model plants such as *Arabidopsis* (Kessler and Sinha, 2004) and it is now possible to utilize information from model systems linked to emerging genomic resources in crops to develop informed molecular plant improvement programmes (Zamir, 2001).

Quantitative trait locus (QTL) analysis is a useful tool in such an approach, not only providing DNA markers linked to agronomic traits, but also for elucidating fundamental mechanisms of genetic control of leaf growth (Asins, 2002; El-Lithy *et al*., 2004). Clear progress has been achieved using QTL mapping to improve several crop agronomic traits, such as rice yield (Xing *et al*., 2002), tomato size and quality (Fridman *et al*., 2002; Frary *et al*., 2000), and bean disease resistance (Kelly *et al*., 2003). There have been only three QTL studies in lettuce, reporting the improvement of root water use efficiency (Johnson *et al*, 2000), seed traits (Argyris *et al*., 2005), and disease resistance (Jeuken and Lindhout, 2002) but none has considered leaf development and post-harvest traits. Recently, lettuce backcross inbred lines have been developed for exploration of the *Lactuca saligna* (wild lettuce) germplasm (Jeuken and Lindhout, 2004).

Cell division and expansion both contribute to final leaf size and shape (Wang *et al*., 2000; Dengler and Kang, 2001; Wyrzykowska *et al*., 2002; Taylor *et al*., 2003; Tsukaya, 2003), providing targets for future manipulation and breeding. Leaf cell expansion is determined at the primary cell wall by loosening and reassembly (Cosgrove *et al*., 2002), and this is driven primarily by internal osmotic pressure generated by water uptake. Expansion also depends on cell wall composition and the degree of association between its different components. The plant cell wall is an important structure, providing essential mechanical strength and rigidity, and protecting against pathogens and dehydration (Cosgrove, 2001). The cell wall has two conflicting characteristics: tensile strength and stability versus structural plasticity. A number of important agronomic properties of plants are influenced by the cell walls, for example nutrient absorption and insect resistance. Hazen *et al*. (2003) reported QTLs for sugar composition of the cell walls in maize. They identified a few candidate genes involved in the essential process of cell wall biosynthesis. It was suggested that xyloglucan endotransgluscosylases/hydrolases (XTHs) serve important roles in both assembly and loosening of the cell wall, together enabling long-term plant cell expansion with minimal loss of wall strength (Thompson and Fry, 2001). Investigating the role of the cell wall is likely to lead to identification of the candidate genes involved in the leaf processability. Similarly, the plant cell cycle may also provide further candidate genes. To date, the coordination of cell division and cell expansion during the growth process is still unclear, but several control points in the plant cell cycle have been shown to have an effect on leaf size (Wang *et al*., 2000).

Substantial genomic resources are now available for lettuce, including >68 197 expressed sequence tags (ESTs), providing a unigene set of 22 185 (http://cgpdb.ucdavis.edu/), but these have yet to be employed to improve our understanding of leaf growth, development, and crop improvement. Recently, a new genetic linkage map has been developed based on the mapping population used in this study. The full mapping population includes 130 F_9 recombinant inbred lines (RILs), derived from a cross between cultivated lettuce (*L. sativa* cv. Salinas) and wild lettuce (*L. serriola* acc. UC96US23). The 60 most informative lines (containing the most recombination events) were used in this study. One advantage of such a genetic resource is the opportunity it provides to examine genotype by environment interactions (G×E)—crucial for any programme of plant improvement, where robust QTLs, irrespective of environment, provide a starting point for candidate gene discovery and testing (Maloof, 2003).

The aim of this study is to develop a new 'ideal ideotype' lettuce with extended shelf life to meet the growing market niche. This study is the first approach to identify QTLs for leaf shelf life (processability) and discuss the possible candidate genes involved in processability, and thus provides the first scientific basis on which future crop improvement strategies may be developed. Here (i) evidence is presented of QTLs for pre-harvest leaf development traits and their relationship to post-harvest shelf life; and (ii) the G×E is assessed by comparing QTL discovery at two contrasting field sites, one in northern, temperate, Europe and the other in southern, Mediterranean, Europe.

Materials and methods

Plant material and field sites

The mapping population was derived from a cross between cultivated lettuce (*L. sativa* cv. Salinas) and wild lettuce (*L. serriola* acc. UC96US23). From 113 F_9 RILs, 60 highly informative recombinant lines were selected from the genetic map development to be used in this study using MapPop (Vision *et al*. 2000) and GenoPlayer (http://compgenomics.ucdavis.edu/genoplayer/). This provided a population that had nearly as many recombination breakpoints and was therefore as informative as a population of ~90 RILs. Two contrasting field sites were used in this study. The

Portugal site was at Boavista farm, near Odemira (latitude 37°36′N, longitude −8°38′N), while the UK site was at Pinglestone Farm, near Winchester (latitude 51°6′N, longitude 1°10′N). The climate during the growing season of each crop was similar in Portugal (average minimum–maximum temperature during the growing season: 6–23 °C, average precipitation: 36–53 mm) to that in the UK (temperature 7–22 °C, precipitation: 41–56 mm) (http://weather.co.uk/). The field trials were designed with three blocks. In each block, three replicates of 62 lines (60 F_9 RILs and two parents) were randomized using the statistical software package Minitab 13.0 for Windows (Minitab Inc., Philadelphia, PA, USA). There were four plants of the same line in each replicate plot, with 10 cm between each plant. Two rows of Cos lettuce were planted around each block to avoid 'edge effects'. In each field trial, an average of six replicates was available following germination and establishment for each of the traits measured. The field trials were in commercial farms with standard industry maintenance.

Leaf traits data recording

The plants in two sites were harvested at similar maturity, 7 weeks after planting. In the Portugal field trial, eight leaves, starting from the youngest leaf which was big enough to take two discs of 10 mm diameter, were sampled and labelled in accordance with the leaf development age from 1 (youngest) to 8 (oldest) in series. In the UK field trial, only leaves 3, 6, 7, and 8 were sampled for further measurements.

Leaf area and growth rate

Images of labelled leaves 1–8 in the Portugal trial were taken using a digital camera (Nikon Coolpix 5000), against a white background and linear scale bar. In the UK trial, images of leaves 3, 6, 7, and 8 were obtained using the same procedure. During the growing period in the UK field trial, at ∼5 weeks after planting, an additional set of digital images from replicate plants was taken on labelled leaves twice, with a 4 d gap. Pairs of leaf images were used to determine absolute growth rates (AGRs) ($mm^2\ h^{-1}$) and relate.113TD.434TD[67re06ar55(1T6768190-TD[403008.pled)-31269i(1.107d/T1_Td[(of)-3/f)-3/fof2hof2hn

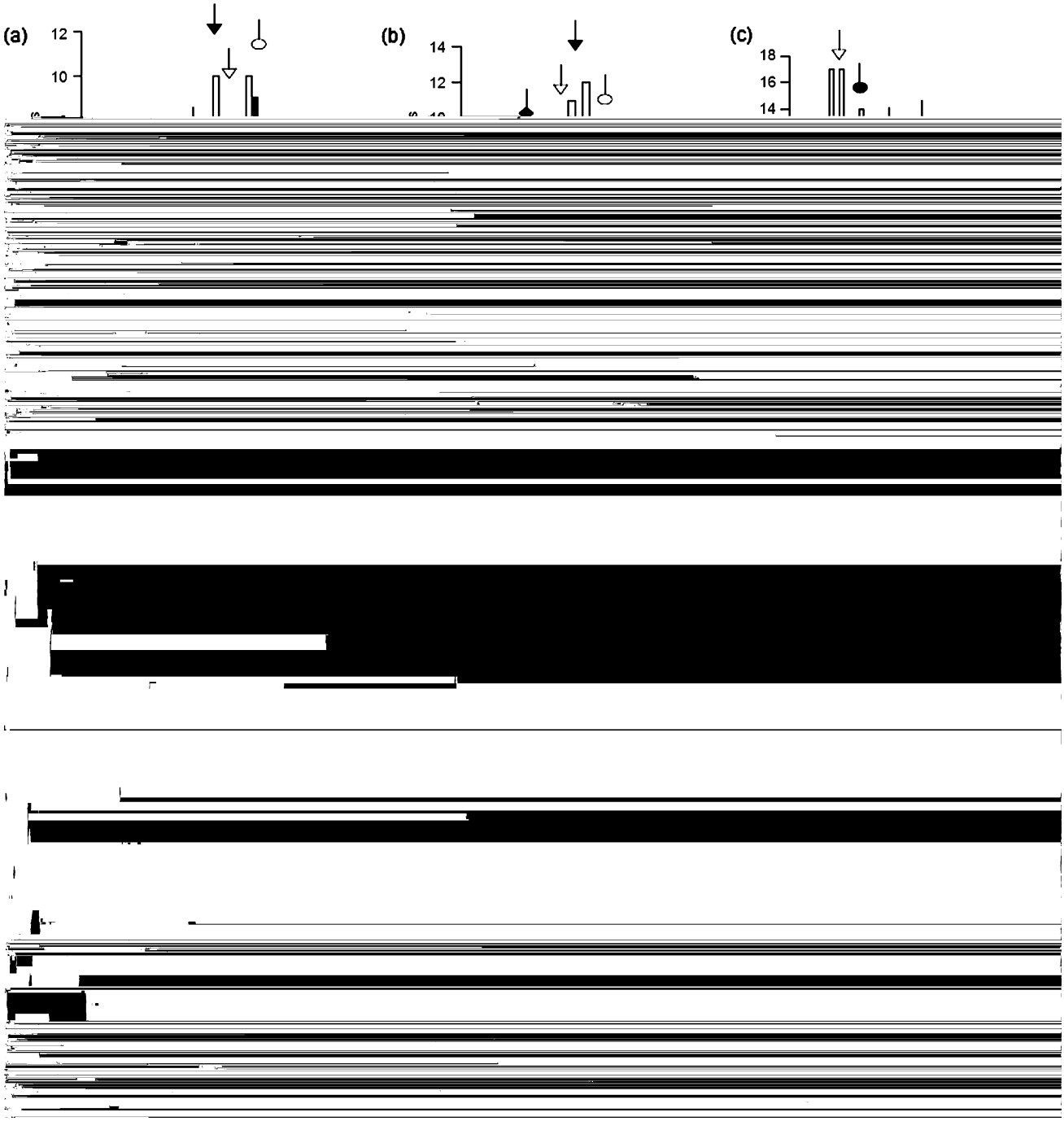

Fig. 1. Distributions of selected traits in the RIL mapping population: (a) Mean leaf area of leaves 3, 6, 7, and 8, (b) Leaf 7 fresh weight, (c) leaf 7 dry weight as a percentage of fresh weight, (d) leaf 7 chlorophyll content, (e) leaf 8 epidermal cell area, (f) leaf 8 cell number per leaf, (g) leaf 8 stomatal density, (h) leaf 8 stomatal index, (i) cell sap osmolality, (j) shelf life, (k) maximum load, (l) plasticity+elasticity, (m) elasticity, and (n) plasticity. The field sites are indicated as either Portugal (filled bar) or the UK (open bar). The mean values of the parents *L. serriola* and *L. sativa*, and of the RILs are indicated by arrows.

poor in previous trials based on visual characteristics such as bruising and waterlogging of the leaf tissue (Fig. 4a). Both parental lines scored well and appeared to last as well as the three good lines. However, membrane leakage assessed by conductivity measurements indicated that the wild parent *L. serriola* had less permeable membranes than *L. sativa* at day 10. This may be due to the growth rate of *L. serriola* being slower than that of *L. sativa*, leading to smaller, more robust leaves at the time of harvest, and therefore a reduced tendency towards membrane leakage. Of the RILs classified as poor, two (19 and 89) had higher conductivity than all the good

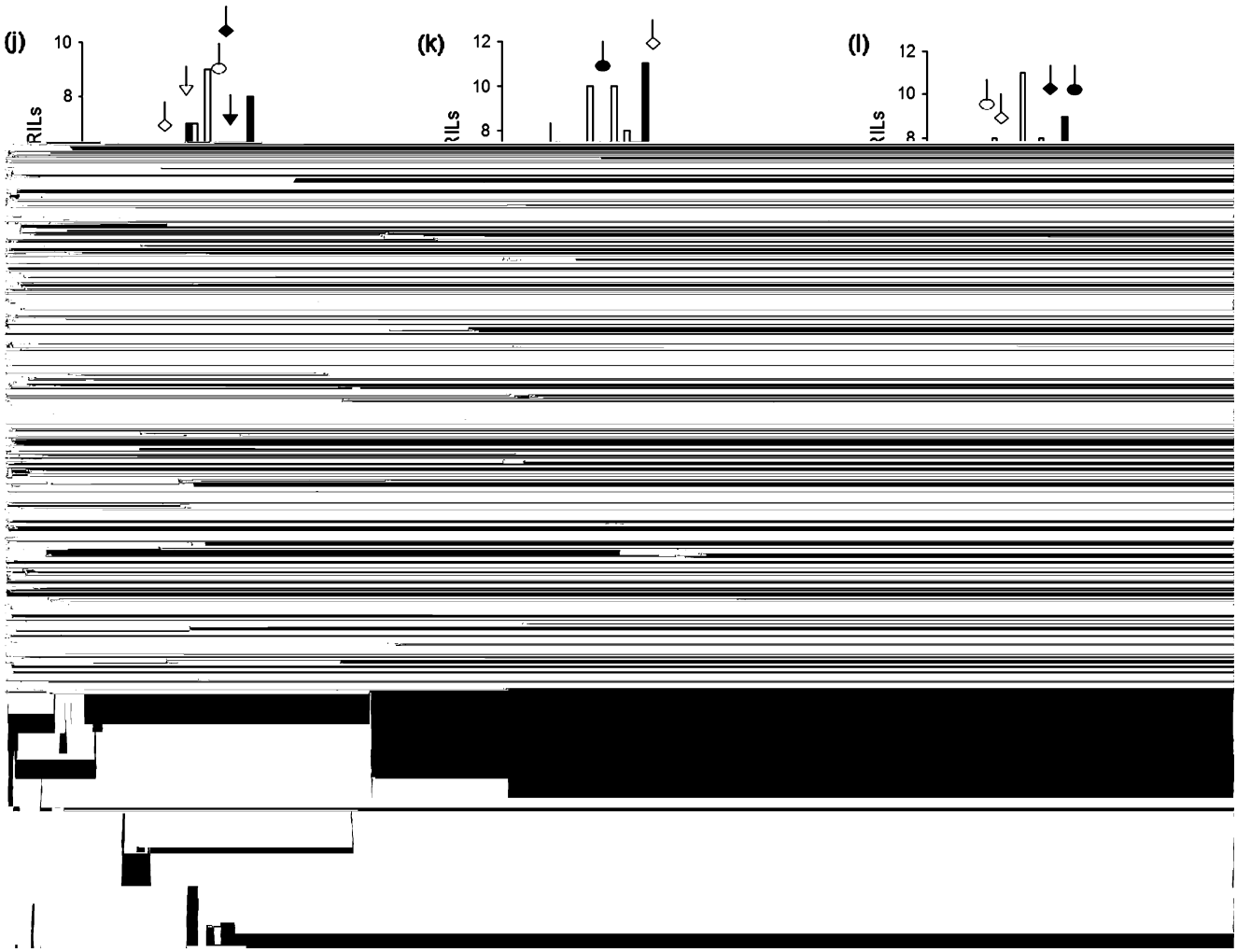

Fig. 1. (*Continued*).

lines, but RIL 32 had very low leakage, despite its poor appearance (Fig. 4b). Examination of coliform units on each RIL showed good correlation with qualitative measurements of appearance, and indicates that bacterial colonization may be a significant contributor to poor shelf life. All of the poor lines showed approximately double the amount of coliform units at day 10 compared with the good lines (Fig. 4c). Prior measurements indicated that coliform units on a leaf line did not change significantly during storage at 4 °C (data not shown); thus, the counts indicated the amount of bacterial load that persisted after processing. Differences between lines may be due either to different levels of colonization in the field, perhaps variable depending on the secondary products produced by each leaf, or to the leaf morphology (e.g. convolutions of the epidermal surface), making the washing process more or less efficient at removing bacteria, or to an interaction of the two factors.

Correlation among the traits

Correlations between the traits in both field trials were calculated using Pearson's correlation coefficient analysis (Table 2). There is a consistent correlation between most pairs of traits in the two field trials. For example, LA had a highly significant positive phenotypic correlation with FW (0.87; 0.85, Portugal and the UK respectively), DW (0.91; 0.86), and ECN (0.61; 0.69), while LA had a negative phenotypic correlation with SI (−0.27; −0.29). FW had the highest positive correlation with DW at both trials (0.90; 0.93), and ECA had the highest negative correlation with ECN (−0.69; −0.70) and SD (−0.65, −0.79) in both trials. Surprisingly, in the UK field trial, SL showed a highly significant positive correlation with LA (0.41), FW (0.36), DW (0.36) (P <0.01), ECN (0.28), and ML (0.30) (P <0.05), and a significant negative correlation with E (−0.38) (P <0.01), CHL (−0.29), SI (−0.27), P (−0.29), and P+E (−0.34) (P <0.05). The only

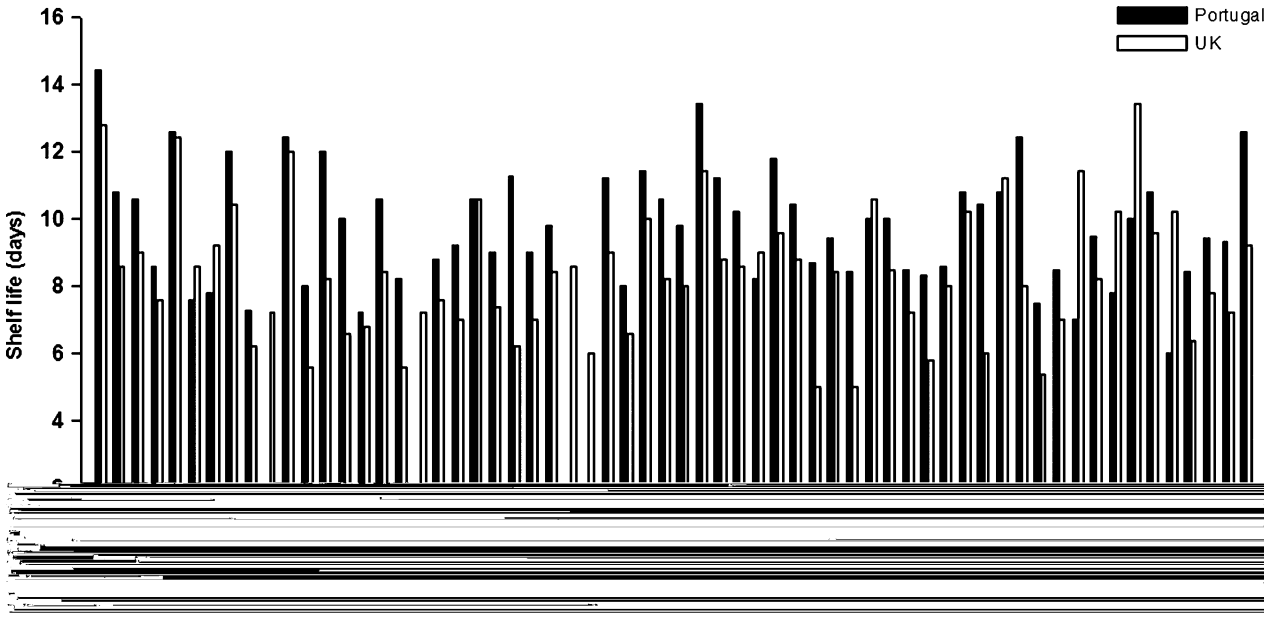

Fig. 2. Shelf life of the RIL mapping population in two field trials: Portugal (filled bar) and the UK (open bar). Five replicates of most lines were kept at 7 °C in a fridge, and shelf life was determined through a visual assessment. When breakdown, bruising, or damage was seen in the pack, the bag was rejected. SER, *L. Serriola*; SAT, *L. Satira*.

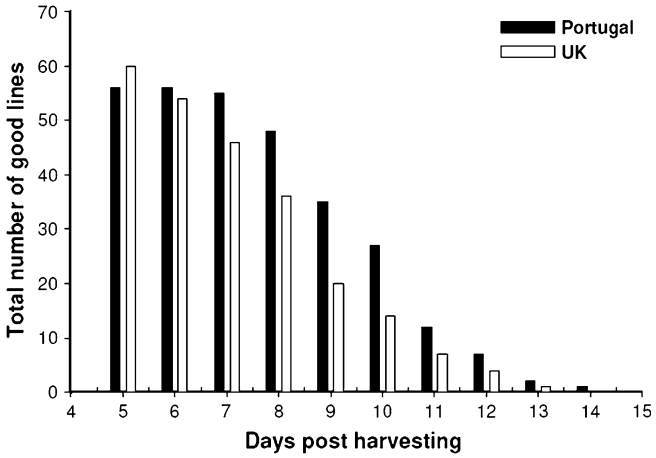

Fig. 3. Post-harvest rejection of the RIL mapping population in two field trials: Portugal (filled bar) and the UK (open bar). The total number of lines in good condition was assessed each day during the shelf life period (n=5).

significant correlation between SL and developmental traits detected in the Portugal trial was for OSM (0.27), but this was not detected in the UK. However, SL also showed a significant negative correlation with elasticity (E; −0.26) in the Portugal trial, correlating with the relationship found in the UK between these two traits.

QTL analysis

A genetic map containing 1334 AFLP (amplified fragment length polymorphism) and SSR (simple sequence repeat) markers was used for composite interval mapping analysis [http://cgpdb.ucdavis.edu/database/supplemental_data (MAP2_JMR3)]. All the traits, including the different leaf development stage measurements, were analysed. A total of 107 QTLs with significant effects were detected for the 29 traits, distributed on all nine of the linkage groups (Table 3, Fig. 5, Supplementary Fig. S5 available at *JXB* online). Individual QTLs accounted for 10.83–66.34% of the phenotypic variation in this population. Among these detected QTLs, 61 significant QTLs were identified for the leaf traits measured in the Portugal trial (filled bars in Fig. 5 and Supplementary Fig. S5, available at *JXB* online) and 45 for the UK trial (open bars in Fig. 5 and Supplementary Fig. S5, available at *JXB* online). Five QTLs located to overlapping regions of the same linkage groups in both field trials. These common QTLs were for SD on LG1, DWP on LG4 and LG8, shelf life at day 8 (SL_d8) on LG8, and ML on LG4. The total number of QTLs identified in the UK trial was less than in the Portugal trial, perhaps due to fewer leaf developmental stage samples being tested in the UK. Three different developmental stage samples were assessed for most of the traits in Portugal, while only one leaf sample was assessed in the UK. The maximum number of QTLs per trait for each leaf age was four.

Leaf growth and development (Table 3)

QTLs for leaf area from the Portugal trial were identified on seven chromosomes, but strong overlapping QTLs for leaves 2, 3, and 4 were found on LG3 (30.0–34.8 cM,

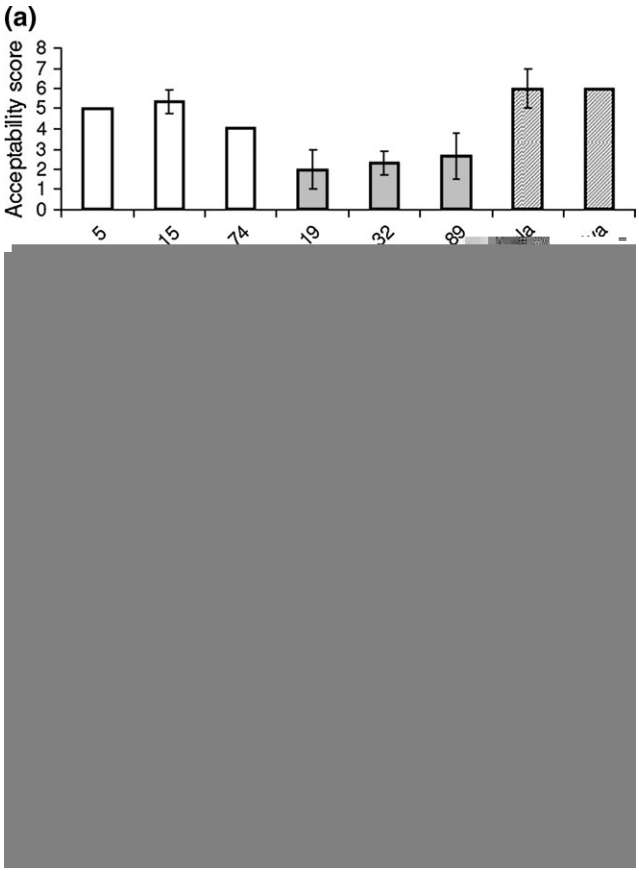

Fig. 4. Quantitative measurements of shelf life from selected RILs 10 d after harvest (7 d after processing). RILs selected as performing well in both previous trials in Portugal and the UK (5, 15, and 74) are indicated by white bars, RILs selected as poor performers in the same trials (19, 32, and 89) as grey bars, and the bars relating to the parental lines are hatched. (a) Acceptability score (out of 10) based on visual assessment (n=3). (b) Conductivity measurements (an an indicator of membrane leakage) (n=6). (c) Bacterial counts (coliform units per g FW) (n=4). Error bars are the SEM.

1A02-270–LK1225) and for leaves 4–8 also on LG3 (82.0–91.8 cM, E45/M49-F-275–LE1011). The increasing allele for leaves 4 and 8 was from *L. serriola*, but from *L. sativa* for leaves 5, 6, and 7. The total phenotypic variance explained by these identified QTLs was between 14% and 21% in young and semi-mature leaves and 29% in mature leaves in the Portugal trial. Only two QTLs were identified for leaf area in the UK trial, one on LG4 (127–138 cM, LE4022) from the *L. serriola* allele for leaves 7 and 8, and another for leaf 8 on LG9 (47.0–48.7 cM, E35/M60-F-157) from the *L. sativa* allele, accounting for 20–25% of the phenotypic variability between them. No QTLs were found for LA from young or semi-mature leaves in the UK trial. AGR and RGR were only measured in the UK trial. A QTL for AGR and one for RGR were both found on LG2 for leaves 3–6 (around markers LE0371 and LE3023, respectively). The QTL for AGR co-located with a QTL for SI, and the QTL for RGR with one for OSM. The QTL for AGR on LG7 also co-located with the first LA measurement taken during development (LAf_UK), ECA in the Portugal trial, and CHL from the UK, spanning a region from 99.1 cM to 111.4 cM (markers LK1513–M6982). The latter two traits were derived from *L. serriola*, the former from *L. sativa*, and accounted for 13–37% of the phenotypic variation.

For FW and DW there were a number of overlapping QTLs. The two traits co-located on LG2 with one for LA (132.4–134.8 cM, E33/M59-F-121–E33/M59-F-226). Again the three traits (FW, DW, and LA) co-located to a QTL on LG3 (82.0–91.8 cM, E45/M49-F-275–LE1011). A QTL for DWP co-located with those for DWP in the Portugal trials on LG4, and also with one for SI (81.8–88.5 cM, E35/M49-F-296–1A04-137), with the increasing alleles coming from *L. serriola* in all cases. The same allele accounted for QTLs for DWP, ECA, and SI on LG6 (123.6–131.1 cM, L2219–LE3085), and a strong overlapping QTL hotspot was identified on LG8 that encompassed the above three traits, plus SLA, ECN, DW, and FW (56.1–70.6 cM, LE1345–1A09-212). With the exception of ECN, DW, and FW, the allele came from *L. serriola* in the remainder, correlating with the allelic origin of the same traits on LG4 and LG6 and accounting for up to 20% of the phenotypic variation.

Leaf biophysical properties (Table 3)

Instron analysis of P, E, and ML (breakstrength) of the leaves revealed several QTLs. One QTL for ML co-located on LG4 in both the Portugal and UK trials (22.2–28.9 cM, LE4021–E33/M59-f-364) and accounted for up to 28% of the phenotypic variation, derived from the *L. sativa* allele in both cases. Another QTL for maximum load in the UK trial co-located with a strong shelf life QTL from the same experiment on LG6. As predicted, QTLs for P+E, representing the total deformation potential of the tissue, co-located with those for either P or E calculated independently, thereby showing which type of stretch the deformation was largely due to. E in both trials co-located with other developmental traits in hotspots on LG7 and LG8, in both cases derived from the *L. sativa* allele. However, QTLs for increased P were attributed to *L. serriola* alleles in the UK trial, but to *L. sativa* in the Portugal trial, and in both cases were clearly associated with hotspots concerned with shelf life on LG5 (21.2–61.0 cM, 1A09-212–LE9015) and LG8 (112.7–124.1 cM, L0248–E35/M49-F-267<N>).

Shelf life (Table 3)

Shelf life QTLs for the Portugal trial were found on LG5 (47.6–61.0 cM, E44/M48-F-085–LE9015) and LG8 (94.4–96.0 cM–E35/M59-F-530). These coincided with QTLs derived from the binary data on days 10 and 12 of shelf life. Additional QTLs were identified for day 9 (LG6) and day 10 (LG2). Chromosome 6 proved to be a hotspot for shelf life data derived from the UK trial,

Table 2. *Pearson's correlation coefficient of trait means of the RIL mapping population in two field trials*

*Correlation is significant at the 0.05 level (two-tailed); **correlation is significant at the 0.01 level; and ***correlation is significant at the 0.001 level.

Field site	Traits[a]	LA	FW	DW	DWP	SLA	CHL	ECA	ECN	SD	SI	OSM	SL	ML	P+E	P	E
Portugal	LA	1															
UK		1															
Portugal	FW	0.87***	1														
UK		0.85***	1														
Portugal	DW	0.91***	0.90***	1													
UK		0.86***	0.93***	1													
Portugal	DWP	−0.16	−0.46***	−0.06	1												
UK		−0.16	−0.41**	−0.07	1												
Portugal	SLA	−0.06	−0.27*	−0.41**	−0.22	1											
UK		0.07	−0.26*	−0.40**	−0.29*	1											
Portugal	CHL	−0.18	−0.14	−0.02	0.26*	−0.35**	1										
UK		−0.36*	−0.02	−0.08	−0.15	−0.41**	1										
Portugal	ECA	0.02	−0.12	0.01	0.32	−0.04	−0.09	1									
UK		−0.07	−0.16	−0.18	0.07	0.21	−0.28*	1									
Portugal	ECN	0.61***	0.62***	0.51***	−0.42**	0.09	−0.20	−0.69***	1								
UK		0.69***	0.67***	0.69***	−0.15	−0.12	−0.09	−0.70***	1								
Portugal	SD	−0.27*	−0.17	−0.28*	−0.18	0.06	0.14	−0.65***	0.32*	1							
UK		−0.16	−0.03	−0.01	0.04	−0.20	0.24	−0.79***	0.48***	1							
Portugal	SI	−0.27*	−0.32*	−0.30*	0.17	−0.02	0.12	0.31*	−0.42**	0.48***	1						
UK		−0.29*	−0.29*	−0.22	0.28*	−0.09	−0.02	0.19	−0.30*	0.41**	1						
Portugal	OSM	−0.18	−0.29*	−0.08	0.58**	−0.27*	0.13	0.43**	−0.38**	−0.23	0.20	1					
UK		−0.22	−0.25*	−0.12*	0.40**	−0.29	0.17	0.17	−0.35**	−0.08	0.23	1					
Portugal	SL	0.14	0.07	0.07	−0.01	0.158	−0.19	0.14	0.11	−0.06	0.03	0.27*	1				
UK		0.41**	0.36**	0.36**	−0.10	0.07	−0.29*	0.04	0.28*	−0.20	−0.27*	−0.09	1				
Portugal	ML	0.02	−0.08	0.09	0.44	−0.25	0.19	0.36	−0.35	−0.40	−0.05	0.11	−0.04	1			
UK		0.19	−0.26*	−0.24	−0.14	0.28*	−0.19	0.30*	0.06	−0.28*	−0.14	−0.09	0.30*	1			
Portugal	P+E	0.18	0.32*	0.14	−0.50***	0.17	−0.21	−0.49***	0.55***	0.27*	−0.33*	−0.47***	−0.23	−0.47***	1		
UK		0.02	0.16	0.18	0.07	−0.27*	0.14	−0.09	−0.24	0.26*	0.33*	0.11	−0.34*	0.10	1		
Portugal	P	0.21	0.34*	0.19	−0.45***	0.11	−0.19	−0.41**	0.50***	0.23	−0.30*	−0.42***	−0.20	−0.48***	0.98***	1	
UK		0.04	0.17	0.19	0.10	−0.22	0.11	−0.07	−0.22	0.25*	0.30*	0.08	−0.29*	0.10	−0.31*	1	
Portugal	E	0.09	0.23	0.03	−0.52***	0.26*	−0.22	−0.56***	0.55***	0.33*	−0.34*	−0.49***	−0.26*	−0.44***	0.91***	0.79***	1
UK		−0.07	0.10	0.14	0.01	−0.33	0.18	−0.11	−0.25*	0.26*	0.34*	0.14	−0.38**	0.08	−0.39**	0.98***	1

[a] Trait abbreviation: LA, mean leaf area of leaves 3, 6, 7, and 8 (mm^2); FW, leaf 7 fresh weight (mg); DW, leaf 7 dry weight (mg); DWP, leaf 7 dry weight as a percentage of fresh weight (%); CHL, leaf 7 chlorophyll content ($\mu g\ mm^{-2}$); ECA, leaf 8 epidermal cell area (μm^2); ECN, leaf 8 epidermal cell number per leaf ($\times 10^6$); SD, leaf 8 stomata density (no. of stomata mm^{-2}); SI, leaf 8 stomata index (%); OSM, leaf 6 cell sap osmolality (mmol kg^{-1}); SL, shelf life period, counted from harvesting to the day before being rejected (d); ML, maximum load of leaf strip before breakage (N); P+E, plasticity+elasticity of leaf strip (% extension); P, plasticity (% extension); E, elasticity (% extension).

Table 3. *QTLs detected by composite interval mapping for all leaf traits assessed in the RIL mapping population in two field trials*

Trait[a]	Position[b] (cM)	Marker[c]	Portugal field trial				UK field trial			
			Leaf no.[d]	LOD[e]	Additive[f]	Variance[g] (%)	Leaf no.	LOD	Additive	Variance (%)
LA	LG1: 98.6–102.3	LK1072	3	5.65	130.03	21.14				
	LG2: 132.9–134.4	E33/M59-F-226	7	4.68	469.04	15.54				
	LG3: 3.7–4.4	LM0075	4	3.74	−171.73	10.83				
	LG3: 30.0–31.3	1A02-270	2,3,4	4.19	119.36	14.62				
	LG3: 34.5–34.8	LK1225	4	3.77	−224.66	21.95				
	LG3: 82.0–84.0	E45/M49-F-275	4,8	5.21	−247.68	21.31				
	LG3: 83.3–91.8	LE1011	5,6,7	7.41	362.33	29.73				
	LG4: 127–138	LE4022					7,8	5.49	−630.39	25.53
	LG7: 78.3–84.1	E44/M49-F-328	1	4.05	33.39	20.77				
	LG8: 73.7–74.6	LE0460	8	4.02	451.26	13.01				
	LG9: 47.0–48.7	E35/M60-F-157					8	5.89	677.96	20.69
	LG9: 75.5–76.4	LE3171	7	5.55	471.59	16.26				
LAf_UK	LG4: 90.6–91.3	E35/M60-F-286						4.46	−210.58	16.48
	LG7: 99.2–102.4	LE0463						8.16	307.24	36.95
LAo_UK	LG7: 93.5–96.8	LK1513						6.31	138.08	29.06
AGR	LG2: 64.5–67.7	LE0371					3–6	3.88	1.10	13.34
	LG7: 99.1–104.0	LK1513						5.97	1.46	22.81
RGR	LG2: 96.5–96.9	LE3023					3–6	3.22	−1.08	21.80
FW	LG2: 132.4–132.8	E33/M59-F-121	4	4.66	−0.1	16.54				
	LG3: 82.9–90.3	LE1011	4,7	6.75	−0.14	24.99				
	LG4: 131.2–141.4	1A09-349<N>					7	4.10	−0.21	16.57
	LG5: 131.2–133.6	1A01-203					7	5.06	0.24	21.45
	LG7: 68.4–70.9	E35/M49-F-590	1	5.33	0.01	24.56				
	LG8: 68.0–69.2	1A20-148	4,7	7.06	0.12	24.49				
DW	LG2: 132.6–134.8	LK0017	7	4.50	−0.03	17.02				
	LG3: 82.8–96.0	LE1011	4,7	8.10	−0.04	31.25				
	LG4: 82.8–96.0	1A15-333	4	5.04	−0.69	21.26				
	LG5: 131.6–138.6	LE0369					7	4.48	0.02	18.50
	LG7: 78.0–84.0	E44/M48-F-328	1	4.33	0.00	19.75				
	LG8: 68.0–68.9	1A20-148					7	4.71	0.02	16.94
DWP	LG1: 92.0–95.1	LE0488	7	3.99	0.46	11.04				
	LG3: 32.6–33.1	1A20-141					7	5.46	−0.33	16.70
	LG4: 100.5–103.0	1A15-333	4	5.04	−0.68	21.26				
	LG4: 81.8–82.8	E35/M49-F-296					7	4.61	−0.30	12.89
	LG4: 85.0–86.8	1A04-137	7	6.48	−0.66	19.99				
	LG6: 123.6–128.6	L2219	7	3.82	−0.44	10.89				
	LG8: 56.1–58.0	LE1345	1	4.06	−0.91	16.06				
	LG8: 69.6–70.2	LE0460	7	5.98	−0.54	19.27				
	LG9: 39.4–40.0	E54/M48-F-307					7	4.11	0.29	12.29
SLA	LG1: 52.2–55.9	1A01-121	4	4.59	−1.17	21.08				
	LG2: 8.1–18.2	M1282	1	4.30	−1.46	17.79				
	LG8: 58.4–59.6	1A02-132<N>					7	5.16	−1.44	19.31
	LG8: 86.2–87.7	E33/M59-F-216	1	4.40	1.81	19.12				
CHL	LG3: 34.5–34.8	LK1225					7	4.79	0.01	20.32
	LG4: 57.4–58.2	Sf2979					7	4.64	0.01	12.56
	LG7: 108.6–111.4	M6982					7	4.85	−0.01	14.86
	LG9: 9.9–12.6	LK1355	1	3.83	0.021	19.02				
ECA	LG1: 84.3–85.5	E33/M59-F-391					8	5.65	248.96	18.14
	LG3: 34.7–34.8	E33/M59-F-208	2	4.30	−60.25	17.63				
	LG4: 105.9–106.7	L1371	5	4.93	−74.20	15.76				
	LG5: 21.2–26.4	1A09-212	8	4.69	−94.45	16.66				
	LG5: 44.5–47.4	LK1046					8	4.28	−203.66	12.37
	LG6: 125.7–131.9	1A09-410<N>	2	4.66	−61.71	15.63				
	LG7: 10.6–20.6	E38/M54-F-304	5	3.96	60.00	10.63				
	LG7: 100.9–110	E35/M60-F-114	2	3.87	−51.55	12.56				
	LG8: 22.6–23.7	LE0040B					8	5.35	−255.93	15.74
	LG8: 66.2–69.3	E33/M59-F-342	5	4.08	−72.72	14.89				
ECN	LG1: 20.4–23.4	LE7032					8	3.77	0.23	11.89
	LG1: 88.5–90.0	1A17-253<N>	2	6.42	−0.49	21.68				
	LG4: 5.3–10.5	1A08-254					8	5.86	−0.30	20.90
	LG7: 87.8–92.0	M2395					8	6.23	0.33	24.71
	LG8: 66.7–69.4	1A20-148	2,8	9.01	0.71	40.66				
	LG8: 70–70.6	1A09-212	5	5.80	0.54	21.45				
SD	LG1: 82.7–85.2	1A17-253< N >					8	7.63	−9.47	25.09
	LG1: 89.6–94.4	E33/M59-F-391	8	6.46	−22.16	21.22				

Table 3. (*Continued*)

Trait[a]	Position[b] (cM)	Marker[c]	Portugal field trial				UK field trial			
			Leaf no.[d]	LOD[e]	Additive[f]	Variance[g] (%)	Leaf no.	LOD	Additive	Variance (%)
	LG4: 0.0–2.5	1A15-253<N>	5	4.83	−23.13	13.91				
	LG7:0.0–1.1	E44/M49-F-248	5	5.68	−25.37	16.71				
	LG8: 9.6–14.8	LE9214	5,8	6.37	20.91	19.33	8	5.52	7.69	17.40
SI	LG1: 68.1–70.3	1A20-191					8	5.82	−0.74	18.52
	LG2: 71.3–74.1	M4833					8	3.94	0.62	14.39
	LG4: 85.2–88.5	1A04-137	2	6.18	−1.88	23.10				
	LG6: 117.6–119.9	E44/M49-F-081	8	4.21	−0.96	17.03				
	LG6: 128.1–131.1	LE3085	2	4.33	−1.44	14.85				
	LG7: 33.9–39.6	E44/M48-F-134	5	4.60	−1.21	17.19				
	LG8: 66.1–66.5	E45/M48-F-490	2	5.29	−1.68	20.08				
OSM	LG2: 97.3–97.8	E35/M59-F-200						7.62	15.52	37.85
SL_P	LG5: 47.6–50.3	E44/M48-F-085		5.49	−0.84	17.93				
	LG6: 84.6–86.5	E44/M49-F-246						6.34	0.98	22.36
	LG8: 94.1–96.0	E35/M59-F-530		4.92	0.78	19.52				
SL_d8	LG5: 34.4–39.3	E44/M48-F-292		5.43	−0.20	22.91				
	LG6:103.8–107.8	LE3092						7.25	0.28	30.19
	LG8: 104.3–106.1	LK1388						4.62	0.25	20.63
	LG8: 121.6–124.1	E35/M49-F-267<N>		4.39	−0.17	19.05				
SL_d9	LG6: 40.0–42.4	1A12-515<N>		4.86	0.21	17.00				
	LG6: 84.8–86.6	E44/M49-F-246						7.97	0.26	29.84
	LG8: 112.7–115.1	LE0248						5.90	0.25	21.91
SL_d10	LG2: 9.6–16.6	M1282		4.94	0.22	16.10				
	LG6:84.8–86.6	E44/M49-F-246						4.51	0.18	17.53
	LG8: 94.1–96.0	E35/M59-F-530		4.66	0.24	16.00				
SL_d11	LG1: 51.8–54.0	LE0243						7.16	0.17	25.62
	LG6: 6.9–10.4	E51/M49-F-206						6.53	0.15	21.25
	LG6: 85.8–87.8	E44/M49-F-246						4.22	0.1251	66.34
SL_d12	LG5: 52.5–61.0	LE9015		6.23	−0.20	28.40				
	LG5: 93.6–95.6	1A18-239<N>						6.26	−0.13	20.15
ML	LG4: 22.2–22.9	LE4021						4.02	0.07	17.46
	LG4: 26.1–28.9	E33/M59-F-364		6.21	0.10	27.58				
	LG5: 30.8–32.1	E35/M59-F-210						3.80	−0.06	14.03
	LG6: 92.1–94.6	LE3108						3.71	−0.06	12.93
	LG8: 0.6–3.5	1A15-569<N>		5.68	−0.09	18.51				
P+E	LG8: 66.7–68.7	E33/M59-F-342		4.12	1.08	16.69				
	LG8: 116.6–119.7	LK1471						4.46	−0.80	19.26
P	LG5: 41.2–42.2	LE1076		4.32	0.77	18.57				
	LG8: 116.6–118.7	LK1471						4.29	−0.52	17.79
E	LG2: 84.2–89.9	E35/M60-F-354		3.87	−0.38	15.32				
	LG7: 97.8–100.1	LE1120						5.34	0.34	25.61
	LG8: 66.7–69.5	E33/M59-F-342		3.99	0.40	17.61				

[a] Trait abbreviation: LA, leaf area at maturity (mm^2); LAf_UK, first leaf area measurement during development in the UK trial (mm^2); LAo_UK, second leaf area measurement during development in the UK trial (mm^2); AGR, absolute growth rate ($mm^2\ h^{-1}$); RGR, relative growth rate ($\times 10^{-3}$ $mm^2\ mm^{-2}\ h^{-1}$); FW, leaf fresh weight (mg); DW, leaf dry weight (mg); DWP, leaf dry weight as a percentage of fresh weight (%); CHL, chlorophyll content ($\mu g\ mm^{-2}$); ECA, epidermal cell area (μm^2); ECN, epidermal cell number per leaf ($\times 10^6$); SLA, specific leaf area ($\mu g/mm^2$); SD, stomatal density (no. of stomata mm^{-2}); SI, stomatal index (%); OSM, cell sap osmolality (mmol kg^{-1}); SL-P, shelf life period, counted from processing to the day before being rejected (days). The day number after trait SL indicated the days after harvesting where the QTL for shelf life was detected; ML, maximum load of leaf strip before breakage (N); P+E, plasticity+elasticity of leaf strip (% extension); P, plasticity (% extension); E, elasticity (% extension).

[b] Position indicated by the linkage group number, and the significant QTL interval over the threshold estimated by permutation analysis of each trait using 1000 iterations.

[c] Markers that are the nearest marker to the QTL.

[d] Leaf no. indicated at which leaf development stage the QTL was detected. NA, not assessed in the field trial for the individual trait.

[e] LOD: log of the odds score. To convert LR to LOD values, LOD=0.217 LR.

[f] Additive effect indicates which parental allele causes an increase in the trait value. Positive values indicate that the cultivated (*L. sativa*) allele increases trait values, and negative values indicate that the wild-type (*L. serriola*) allele increases trait values.

[g] Variance indicated the percentage of phenotypic variance in the mapping population explained by the detected QTL.

with overall shelf life plus binary data for days 9–11 accounted for by a region between 84.6 cM and 87.8 cM (E44/M49-F-246 the closest marker in all cases) derived from the *L. sativa* allele. This hotspot was also associated with ML of the tissue in the same trial. QTLs for SL_d8 were found on LG8 in both the Portugal and UK trials, although there was a gap of ~15 cM between the two QTLs. These QTLs were located slightly below the hotspot for developmental traits discussed above, but co-located with one associated with leaf P. QTLs for SL_d12

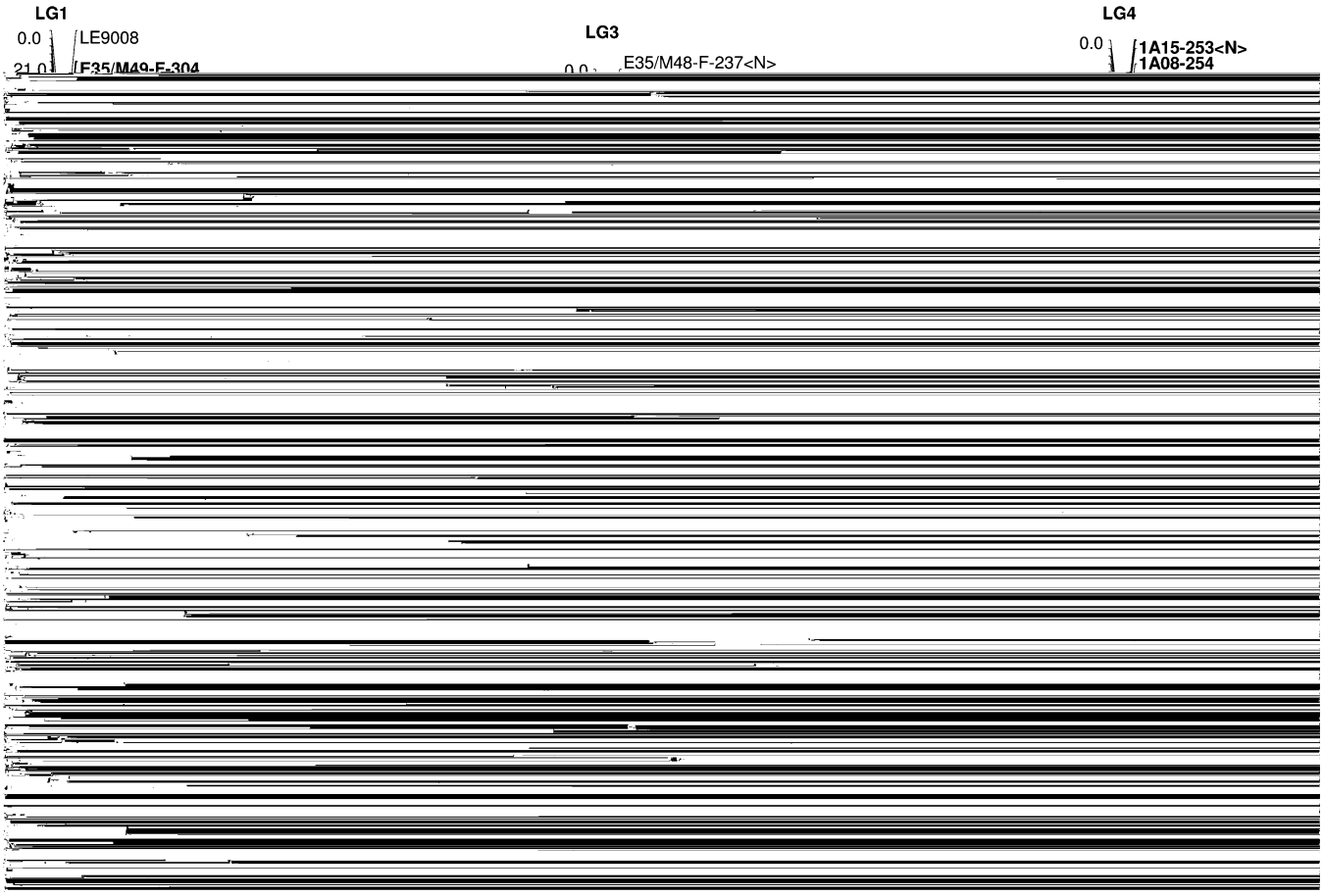

Fig. 5. QTL distributions on the molecular linkage map of the RIL mapping population based on composite interval mapping. Map positions are given in cM, listed on the right of each linkage group. A marker in bold indicates the nearest marker to the QTL for the trait of interest. For trait abbreviations, see Table 3. The leaf number is given after each trait where trait is dependent on development stage. The day number given after the trait SL indicates the days after harvesting where the QTL for shelf life was detected. The length of the bars indicates the LOD interval over the significant threshold for each QTL, and the line extensions of each bar indicate a one LOD lower than significant LOD support confidence interval for each QTL threshold. Filled bars represent the QTLs detected in the Portugal trial, open bars represents the QTLs detected in the UK trial, and

also located to the same chromosome (LG5) in both trials, with a 32 cM interval between them, but both were derived from the *L. serriola* allele and accounted for 20–28% of the phenotypic variation. Hotspots for shelf life thus show correlation with biophysical traits, but they locate to different regions of the genome due to the effect of the climate in which they are grown.

QTL hotspots

From the above analysis of multiple phenotypic traits associated with leaf development and post-harvest shelf life performance, several regions have been identified where there are overlapping QTLs, known as hotspots (Fig. 5). There is one such region on LG1 accounting for developmental traits spanning 19 cM and another 4 cM QTL for shelf life located 16 cM away. LG2 has several small QTLs accounting for developmental traits. There are two major hotspots for developmental traits on LG3 that are both highly discrete, spanning 5 cM and 10 cM, the first one accounting for traits from the Portugal and UK trials. LG8 is another major hotspot for developmental traits from both countries, with several QTLs for shelf life located on the same LG at 15–53 cM distance from the developmental one. QTLs for developmental traits rarely co-located with those for shelf life in large hotspots, although there were small regions on LG1, 2, and 5 with overlapping QTLs. A QTL hotspot for shelf life traits was identified on LG5 that co-located with biophysical properties of plasticity and breakstrength. Leaf biophysical properties and shelf life again co-located in hotspots on LG6 and LG8, in all cases co-locating with QTLs for ECA, indicating that small cell size is a major contributing factor to increased leaf strength.

Discussion

Shelf life and leaf development characteristics

This study is the first to report QTLs for leaf shelf life in lettuce and to link this post-harvest trait to pre-harvest leaf

was measured in the oldest leaves rather than the newly emerged leaves in this lettuce population as it was harvested before the rosette could form a 'head' typical of an iceberg-type lettuce in which light is excluded from the youngest leaves. Measurements suggested that the older leaves were in the process of senescence. Several genetic mutants and potential regulator components have been identified for leaf senescence in *Arabidopsis* (Lim *et al*., 2003), which will assist the future beneficial analysis of the senescence rate and its genetic basis in lettuce.

Clusters of QTLs

The relatively small population size necessitated by the operational constraints of the experiment and the consequent significance level of this study only allow the detection of QTLs of large effect (Kearsey and Farquhar, 1998). Only 60 RILs of the most informative lines were analysed in this study, although there are 130 RILs in the whole population. The fact that much of the variation was left unexplained in this study suggested that there are probably more QTLs with smaller effects that could not be detected. If QTL peaks are situated close to each other and the LOD support intervals overlap, multiple QTLs can be regarded potentially as a single QTL with pleiotropic effects. However, in most studies, each QTL has been counted independently as this does not imply functional or genetic associations between genes on a similar chromosome location (Xu *et al*., 2004). Several clusters of QTLs were detected in this study (Fig. 5), on linkage groups 1, 3, 5, 6, 7, and 8, and each QTL has been counted separately. For example, QTLs for traits of LA, DWP, SLA, ECA, ECN, and SD mapped to a region on LG1 (83–102 cM), while QTLs for traits of LA, DW, CHL, ECA, ECN, and AGR all map to a similar position on LG7 (78–111 cM). Similarly clustered QTLs have been reported in other studies for leaf traits (Rae *et al*., 2004), rice seed traits (Xu *et al*., 2004), and fruit quality traits (Causse *et al*., 2002). The result showing the clustering of QTLs is consistent with the high correlation coefficients among the traits (Table 2). For example, LA, AGR, SLA, FW, and DW are groupings of physiologically associated traits. CHL is presented as total chlorophyll content per mm^2. The larger (LA or SLA) leaves, with greater thickness, usually had higher chlorophyll content. Therefore, it is not surprising that QTLs for CHL were clustered with QTLs for LA, SLA, and DW leaf traits. It is consistent with the report that growth traits (plant area, dry weight, and relative growth rate) co-located at five genomic regions in an *Arabidopsis* RIL population (El-Lithy *et al*., 2004) in addition to the finding of co-location of QTLs for leaf area, specific leaf area, and chlorophyll fluorescence.

This clustering of QTLs has important implications for plant breeding programmes. For example, clustered QTLs on LG1 showed pleiotropic effects on LG1 for leaf area, dry weight (as a percentage of fresh weight), cell area, and cell number. In *L. sativa*, all of the QTLs showed increased values for desirable traits. For this kind of QTL cluster, the selection of the ideal genotype of one QTL region could simultaneously improve several other traits positively. For other QTL clusters, where both desirable and undesirable traits map together, fine mapping and analysis of near-isogenic substitution lines is necessary to determine whether there are multiple QTLs or a single QTL with pleiotropic effects. If the latter is the case, it would be difficult to select for an improved genotype.

Leaf development-specific QTLs

In the Portugal field trial, QTL effects for 10 leaf traits (LA, FW, DW, DWP, SLA, CHL, ECA, ECN, SD, and SI) were analysed in three different developmental stages (young, semi-mature, and mature). None of the 75 QTLs was identified in all three different developmental stage leaves, although young and semi-mature leaves did map QTLs for LA on LG3. QTLs are therefore dependent on leaf development stage, indicating that some loci may have an overall effect on plant growth, while others only specifically regulate certain processes during a specific phase of growth. Similarly, QTLs for growth-related traits at the top of chromosome 3 of *Arabidopsis* were found mainly for the earlier phase (El-Lithy *et al*., 2004). In this study, QTLs for ECN and/or ECA co-located with QTLs for LA in several places on the genome, but rarely for the same leaf developmental stages; however, the co-location of QTL for LA and FW/DW was commonly for the same leaf developmental stage. This suggests that the leaf size at the stage measured was largely changing as a consequence of cell expansion through water uptake. Research on *Arabidopsis* mutants has also confirmed the importance of both cell production and cell expansion in leaf growth (Kim *et al*., 2002), and Donnelly *et al*. (1999) showed that cell division occurs only after cells have reached a certain size, supporting the view from this study that the leaves are growing by means of cell expansion rather than division. The relative importance of cell division and cell expansion varies among plant species and is under genetic control (Taylor *et al*., 2003). The present results show that cell expansion is highly responsive to environmental conditions, as the epidermal cell area was significantly different between two trials (Table 1). Other studies have reported that leaf cell expansion appeared extremely sensitive to environmental conditions, and QTLs for different environments were identified (Ferris *et al*., 2001, 2002).

QTL×environment interaction

QTL effects may be environmentally sensitive, and this sensitivity results in phenotypic plasticity (Gurganus *et al*., 1999). In this study, QTLs for leaf development, biophysical properties, and shelf life have been studied in two different environments. In the Portugal trial, 61 QTLs

were detected, and in the UK trial 45 were found. Of these, five QTLs were common to both trials and were assumed to be independent of the environment (Table 3, Fig. 5). The number and contribution of each QTL that has significantly different effects across the environments would be associated with substantial G×E interaction effects. Further analyses would reveal whether a QTL detected in one environment but not in the other indicates a real QTL×E interaction.

In summary, this study demonstrated substantial progress in using QTL mapping to understand the genetic basis of variation in plant growth and morphology. The ideal ideotype lettuce for improved shelf life is one with strong cell walls, and low levels of leaf plasticity, traits that are linked to, or possibly even brought about by, small cell area. These physiological traits were associated with the best performing lines with regard to shelf life in the Spanish trial. The QTL mapping provides opportunities for future functional research of not only specific loci but also their interactions with other loci (epistasis) and the environment (G×E interactions). The most important finding in this study was the detection of QTLs for shelf life and the association with leaf biophysical traits. Although the minor QTLs were subject to variable genomic locations in different climactic growing conditions, the trait associated with shelf life, leaf strength, and cell area was consistent in each environment. Mapping of candidate genes, such as members of the XTH gene family that are linked to cell wall biosynthesis, is currently in progress in order to identify the genetic basis of processability. The QTL information offers new targets for investigating the molecular regulation of shelf life, and the marker density of the lettuce map allows transference of significant QTL regions to other mapping and breeding populations, if the selected markers are polymorphic, after QTL stability is confirmed. Further fine mapping will enable a greater precision of QTL location and, as more ESTs are positioned on the map, the underlying genetics of shelf life control will be elucidated.

Supplementary data

The supplementary data mentioned herein are available at *JXB* online.

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