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ORIGINAL PAPER

An intra-specific linkage map of lettuce (*Lactuca sativa*) and genetic analysis of postharvest discolouration traits

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Abstract Minimally processed salad packs often suffer from discolouration on cut leaf edges within a few days after harvest. This limits shelf life of the product and results in high wastage. Recombinant inbred lines (RILs) derived from a cross between lettuce cvs. Saladin and Iceberg were shown to be suitable for genetic analysis of postharvest discolouration traits in lettuce. An intra-specific linkage map based on this population was generated to enable genetic analysis. A total of 424 markers were assigned to 18 linkage groups covering all nine chromosomes. The linkage map has a total length of 1,040 cM with an average marker distance of 2.4 cM within the linkage groups and was anchored to the ultra-dense, transcript-based consensus map. Significant genetic variation in the postharvest traits 'pinking', 'browning' and 'overall discolouration' was detected among the RILs. Seven significant quantitative trait loci (QTL) were identified for postharvest discolouration traits providing markers linked to the QTL that can be used for marker-assisted selection. Phenotypic stability was confirmed for extreme lines possessing the corresponding QTL parental alleles and which had shown transgressive segregation. This study indicates that a desired phenotype with reduced levels of postharvest discolouration can be achieved by breeding using natural variation.

Introduction

There is an escalating demand for 'ready-to-eat' salad products. The UK processed bag and dressed salad sector has been steadily growing over the last decade and was

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reported to be worth approximately £823 million in 2008 (Altunkaya and Gokmen 2008; Mintel International 2009), while UK lettuce production/imports had an estimated farm-gate value of £266 million in 2011 (Defra 2012). An annual growth of 15–20 % has been reported by multiple retailers to cater for increased consumer demand for prepared fresh produce.

In today's market of 'food perfection', any deterioration in quality characteristics of fresh produce elicits an unfavourable consumer response. A prime example is pre-packed cut salads where postharvest loss of quality leads to high wastage levels and it has been reported that almost 50 % of salads purchased in the UK are thrown away (WRAP 2009). Pre-packed salads generally have a relatively short designated shelf life of 5–6 days (Wagstaff et al. 2010). If a salad pack does not fulfill its required shelf life when in store with the retailer it is discarded as waste; however, all processes used to generate the product still have to be accounted for by all prior partners in the food chain which results in a monetary loss. Therefore, there is a need to improve postharvest quality and extend shelf life to reduce waste and deliver a product of consistently high quality to the consumer.

Consumers expect the produce to be fresh, visually uniform, without detrimental change to organoleptic characteristics, and to be at the correct stage of maturity depending on salad type throughout the whole of the products recommended shelf life (Watada and Qi 1999). The shelf life of salad products can be limited by microbial spoilage, discolouration, textural changes and the development of 'off-flavour' or 'off-odour' characteristics (Barrett et al. 1998). Cut salad packs, in particular, tend to suffer from discolouration on cut leaf surfaces within a few days after harvest, limiting their shelf life. De novo biosynthesis of polyphenols through the phenylpropanoid pathway causes the materialisation of brown and/or pink/red pigments at cut leaf edges, which is known as 'browning' and 'pinking', respectively (Joslin and Pointing 1951; Zawistowski et al. 1991; Martinez and Whitaker 1995; Toivonen and Brummell 2008; Hilton et al. 2009).

Discolouration is initiated by the breakdown of membranes within cells of plant tissue (Toivonen 2004). Subcellular compartmentalisation is disrupted when the leaf is wounded resulting in the mixing of substrates and enzymes which are normally separated, thus initiating reactions which would not normally occur, such as the discolouration response (Degl'Innocenti et al. 2005; Toivonen and Brummell 2008). Phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5) catalyzes the initial and core step of the phenylpropanoid pathway, controlling the flux of primary metabolites into this secondary metabolic pathway (Wanner et al. 1995). PAL is induced upon wounding of plant tissue, increasing activity in vascular tissue and subsequently increasing downstream biosynthesis of polyphenols for oxidation (Lopez-Galvez et al. 1996; Peiser et al. 1998; Hisaminato et al. 2001). Polyphenol oxidase (PPO) (EC1.14.18.1) and phenol peroxidase (POD) (EC 1.11.17) are the main agents responsible for discolouration via the oxidation of polyphenols (Nicoli et al. 1991; Hisaminato et al. 2001; Tomas-Barberan and Espin 2001). The resulting quinones subsequently undergo non-enzymatic polymerisation (with amino acids or proteins) to form brown and red pigments known as melanins. The accumulation of these pigments is associated with the 'browning' and 'pinking' responses (Joslin and Pointing 1951; Zawistowski et al. 1991; Martinez and Whitaker 1995; Solomon et al. 1996; Toivonen and Brummell 2008; Hilton et al. 2009).

Postharvest discolouration of cut salad packs is currently controlled by combinations of various postharvest treatments; however, modified atmospheric packaging (MAP) is the most common (Brecht et al. 2003; Hilton et al. 2009). For passive MAP, the internal atmosphere is modified by the respiration of the product and equilibrium is reached when the gas composition within the bag stabilises (when the quantity of gas exchanged through the product surface is the same as that exchanged through the packaging) (Zagory and Kader 1988), while for active MAP the addition of gases at specific concentrations results in an earlier equilibrium (Yahia and Gonzalez-Aguilar 1998). MAP reduces the respiration rate and water loss of the enclosed product thus slowing the metabolic rate of tissue and delaying postharvest discolouration (Hilton et al. 2009). However, in the case of postharvest discolouration as soon as the packet is opened, the atmospheric equilibrium is lost and oxygen comes into contact with the tissue, which induces PPO and the specific branch of the phenylpropanoid pathway resulting in discolouration.

Several genetic maps for lettuce have been published previously. Landry et al. (1987), Kesseli et al. (1994), Waycott et al. (1999) and Hayashi et al. (2008) described the generation of linkage maps based on populations derived from intra-specific crosses within *Lactuca sativa* spp. However, the number of linkage groups (LG) for each map (with the exception Landry et al. (1987)) exceeded the chromosomal number for lettuce. Genetic maps with nine chromosomal LG based on populations derived from *L. sativa* crossed with a wild relative (*L. serriola* or *L. saligna*) have been reported by Johnson et al. (2000), Jeuken et al. (2001), Syed et al. (2006) and McHale et al. (2009). These genetic maps based on populations derived from inter-specific crosses are more complete than those derived from intra-specific crosses as they have higher levels of polymorphism and fewer monomorphic regions between the parents. However, the majority of polymorphisms segregating in such populations are not present in the cultivated crop (Truco et al. 2007). Truco et al. (2007) constructed a

high density integrated map of lettuce with 2,744 loci that included data from the majority of the published maps. Recently, an ultra-dense, transcript-based consensus map based on the *L. sativa* cv. Salinas × *L. serriola* US96UC23 population comprising 13,788 unigenes has been developed using a custom Affymetrix GeneChip® microarray designed to detect single position polymorphisms (SPP) in more than 35,000 unigenes (Truco et al. 2007; Truco et al. 2013). These genetic maps provide resources for mapping agronomically important traits leading to marker-assisted selection and candidate gene identification as well as evolutionary studies of the Compositae. The intra-specific cross between cvs. Saladin and Iceberg used in this study are of significance for lettuce breeding research because it is derived from two lettuce cultivars. The resultant markers will, therefore, have a direct application in lettuce breeding, in contrast to linkage maps based on inter-specific crosses. Previous studies by Atkinson et al. (2013) confirmed that cvs. Saladin and Iceberg differed significantly for both the degree and intensity of postharvest discolouration in processed leaves. In addition, this variation has subsequently been confirmed and shown to be representative of the range of natural variation observed in the lettuce genepool (Atkinson et al. 2013). This study aimed to provide the tools and knowledge to breed lettuce cultivars with reduced levels of postharvest discolouration through the generation of an intra-specific linkage map and identification of quantitative trait loci (QTL) for postharvest traits to underpin development of a breeding programme that incorporates marker-assisted selection.

Materials and methods

Plant material

Lettuce cultivars 'Saladin' (syn. Salinas) and 'Iceberg' (syn. Batavia Blonde à Bord Rouge) were used as parents for generation of a recombinant inbred line (RIL) mapping population. Iceberg is a traditional Batavian variety bred in France during the late 1850s; it has pale green leaves with variable red edges (Rodenburg and Basse 1960). Saladin is a crisphead type and has dark green leaves; it is synonymous with cv. Salinas, which was released in the 1970s by the United States Department of Agriculture (Ryder 1979). The majority of modern European and many American crisphead cultivars are derived from Saladin.

The genetically most informative 125 F_7 RILs were identified by analysing the genotype data for the F_5 mapping population. A total of 254 F_5 individuals were genotyped with 232 amplified fragment length polymorphisms (AFLP) and 6 simple sequence repeat (SSR) markers to reveal 237 cross-overs in 15 linkage groups. Individuals were ranked by number of cross-overs using 'Genoplayer' software (http://compgenomics.ucdavis.edu/archive/genoplayer/). The subset of selected 125 F_7 RILs included 73.5 % of the mapped cross-overs in the whole mapping population. A subset of 94 RILs, containing 1,451 known cross-overs, was also identified (59.5 % of the total).

Linkage map construction

Genomic DNA of the two parents and all 125 RILs was extracted from a young, fully expanded leaf from a seedling for each line using the QIAGEN DNeasy 96 Plant Kit (QIAGEN Ltd., West Sussex, UK). Individuals were scored as 'A' when they were homozygous for the female parental allele Iceberg, 'B' when they were homozygous for the male parental allele Saladin and 'H' when they were heterozygous.

AFLP markers were used for genotyping according to Vos et al. (1995) using the enzyme combination *Eco*RI/*Mse*I. A total of 46 publically available primer combinations were assayed (Vos et al. 1995; Vuylsteke et al. 1999). Samples were subsequently run on an ABI DNA Sequencer 3100 and analysed using GeneMarker v1.6 software (SoftGenetics®, California, USA).

Twenty-six expressed sequence tag (EST) markers based on the *L. sativa* cv. Salinas × *L. serriola* US96UC23 population (Truco et al. 2007) were used for PCR-based genotyping of all RILs by the breeding company Rijk Zwaan using their in-house genotyping facilities.

Twenty-six conserved ortholog set (COS) markers (http://cgpdb.ucdavis.edu/database/genome_viewer/viewer/) were also used for PCR-based genotyping. PCR was carried out in a 10 µl reaction mixture consisting of 1 µl 10 × PCR buffer (Invitrogen™, Paisley, UK), 1.3 µl 10 mM dNTP mix, 1 µl forward primer (5 mM), 1 µl reverse primer (5 mM), 4.6 µl sterile H_20, 0.1 µl *Taq* polymerase and 1 µl template DNA. PCR was performed in a Gene Amp® PCR system 9700 (Applied Biosystems™, California, USA), with an initial denaturation at 95 °C for 5 min followed by 34 cycles of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C, with a 10 min extension step at 72 °C. PCR samples were ran via electrophoresis in a 1 % agarose gel containing GelRed (Biotium Inc., California, USA).

Illumina GoldenGate (IGG) single nucleotide polymorphism (SNP) analysis of the RILs using the OPA3 and OPA4 assays (384 SNPs in each OPA) (online resource 1) (http://compgenomics.ucdavis.edu/compositae_SNP.php) was conducted by The DNA Technologies Core, UC Davis Genome Center, USA (http://dnatech.genomecenter.ucdavis.edu/). SNP markers were scored using BeadStudio software v3.1.3.0 (Illumina Inc., San Diego, USA).

An initial 'best fit' linkage map from the RIL population (see method below) was compared to the consensus map

by IGG anchor markers (Truco et al. 2013) and oligonucleotide primers were designed for EST/contig sequence(s) possessing single position polymorphisms (SPP) between Saladin and Iceberg where gaps were present in the map (>100 bins) (Stoffel et al. 2012). PCR-based primers were designed using Primer3 v.0.4.0 design software (Rozen and Skaletsky 2000) specifically around SPP sites of the EST/contig associated with each marker at the designated bin (Cui et al. 2010) (online resource 2). PCR was carried out in a 20 µl mixture consisting of 4 µl 5× iProof HF buffer (BIO RAD laboratories, California), 0.4 µl 10 mM dNTP mix, 0.5 µl forward primer (0.5 µM), 0.5 µl reverse primer (0.5 µM), 12.4 µl sterile H_20, 0.2 µl iProof polymerase and 2 µl template DNA. PCR was carried out in a DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO RAD laboratories, California), with an initial denaturation at 98 °C for 30 s followed by 29 cycles of 5 s at 98 °C, 10 s at 57 °C and 25 s at 72 °C, with a 5 min extension step at 72 °C. Amplified products were analysed by SSCP and silver stained as described by Sentinelli et al. (2000).

The linkage map was constructed using Joinmap® 4 (Van Ooijen 2006). Markers were assigned to linkage groups by increasing the LOD [logarithm (base 10) of odds] score for grouping in steps of 1 LOD. Mapping was carried out using REC (recombination frequency) of 0.49, LOD of 0.01, JUMP (goodness-of-fit threshold) of 5 and the regression mapping algorithm. Recombination frequencies were converted to map distance in centimorgans (cM) using Kosambi's mapping function (Kosambi 1944). After initial generation of a 'best fit' linkage map with all markers, markers within a linkage group were consequently assigned to a known LG based on EST, COS, IGG and SPP anchor markers. The map was regenerated as individual LGs independently. Fifty-seven selected key IGG markers with known marker order on the consensus lettuce linkage map (out of 120 markers) were mapped with an imposed fixed order to generate a scaffold map. The remaining markers were assigned to individual linkage groups without any other constraints.

Phenotypic assessment of postharvest discolouration traits

The 94 genetically most informative F_7 RILs were selected for phenotypic analysis during 2008 in the UK and the Netherlands (NL). A subset of 11 'extreme discolouration' RILs representing the extreme tails of the distribution was subsequently identified based on the results from the phenotypic analysis of RILs in 2008 for further phenotypic analysis in 2009. RILs were selected based on phenotype (they showed significant transgressive segregation for all measures of pinking, browning or overall discolouration in both the UK and NL trials in a single direction) and genotype [they had all beneficial alleles underlying QTL corresponding to the discolouration selected for phenotype (i.e. if they were selected for a high pinking phenotype they also had the alleles that enhanced pinking)]. RILs were selected for low pinking (RILs 5023, 5051), high pinking (RILs 5045, 5075), low browning (RILs 5022, 5055), high browning (RILs 5043, 5053), low overall discolouration (RILs 5002, 5042) and high overall discolouration (RIL 5066).

In 2008, two trials were carried out on 96 lines (these included the mapping population parents and 94 genetically most informative RILs), at the University of Warwick Wellesbourne campus, formerly Warwick HRI, Warwick, UK, (latitude: 52.21, longitude: −1.6), and Rijk Zwaan Breeding Station, Fijnaart, the Netherlands (latitude: 51.97, longitude: 4.25). Each trial consisted of 2 replicates of 96 lines. In the UK, the trial was laid out as a resolvable, incomplete-block, row-column design, in 4 rows and 24 columns, taking row to be a bed. The first trial replicate was sown 24th March 2008 and the second replicate was sown 5th May 2008. In the NL, it was laid out as a resolvable incomplete-block design, with 14 sub-blocks per replicate. The trial was sown end of June 2008. In 2009, another trial was carried out with 3 replicates of 13 lines (these included the mapping population parents and the subset of 11 extreme discolouration RILs) which were laid out as a resolvable incomplete-block design, with 4 sub-blocks per replicate. The trial was sown 18th March 2009. Plots contained 12 plants of the same line in a 3 × 4 rectangle and plots were surrounded by a commercial lettuce cultivar to avoid edge effects. Field trials grown in the UK were irrigated and treated with appropriate pesticides when required according to current good agricultural practice. Field trials grown in the NL were subjected to Rijk Zwaan's normal field trial maintenance.

Plants were harvested when heads reached maturity level for whole head harvest (for individual plots) to simulate commercial practice. For the UK trial during 2008, harvests occurred on 24th and 26th June, 8th and 15th July for replicate 1 and on the 5th, 12th and 20th August for replicate 2. For the NL trial during 2008, harvests occurred on 15th, 17th, 22nd and 24th September. For the UK trial during 2009, harvests occurred on 27th and 28th July. The two central heads per plot were harvested and processed as in Atkinson et al. (2013) with slight modifications. Processed leaf material (~4 cm^2 pieces) was mixed thoroughly and 75 g of material put into a P + 35PA240 semi-permeable bag, 35 µm thick (Amcor Flexibles, Bristol, UK) to create passive MAP conditions. The bags were then heat-sealed and stored in the dark at 5 °C. All lettuce heads were handled separately with material from one head used to fill two replicate 'salad bags'.

Postharvest discolouration of the cut lettuce leaves was assessed visually after 1 and 3 days (for the 94 RILs) and

1, 2, 3 and 4 days (for the 'extreme discolouration' RILs grown in 2009) using the methods described by Hilton et al. (2009) and Atkinson et al. (2013). Bags were removed from storage and arranged under a halogen light source for each assessment. A 12-square 4 × 3 acetate grid was overlaid on the bag and the discolouration of the tissue in each grid square was then scored visually on a five-point scale by two independent assessors. When no discolouration was observable, the grid square was classified as clean. Pinking and browning were each split into two categories of severity of slight and intense. The most intense colour of the tissue in each grid square was recorded for both pinking and browning. Each type of discolouration for individual bags per day was quantified. Mean discolouration scores were calculated that represented the intensity of the discolouration per bag, while percentage discolouration per bag represented the extent of discolouration on the leaves per bag. Scores for pinking and browning were also combined to describe overall discolouration.

Statistical analyses

Segregation ratios for all markers were calculated using Chi-square (χ^2) tests with expected Mendelian ratios of 1:1 in Joinmap® 4 (Van Ooijen 2006). Markers were considered distorted if they deviated at a level of significance of $P < 0.05$.

All statistical tests and transformations for phenotypic data were made using GenStat 10th edition software (Payne et al. 2009). Discolouration was analysed as pinking, browning and overall discolouration (i.e. regardless of 'colour'). Data were transformed and mean discolouration scores per bag and percentage discolouration per bag for each type of discolouration were calculated (for each day) as described by Hilton et al. (2009) and Atkinson et al. (2013); these represented the intensity of the discolouration and the extent of discolouration on the leaves. Data were analysed using restricted estimates maximum likelihood (REML) method; REML is a generalisation of ANOVA which is suitable for unbalanced data. The data from 2008 trials were initially analysed for the two sites separately. For each site, the period of shelf life (day 1 and day 3), the genotype (RIL), and the interaction between them were taken as fixed factors. For the UK trial, the effects of replicates, of rows, columns and plots within replicates, of heads within plots and of bags within heads were taken as random factors. The analysis of the NL trial was almost identical, the only difference being that due to the different design, the effect of sub-blocks within replicates replaced the effects of rows and columns within replicates. The data from the two sites were then analysed jointly in two separate analyses, one for each period of shelf life. For these analyses, the effects of site, genotype and the interaction between them were taken as fixed factors and the effects of replicates within sites, of the incomplete blocking factors of the separate trials, of plots within sub-blocks, of heads with plots and of bags within heads were again taken as random factors. The sizes of the equivalent variance components were allowed to differ between sites. The analysis of the 2009 trial was similar. Separate analyses were carried out for each of the four periods of shelf life (days 1, 2, 3 and 4). For each analysis, genotype was taken as the only fixed factor, and the effects of replicates, incomplete-blocks within replicates, plots within sub-blocks, heads within plots and bags within heads were taken as random factors.

The correlation between discolouration traits of the 94 RILs from the 2008 trial was analysed and correlation coefficients (r) were calculated using adjusted means from the REML analyses (using RIL means based on 94 pairs of values). Due to the large data sets (i.e. high degrees of freedom), only r values with $P < 0.001$ (using Pearson's correlation coefficient) were considered to indicate a possible relationship of practical significance between traits. The correlation heatmap was created using matrix2png v. 1.2.2. (Pavlidis and Noble 2003). The correlation between discolouration traits of the extreme RILs in both the 2008 and 2009 trials was calculated as above (using RIL means based on 11 pairs of values).

QTL analyses

QTL analysis was conducted using MapQTL® 4.0 software (Jansen 1993; Jansen and Stam 1994; Van Ooijen et al. 2002) using all genotypic markers and phenotypic data. Interval mapping was used initially to increase resolution and reduce background marker effects (Zeng 1994), followed by multiple QTL model (MQM) mapping where significant co-factors were selected to control the genetic background (Jansen 1993, 1994; Jansen and Stam 1994). A genome-wide logarithm of odds (LOD) threshold for QTL significance at $P \leq 0.05$ was estimated using 1,000 permutations per trait. A two LOD support interval was used. The graphical representation of the linkage map and QTL was prepared using MapChart® 2.2 software (Voorrips 2002).

Results

In total, 673 markers (including 335 AFLPs, 3 COSs, 21 ESTs, 305 IGGs and 9 SPPs) were polymorphic in the Saladin × Iceberg mapping population. Of these, 424 markers (including 163 AFLPs, 2 COSs, 18 ESTs, 237 IGGs and 4 SPPs) were assigned to 18 LGs distributed over all nine chromosomes, with marker order, marker LG assignment and LG naming based on the positions of anchor markers on the integrated and consensus maps (Truco et al. 2007,

2013). The majority of the remaining 249 markers that did not map were AFLPs that could not be assigned a linkage group based on their linkage with anchor markers. The 18 LGs had a total length of 1,039.6 cM and an average distance between adjacent loci of 2.4 cM. The length of the LGs did not correlate with the numbers of mapped markers on them. The largest LG (LG5) consisted of 41 markers and had a length of 225.6 cM and the smallest (LG6) consisted of 22 markers with a length of 54.3 cM.

A total of 147 (34.7 %) out of 424 loci showed significant segregation distortion ($P < 0.05$), including 81 AFLPs, 2 COSs, 5 ESTs and 59 IGG mapping in 15 LGs. Of those distorted loci, 78 (53.1 %) were skewed towards the Saladin allele, 61 (40.8 %) favoured the Iceberg allele and 8 (5.4 %) had an excess of heterozygotes. Segregation distortion regions skewed towards the Saladin genotype totalled 217.8 cM (ranging from 0.6 to 65 cM); while segregation distortion regions skewed towards the Iceberg genotype totalled 180.3 cM (ranging from 0.5 to 54). The distorted markers were unevenly distributed among the nine chromosomes of lettuce. No distorted markers were detected on 3 LGs (1b, 3a and 6a). Nine LGs (LGs 2, 3b, 4a, 5b, 6c, 7, 8, 9a and 9b) were skewed towards the Saladin allele, five LGs (LGs 4b, 5a, 5c, 6b and 6d) were skewed towards the Iceberg allele, and 1 LG (LG 1a) was skewed towards the heterozygous genotype. Clusters of markers (where markers mapped to the same position) were present on 11 LGs. The clustering of distorted loci suggested that the distortions are unlikely to be due to the miss scoring of loci which would most probably be randomly distributed. The average heterozygosity in the population was 3 %, which is double that of the 1.5 % expected heterozygosity for RILs in the F_7 generation.

Comparison of the Saladin × Iceberg linkage map to other published maps of lettuce suggests that it is a good quality map. The map contains 261 anchor markers from the lettuce consensus map (Truco et al. 2013) and integrated map (Truco et al. 2007). Of these anchor markers, 237 IGG and 4 SPP loci mapped to the associated LGs as in the consensus map (Truco et al. 2013), while 2 COS and 18 EST loci mapped to the same LGs as for the integrated map (Truco et al. 2007). Although 57 selected IGG markers were mapped with an imposed fixed order to provide a scaffold map, 58 of the remaining 63 anchor markers with known positions on the consensus map were mapped in the same marker order. Region 146.6–167.4 cM of LG 2 (encompassing markers BIAS, AKGO and BVZJ) has been translocated within the LG. Based on anchor marker positions, the section should be situated within the estimated region of 40 cM on LG 2. While marker BHXM on LG 2 and BIDO on LG 4a also do not locate at the correct estimated positions within their associated LG. The marker order allowed the correct orientation of each LG relative to the consensus map. All LGs, with the exception of those with a single anchor marker (LGs 3b, 6d and 9b), are in the correct orientation. There were also 57 and 22 common AFLP markers (within 5 bp) with linkage maps by Jeuken et al. (2001) and Syed et al. (2006).

The Saladin × Iceberg map captures 57 % of the consensus map (1,040 cM out of 1,842 cM). Genetic distances between the termini of the LGs were compared between maps, with LGs from the Saladin × Iceberg map covering between 33.2 and 89 % of the respective LGs from the consensus map (Table 1). The supra-chromosomal number of linkage groups in the Saladin × Iceberg map is probably due to monomorphic regions between the parental lines, although over half of the consensus map was covered by this map despite the relatedness of the parents.

Postharvest discolouration traits

Iceberg was consistently more susceptible to postharvest discolouration than Saladin, with significantly

Table 1 Comparison of Saladin × Iceberg lettuce map to consensus lettuce map

Consensus lettuce map			Saladin × Iceberg lettuce map			Consensus map V Saladin × Iceberg map
LG	Full map # bins	Framework map length, cM (# loci)	# Component LGs	Component LG length, cM (# loci)	Total LG 1ength, cM (# loci)	Map captured [length (%)]
1	1,590	170.2 (56)	2	61.9 (12)/9.4 (18)	71.3 (3)	41.9
2	1,749	188.4 (64)	1	167.4 (77)	167.4 (77)	89
3	947	127.9 (42)/50.0 (17)	2	21.5 (5)/43.6 (7)	65.1 (12)	36.6
4	2,051	277.7 (86)	2	86.7 (40)/107.5 (46)	194.2 (86)	69.9
5	2,090	260.1 (89)	3	128 (24)/58 (10)/39.6 (7)	225.6 (41)	86.7
6	1,310	161.1 (55)	4	5.8 (3)/21.7 (4)/3.3 (12)/23.5 (3)	54.3 (22)	33.7
7	1,299	167.7 (49)	1	95.3 (84)	95.3 (84)	56.8
8	2,113	251.5 (88)	1	104.2 (55)	104.2 (55)	41.4
9	1,227	187.3 (53)	2	50.8 (11)/11.4 (6)	62.2 (17)	33.2
Total	14,376	1,842 (599)	18	1,039.60 (424)		56.4

Table 2 Mean value and standard error (SE) of postharvest discolouration traits of minimally processed lettuce RIL population ($n = 94$) stored in semi-permeable packaging over 13 days at 5 °C in the dark

Postharvest discolouration trait	Days	Mean and standard error (SE) for lettuce RIL population											
		UK trial site				NL trial site				Across site			
		Minimum	Maximum	Mean	SE	Minimum	Maximum	Mean	SE	Minimum	Maximum	Mean	SE
Pinking intensity	1	0.05	40.18	18.53	5.4	7.8	43.78	27.74	5.86	7.68	37.74	23.14	3.98
	3	33.55	74.93	54.18	7.22	39.17	57.84	57.84	5.66	42.94	72.62	56.01	4.59
	1–3	20.16	52.79	36.34	5.61	25.61	56.94	42.77	4.94	25.61	51.77	39.57	30.13
Extent of pinking	1	0.31	58.96	27.04	8.3	10.99	73.97	42.03	9.65	10.29	58.24	34.54	6.36
	3	46.19	91.57	74.61	7.64	60.99	90.15	83.68	5.24	63.34	88.95	79.13	4.63
	1–3	27.73	69.39	50.8	6.73	42.77	82.23	62.82	6.06	37.69	73.07	56.84	36
Browning intensity	1	7.5	32.87	19.1	6.35	5	26.72	17.09	4.23	10.85	26.93	18.09	3.82
	3	22.31	58.14	39.83	7.29	10.65	43.64	26.17	6.41	19.18	50.89	33	4.85
	1–3	17.49	42.75	29.47	6.06	11.16	34.08	21.63	4.39	16.4	36.6	25.55	19.69
Extent of browning	1	9.52	48.65	26.96	9.44	5.88	34.08	22.47	5.34	14.97	37.32	24.72	5.44
	3	32.17	78.47	55.61	10.89	14.8	60.42	32.63	7.57	28.01	61.95	44.12	6.63
	1–3	24.89	63.13	41.3	8.99	14.99	45.72	27.55	5.28	21.76	47.06	34.42	27.77
Overall discolouration intensity	1	9.02	31.99	20.25	4.91	15.21	35.66	24.08	3.56	14.09	30.81	22.17	3.04
	3	33.12	61.12	46.67	5.56	31.97	54.52	42.63	3.54	38.07	54.8	44.65	3.3
	1–3	22.4	44.92	33.46	4.77	24.77	42.59	33.35	2.88	26.21	40.03	33.41	21.47
Extent of overall discolouration	1	17.96	68.77	40.76	10.41	28.75	76.75	49.57	8.32	26.33	64.54	45.16	6.66
	3	60.89	91.13	82.05	6.15	67	90	86.36	3.26	71.48	90.56	84.2	3.48
	1–3	39.7	76.75	61.4	7.28	48.88	82.8	67.95	4.75	48.98	76.21	64.68	30.75

Days 1–3 averaged across days 1 and 3

Table 3 Restricted estimates maximum likelihood (REML) analyses of postharvest discolouration traits of minimally processed lettuce RIL population ($n = 94$) grown in two field sites (UK and NL) then stored in semi-permeable packaging over 3 days at 5 °C in the dark

Postharvest discolouration trait	Site	Days	Fixed term	Wald statistic	n*df*	F statistic	d*df*	F probability
Pinking intensity	UK	1	RIL	198.19	95	2.11	34.5	**0.007
		3	RIL	121.82	95	1.29	37.2	0.194
		1–3	RIL × day	148.87	95	Chi	1.57	***<0.001
	NL	1	RIL	209.04	95	2.2	66.9	***<0.001
		3	RIL	179.94	95	1.91	53.5	**0.005
		1–3	RIL × day	309.82	95	Chi	3.26	***<0.001
	UK-NL	1	RIL	294.91	95	3.1	107.1	***<0.001
		1	Site	29.43	1	29.43	22.5	***<0.001
		1	Site × RIL	112.32	95	1.18	112.3	0.199
		3	RIL	201.1	95	2.11	117.5	***<0.001
		3	Site	1.59	1	1.59	1.4	0.378
		3	Site × RIL	100.65	95	1.05	112	0.395
Extent of pinking	UK	1	RIL	186.29	95	2.11	34.9	**0.01
		3	RIL	132.4	95	1.29	1.39	**0.007
		1–3	RIL × day	153.63	95	Chi	1.62	***<0.001
	NL	1	RIL	212.13	95	2.2	67.3	***<0.001
		3	RIL	153.54	95	1.91	94.8	**0.01
		1–3	RIL × day	4.07.52	95	Chi	4.29	***<0.001
	UK-NL	1	RIL	288.47	95	3.1	107.1	***<0.001
		1	Site	31.2	1	29.43	22.1	***<0.001
		1	Site × RIL	109.95	95	1.18	112.9	0.23
		3	RIL	185.24	95	2.11	163	***<0.001
		3	Site	17.68	1	1.59	1.4	0.094
		3	Site × RIL	100.7	95	1.05	153.6	0.37
Browning intensity	UK	1	RIL	67.8	95	Chi	0.71	0.984
		3	RIL	100.39	95	1.06	87.6	0.397
		1–3	RIL × day	168.14	95	Chi	1.77	***<0.001
	NL	1	RIL	119.23	95	1.26	60.4	0.166
		3	RIL	132.53	95	1.4	58.7	0.081
		1–3	RIL × day	154.23	95	Chi	1.62	***<0.001
	UK-NL	1	RIL	105.28	95	1.11	119.9	0.295
		1	Site	2.66	1	2.66	1.9	0.249
		1	Site × RIL	81.76	95	0.86	140.8	0.782
		3	RIL	139.57	95	1.47	140	*0.019
		3	Site	13.86	1	13.86	2	0.066
		3	Site × RIL	93.36	95	0.98	152.1	0.531

Table 3 continued

Postharvest discolouration trait	Site	Days	Fixed term	Wald statistic	n*df*	F statistic	d*df*	F probability
Extent of browning	UK	1	RIL	69.47	95	Chi	0.73	0.977
		3	RIL	86.13	95	0.91	87.6	0.681
		1–3	RIL × day	153.63	95	Chi	1.62	***<0.001
	NL	1	RIL	116.22	95	1.23	59.8	0.195
		3	RIL	139.96	95	1.48	58.5	*0.05
		1–3	RIL × day	165.42	95	Chi	1.74	***<0.001
	UK-NL	1	RIL	103.17	95	1.09	107	0.336
		1	Site	6.31	1	6.31	1.5	0.164
		1	Site × RIL	82.51	95	0.87	130.8	0.765
		3	RIL	132.81	95	1.4	119.3	*0.041
		3	Site	20.79	1	20.79	2	*0.047
		3	Site × RIL	93.28	95	0.98	140.2	0.533
Overall discolouration intensity	UK	1	RIL	103.79	95	1.1	48	0.363
		3	RIL	96.46	95	1.02	87.3	0.472
		1–3	RIL × day	196.66	95	Chi	1.96	***<0.001
	NL	1	RIL	170.17	95	1.79	67.1	**0.006
		3	RIL	152.79	95	1.62	61.7	*0.022
		1–3	RIL × day	293.73	95	Chi	3.09	***<0.001
	UK-NL	1	RIL	174.99	95	1.84	111.4	***<0.001
		1	Site	13.37	1	13.37	15.9	**0.002
		1	Site × RIL	98.97	95	1.04	109.3	0.419
		3	RIL	145.97	95	1.54	113.8	**0.01
		3	Site	2.88	1	2.88	1.7	0.25
		3	Site × RIL	103.28	95	1.09	137.8	0.324
Extent of overall discolouration	UK	1	RIL	107.69	95	1.14	56.4	0.298
		3	RIL	98.32	95	Chi	1.03	0.387
		1–3	RIL × day	211.83	95	Chi	2.23	***<0.001
	NL	1	RIL	196.44	95	2.07	68.4	***<0.001
		3	RIL	205.9	95	2.17	95.8	***<0.001
		1–3	RIL × day	353.46	95	Chi	3.72	***<0.001
	UK-NL	1	RIL	207.23	95	2.18	123.7	***<0.001
		1	Site	11.68	1	11.68	17.3	***0.003
		1	Site × RIL	96.89	95	1.02	123.8	0.456
		3	RIL	201.56	95	2.12	144.2	***<0.001
		3	Site	8.42	1	8.42	1.1	0.193
		3	Site × RIL	102.66	95	1.08	133.8	0.337

Significant effects shown as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

x interaction, *df* degree of freedom, *UK-NL* averaged across the two sites, *1–3* averaged across the two time points, *ndf* numerator *df*, *ddf* denominator *df*

higher levels of pinking, browning and overall discolouration at all sites and on all occasions. All RILs showed both pinking and browning on day 1 and day 3, scores for extent were always higher than for intensity (Table 2). Mean scores for pinking were higher in the NL trial, while mean scores for browning were higher in the UK; however, the ranges of scores were similar in both trials. As would be expected, day of assessment was a significant factor in variation for all types of discolouration ($P \leq 0.001$). There was also a significant interaction between day and RILs (i.e. genotype), indicating an uneven rate of discolouration between RILs. The partitioning of the phenotypic variation by REML analysis showed that the effect of RILs was significant at both sites for pinking and overall discolouration (Table 3). However, significant variation for browning was site specific and only found in the NL trial. There were also significant site effects for pinking scored on day 1 and for browning day 3, indicating that the plants' growing environment influences postharvest performance (Table 3).

Transgressive segregation for all traits was observed in the RIL population. Fifty-two RILs expressed some form of transgressive segregation for pinking, browning and/or overall discolouration for at least 1 day per site. Thirty-two RILs had lower levels of pinking in comparison to the best performing parent Saladin, while five RILs had higher levels of browning in comparison to the worst performing parent Iceberg. Forty RILs had lower levels of overall discolouration than Saladin and eight RILs had higher levels of overall discolouration than Iceberg.

Correlations between discolouration traits are presented as a heatmap (Fig. 1). Positive correlations are represented by darkness of colour (i.e. high heat), while negative correlations are represented by lightness (i.e. low heat). As expected there is high heat along the diagonal; however, there are hot spots spread across the map. The two measures of pinking (intensity and extent) were positively correlated with one another (mean UK site $r_{[df=90]} \geq 0.95$, mean NL site $r_{[df=90]} \geq 0.94$ and mean across sites $r_{[df=90]} \geq 0.95$), as were measures of browning (mean UK site $r_{[df=90]} \geq 0.96$, mean NL site $r_{[df=90]} \geq 0.96$ and mean across sites $r_{[df=90]} \geq 0.95$). Only data collected from the NL site showed a positive correlation between the measures of overall discolouration (mean NL site $r_{[df=90]} \geq 0.85$). Postharvest discolouration scores from the UK and NL trial sites were not correlated indicating an effect of site on the traits.

Broad sense heritability ($V_G/(V_G + V_E)$ where V_G is among genotype variance and V_E is residual variance) for the pinking, browning and overall discolouration responses were calculated as 0.83 ± 0.1, 0.66 and 0.76 ± 0.06, respectively (as a mean across all measures of each discolouration type over days and site). Phenotypic stability was

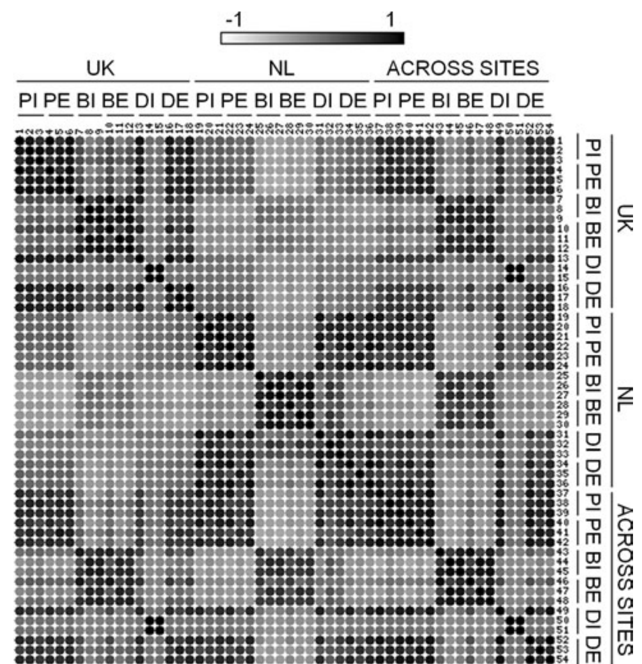

Fig. 1 Correlation heatmap for postharvest discolouration traits assessed in the lettuce RIL population grown in two field sites. Where pinking intensity (*PI*) in the UK trial on days 1, 3 and across days (1, 2, 3); extent of pinking (*PE*) in the UK trial on days 1, 3 and across days (4, 5, 6); browning intensity (*BI*) in the UK trial on days 1, 3 and across days (7, 8, 9); extent of browning (*BE*) in the UK trial on days 1, 3 and across days (10, 11, 12); overall discolouration intensity (*DI*) in the UK trial on days 1, 3 and across days (13, 14, 15); extent of overall discolouration (*DE*) in the UK trial on days 1, 3 and across days (16, 17, 18); pinking intensity (*PI*) in the NL trial on days 1, 3 and across days (19, 20, 21); extent of pinking (*PE*) in the NL trial on days 1, 3 and across days (22, 23, 24); browning intensity (*BI*) in the NL trial on days 1, 3 and across days (25, 26, 27); extent of browning (*BE*) in the NL trial on days 1, 3 and across days (28, 29, 30); overall discolouration intensity (*DI*) in the NL trial on days 1, 3 and across days (31, 32, 33); extent of overall discolouration (*DE*) in the NL trial on days 1, 3 and across days (34, 35, 36); pinking intensity (*PI*) across trials on days 1, 3 and across days (37, 38, 39); extent of pinking (*PE*) across trials on days 1, 3 and across days (40, 41, 42); browning intensity (*BI*) across trials on days 1, 3 and across days (43, 44, 45); extent of browning (*BE*) across trials on days 1, 3 and across days (46, 47, 48); overall discolouration intensity (*DI*) across trials on days 1, 3 and across days (49, 50, 51); extent of overall discolouration (*DE*) across trials on days 1, 3 and across days (52, 53, 54)

also confirmed in the subset of RILs with 'extreme' phenotypes tested the following year. There was little change in ranking of extreme RILs between days for pinking and browning (Table 4), which would be expected if the phenotypes had a significant genetic component. Extreme RILs performed as they did during the original 2008 RIL trial [each measure of discolouration were significantly correlated ($r_{[df=9]} \geq 0.7$, $P < 0.05$)]; those selected for an extreme discolouration type (i.e. pink, brown or overall discolouration) showed the same phenotype in the 2009 trial (Table 4).

Table 4 Mean value and standard error (SE) of postharvest discolouration traits of minimally processed extreme lettuce RILs stored in semi-permeable packaging over 4 days at 5 °C in the dark

Postharvest discolouration trait	Days	RIL mean and standard error (SE)											
		5002	5022	5023	5042	5043	5045	5051	5053	5055	5066	5075	SE
Pinking intensity	1	18.8	30.6	18.5	17.8	24.5	35.3	14.6	36.0	46.9	48.8	20.1	5.17
	2	33.3	44.8	40.9	30.5	41.6	44.3	31.6	48.9	54.9	56.3	42.5	4.69
	3	44.3	53.5	46.4	41.0	49.1	51.3	37.1	64.1	61.0	64.0	51.8	4.67
	4	50.4	56.3	55.2	50.0	53.0	68.4	45.7	59.7	73.7	78.4	60.3	5.54
Extent of pinking	1	27.1	50.0	25.7	26.7	35.9	51.3	21.0	53.4	70.8	72.4	29.1	7.74
	2	52.0	72.4	64.4	46.7	69.0	66.8	47.9	74.9	87.8	81.3	67.6	7.82
	3	73.3	84.5	71.3	60.7	77.8	80.3	60.1	82.8	89.2	82.3	79.7	5.28
	4	78.2	87.4	81.8	77.0	75.3	89.4	73.7	80.9	89.4	87.4	87.7	5.57
Browning intensity	1	19.7	17.4	5.7	21.9	33.1	16.5	17.7	29.1	15.3	24.5	10.5	4.88
	2	28.6	25.9	18.0	31.6	40.1	13.2	29.1	36.8	14.0	18.8	15.7	5.76
	3	35.4	24.4	15.1	35.6	45.7	18.1	32.3	42.6	15.6	19.9	18.5	6.21
	4	37.2	31.9	18.7	43.3	48.5	27.5	38.2	46.6	26.1	26.8	25.1	7.48
Extent of browning	1	24.6	21.8	7.6	26.3	40.9	22.4	23.1	36.0	21.4	29.3	13.3	5.87
	2	34.0	28.6	23.4	36.1	46.2	17.6	33.0	41.9	19.8	24.5	19.7	6.55
	3	39.8	30.0	19.5	41.5	52.9	24.6	37.7	49.0	22.8	25.3	21.9	7.08
	4	40.7	35.5	22.4	46.3	52.7	31.4	42.9	52.8	35.2	32.3	27.9	8.25
Overall discolouration intensity	1	20.5	26.4	14.7	21.0	29.8	26.6	17.2	32.7	33.5	37.8	17.8	3.52
	2	32.5	36.8	31.6	31.9	41.3	31.4	30.9	42.9	37.5	39.2	31.3	2.63
	3	40.9	40.1	33.7	38.8	46.9	36.9	35.2	51.1	40.8	43.0	37.6	2.58
	4	44.4	44.4	39.5	46.8	48.9	48.0	41.6	52.1	47.8	49.5	43.0	3.31
Extent of overall discolouration	1	36.8	54.4	29.2	37.8	54.9	52.8	31.4	60.1	72.4	73.0	34.2	7.18
	2	61.7	77.0	66.9	56.4	85.3	68.0	56.7	85.4	87.0	82.2	69.4	5.58
	3	80.1	86.7	74.2	70.3	85.8	81.4	71.1	88.7	91.0	85.0	79.6	4.40
	4	82.8	89.4	85.5	83.8	90.0	87.2	79.6	88.6	90.0	88.5	85.8	2.93

QTL for postharvest discolouration traits

A genetic map containing 424 markers was used for QTL analyses. All discolouration traits were analysed initially using interval mapping and a total of ten putative QTL were detected. Subsequent MQM mapping increased robustness of QTL identification and increased the number of putative QTL to 14 (Table 5). Individual putative QTL accounted for between 11.3 and 22.6 % of the phenotypic variation in this population and were distributed on three LGs (LGs 4a, 7 and 9b). Many of the putative QTL co-located to the same position and, therefore, are most likely identifying the same underlying genetic factor affecting postharvest discolouration in lettuce. The 14 putative QTL could, therefore, be reduced to seven QTL based on their co-location on the map (2 LOD threshold overlap) and allelic contribution (Fig. 2; Table 6).

Three significant QTL for postharvest pinking were distributed on two LGs [LGs 7 (*Pink1* and *Pink2*) and 9b (*Pink3*)] (Fig. 2; Table 6). The phenotypic variation explained by QTL for pinking traits ranged from 11.3 to 22.6 % (Table 5). The beneficial allele (i.e. for reduced pinking) was derived from Saladin at two QTL and from Iceberg at a single QTL. One significant QTL affecting postharvest browning was identified on LG7 (*Br1*) (Table 6) and 14.8 % of phenotypic variation for browning traits was explained by the QTL (Table 5). The beneficial allele (i.e. for reduced browning) was derived from Saladin. Three significant QTL specific to the 'overall discolouration' trait were identified on LG4a (*Dis1*, *Dis2* and *Dis3*) (Table 6). The phenotypic variation explained by QTL for overall discolouration ranged from 13.1 to 17.4 % (Table 5). The beneficial allele (i.e. for reduced overall

Table 5 Putative QTL detected by MQM mapping for postharvest discolouration traits assessed in the lettuce RIL population grown in two field sites

Trait	Days	Position (cM)	Marker	UK trial site			NL trial site			Across site		
				LOD	Additive	Var. (%)	LOD	Additive	Var. (%)	LOD	Additive	Var. (%)
Pinking intensity	1	LG7: 2.3–3	BLJI	3.63	3.18	16.3				3.53	2.74	15.9
	1–3	LG9b: 0–3.5	RZ.A	3.16	2.52	11.3						
Extent of pinking	1	LG7: 2.3–3	BLJI	3.8	4.86	17				3.6	4.37	16.2
	1	LG7: 21.3–23.7	E33M59.204				4.66	−8.07	22.6			
	1–3	LG7: 2.3–3	BLJI							3.37	3.14	15.2
Browning intensity	1	LG7: 49.4–59.2	QGE10B18				3.41	1.85	14.8			
Overall discolouration intensity	1	LG4a: 8.6–9	RZ.X				4.77	1.96	15.4			
	1	LG7: 19.7–21.9	E44M59.205				4	−1.93	13.3			
	1	LG7: 2.3–3	BLJI							4.25	1.63	16.9
	1	LG7: 49.4–59.2	QGE10B18				5.19	2.3	17.4			
	3	LG4a: 83.1–86.7	RZ.I	4.07	2.54	17						
Extent of overall discolouration	1	LG4a: 0–4.4	E37M61.83				3.21	4.39	13.1			

The day number indicates the days after harvesting when assessed. Position indicated by the LG number and the significant QTL interval over the threshold based on a 2 LOD support interval. Additive effect indicates which parental allele causes the increase in trait value and its magnitude. Positive value indicates that the Saladin allele increases the trait values, and negative values indicate that the Iceberg allele increases the trait value. Variance (Var.) indicates the percentage of variance in the mapping population which is explained by the detected QTL. Markers are the nearest marker to the QTL

LOD logarithm of the odds score, *days 1–3* across day's value

discolouration) was derived from Saladin at all QTL. The remaining putative QTL for overall discolouration identified through MQM co-located with either pink or brown QTL so it was assumed that the more specific colour QTL were the underlying genetic factor for the discolouration.

Discussion

This study has provided an understanding of the genetics of postharvest discolouration in lettuce and thereby provided the tools and knowledge to underpin a breeding programme to improve postharvest shelf life.

The Saladin × Iceberg linkage map

Comparison of the Saladin × Iceberg linkage map to other published lettuce maps suggests that it is a good quality map. The map contains 341 markers common with four lettuce maps (Jeuken et al. 2001; Syed et al. 2006; Truco et al. 2007, 2013). The large number of common anchor markers not only allowed us to determine the quality of the map generated in this study, but also allowed accurate cross referencing between maps. This provides the opportunity to utilise the large number of markers in the ultra-dense map (Truco et al. 2013).

The map presented in this study contains five types of markers (AFLP, COS, EST, IGG and SPP markers) to try to attain full genome coverage. Markers were generally evenly distributed across the 18 linkage groups with an average distance of 2.4 cM, although a few distances exceeded 20 cM (present on LG 1a, 2, 4b, 5a, 6d and 9a), with the largest distance of 37.9 cM recorded on LG 5a. The maximum number of markers mapped at any locus was eight on LG 2 which were all IGG markers. There were many small clusters of IGG markers distributed across the map, which could suggest that the markers had been developed from identical or similar sequences. High numbers of double AFLP clusters were also observed; however, the majority of these appear to be the product of stutter peaks as there is a single base pair difference; E41M49.212 and E41M49.213 on LG 1a. Clustering of AFLP markers has also been recorded by Truco et al. (2007) and van Os et al. (2006) in ultra-dense maps of lettuce and potato, respectively.

The segregation distortion seen in some regions of the map indicates that some inadvertent selection may have occurred during the production of the RILs, e.g. some lines

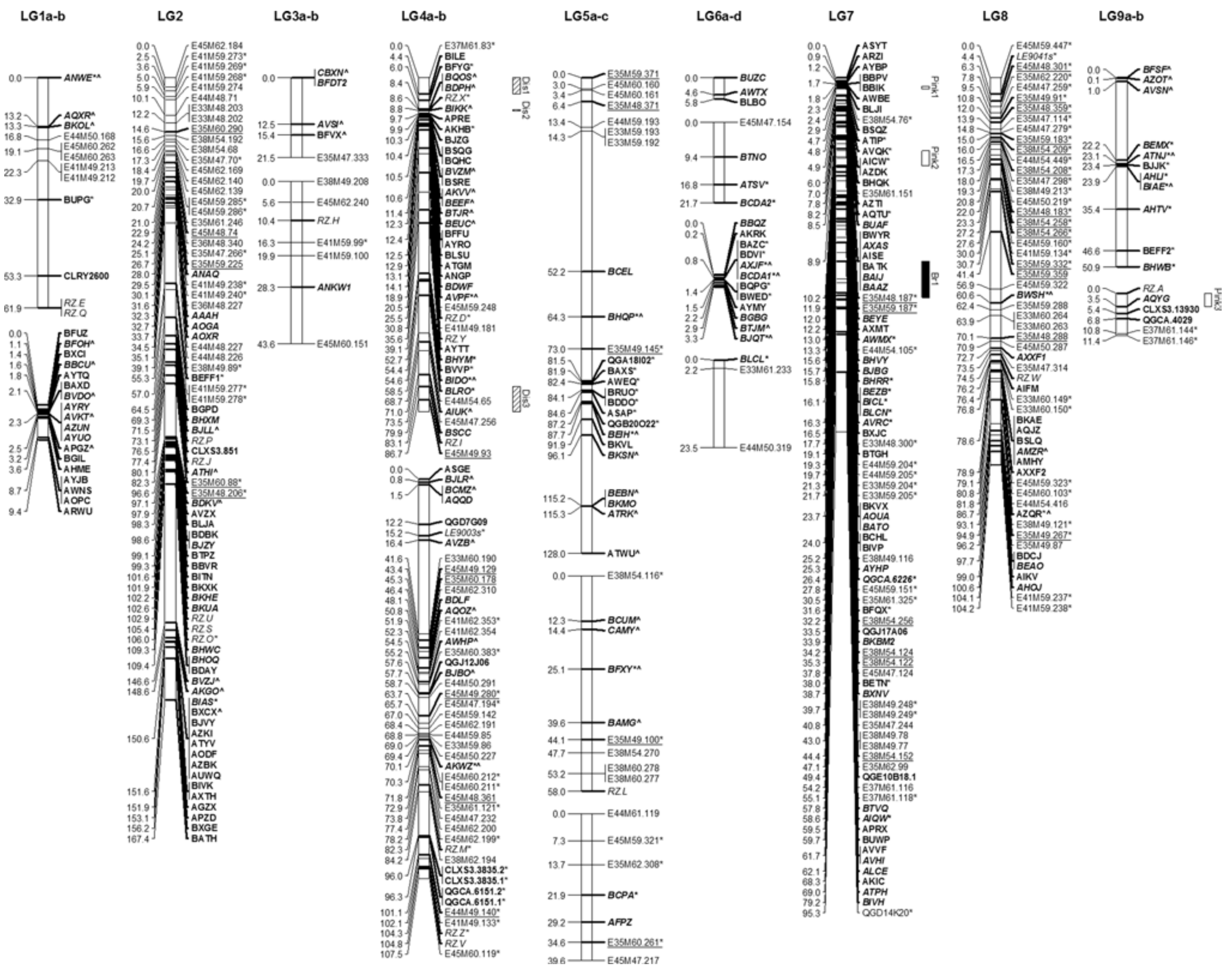

Fig. 2 Linkage map for Saladin × Iceberg RIL population showing the locations of QTL identified for lettuce postharvest discolouration (based on co-location of putative QTL detected via MQM mapping) from two different growing environments (UK and NL). *Br* browning, *Pink* pinking, *Dis* overall discolouration. *Number* after discolouration parameter refers to QTL number for that specific trait. Anchor markers with the ultra-dense transcript-based consensus map (Truco et al. 2013) and the integrated map (Truco et al. 2007) are in *bold and italics,* respectively, while anchor markers with a known marker order from the consensus map are in *bold and italics*. Common markers with the maps by Jeuken et al. (2001) and Syed et al. (2006) are *underlined*. Markers displaying significant segregation distortion are marked with *asterisk*. Markers given a fixed position during mapping are marked with *hat symbol*

Table 6 Significant QTL based on co-location of putative QTL detected by MQM mapping for postharvest discolouration traits assessed in the lettuce RIL population

QTL	Position (cM)	Markers underlying QTL	Allelic contribution
Pink1	LG7: 2.3–3	BLJI/E38M54.76/BSQZ	Saladin
Pink2	LG7: 19.7–23.7	E44M59.205/E33M59.204/E33M59.205/BKVX/AOUA/BATO	Iceberg
Pink3	LG9b: 0–3.5	RZ.A/AQYG	Saladin
Br1	LG7: 49.4–59.2	QGE10B18/E37M61.116/E37M61.118/BTVQ/AIQW	Saladin
Dis1	LG4a: 0–4.4	E37M61.83/BILE	Saladin
Dis2	LG4a: 8.6–9	RZ.X/BIKK	Saladin
Dis3	LG4a: 83.1–86.7	RZ.I/E45M49.93	Saladin

Br postharvest browning, *Pink* postharvest pinking, *Dis* postharvest overall discolouration

were lost to infection by *Botrytis* in the early generations, while others were excluded due to inadequate seed production during generation of the F_7 from the F_6 population.

In addition to its quality, a major advantage of the Saladin × Iceberg map is that it is based on a cross between two lettuce accessions which show significant different phenotypic variation for a large number of agronomically important traits in addition to postharvest discolouration, including field resistance to *Bremia lactucae* and *Myzus persicae*. Genetic analysis of these traits can be carried out within a cultivated background without any of the potentially complicating effects which may be associated with using interspecific crosses, e.g. pleiotropic effects due to undesirable morphological and biochemical traits associated with wild *Lactuca* spp. Any polymorphic markers linked to agronomically important traits are likely to be of direct value to lettuce breeders for marker-assisted selection.

QTL analysis for postharvest discolouration traits

This is the first study to report QTL for postharvest discolouration in lettuce. Postharvest discolouration is a complex trait and, therefore, likely to be controlled by many small effect QTL (Zhang et al. 2007). Seven QTL for postharvest discolouration were identified: one QTL for browning, three QTL for pinking and three QTL which affected overall discolouration. The QTL all had relatively moderate effects explaining between 11.3 and 22.6 % of the observed genetic variance [suggesting that there are probably other QTL that were not detected in this experiment (Zhang et al. 2007)]. A proportion of the observed phenotypic variance was left unexplained by the QTL analysis (heritabilities of 0.83, 0.66 and 0.76 for pinking, browning and overall discolouration responses, respectively) indicating a significant environmental effect on the traits. For six of the QTL the beneficial alleles were from parent Saladin and for the remaining QTL (*Pink2*) the Iceberg alleles were the beneficial alleles. This explains the transgressive segregation observed in the RILs. QTL hotspots were identified for these traits on LGs 7 and 4a. This is most likely due to the presence of one QTL controlling multiple traits for example by common metabolic regulation. However, correlation analysis between the traits indicates only a weak association between pinking and browning suggesting although some of the genetic control may be common, there are also separate genetic factors controlling each trait. This was confirmed by the presence of QTL influencing only a single trait.

Significant site effects were recorded for pinking and browning, indicating that the plants' growing environment significantly influences their postharvest performance. This is in agreement with Hilton et al. (2009) who showed that postharvest discolouration of lettuce could be manipulated by changes in growing conditions. Preliminary investigations of meteorological data suggested that rainfall could affect postharvest discolouration. Both irrigation methods and rainfall have been shown to affect general lettuce postharvest visual quality (Fonseca 2006). However, no effect of weather was recorded for any experimental trials in this study (data not shown). Time of transplanting, timing of harvest and crop maturity have also been shown to have an effect on postharvest discolouration; however, in this study, RILs were sown and transplanted on the same day and harvested within a restricted period for each trial. The trials in the UK and NL were designed for genetic analysis with the aim to provide robust phenotypes for QTL analysis and to determine whether there was any phenotypic plasticity (Gurganus et al. 1999) over environments, which was achieved. Therefore, more research into the effect of environmental factors on postharvest discolouration is required.

Marker loci associated with the seven QTL could be exploited by breeders using marker-assisted selection (MAS) to extend the shelf life of cut salad products by breeding for reduced postharvest discolouration. MAS is particularly useful for traits that are difficult for breeders to assess, e.g. because they are expressed late in developmental stages, or require destructive phenotyping such as postharvest discolouration traits. Desired genotypes can be effectively selected with MAS at the seedling stage, therefore, independent of phenotypic selection and consequently environmental effects, resulting in faster line development and variety release (Collard and Mackill 2008). The current study provides the basis for developing a MAS breeding strategy to improve postharvest discoloration in lettuce.

Before going to the expense of applying MAS, QTL should be confirmed or validated. Fine/high resolution mapping may also be required to have tightly linked markers (i.e. to reduce the possibility of recombination between the marker and QTL) (Langridge et al. 2001). QTL validated as RIL phenotypes were shown to be stable over environment and years, so therefore they were largely determined by genotype [RILs selected for an extreme discolouration type (i.e. pink, brown or overall discolouration based on phenotype and genotype underlying QTL) showed the same phenotype in both the 2008 and 2009 trials]; however, this must be confirmed in subsequent generations. The subsequent step would be to validate QTL by identifying candidate genes, where known function genes could correspond to loci controlling the traits of interest (Pflieger et al. 2001). For example, a candidate gene for the enzyme leucoanthocyanidin dioxygenase (PpLDOX) has been identified as responsible for a QTL (qP-Brn5.1^{m}) affecting browning in peach (Ogundiwin et al. 2008) while a candidate gene for PPO (POT32) has been mapped to a major QTL causing browning in potato (Werij et al. 2007). In lettuce, PPO is located on LG 9 based on EST/contigs in

the consensus map; however, no QTL for postharvest discoloration in lettuce were mapped to this LG in this study.

In conclusion, we have successfully initiated investigation of the genetic control of postharvest discolouration in lettuce and provided the tools and knowledge for breeding programmes. However, it is necessary to approach breeding for reduced postharvest discolouration with a note of caution. Future studies should include an assessment of potential pleiotropic effects of reducing postharvest discolouration on other abiotic and biotic stress responses, particularly pest and disease resistance. Browning is thought to be a wound response which deters pests (Lattanzio et al. 2006); if lines were bred for reduced discolouration, it is important to ensure that resistance to pests and/or disease is not compromised. It is also important to have some understanding of any relationships between postharvest discolouration and agronomic traits to be able to optimise shelf life while retaining agronomic quality (such as disease resistance, yield and quality traits for instance leaf shape and colour). These factors must be investigated so that breeders can take an informed approach.

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