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ORIGINAL PAPER

Quantitative trait loci associated with tipburn, heat stress-induced physiological disorders, and maturity traits in crisphead lettuce

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Abstract Crisphead lettuce (*Lactuca sativa* L.) crops exhibit several economically important, physiological disorders when grown in high temperature conditions. These include tipburn, rib discoloration, premature bolting, ribbiness, and internal rib cracking. We evaluated seven physiological disorders and three agronomic traits segregating in a recombinant inbred line (RIL) population consisting of 152 F_7 RILs derived from an intra-specific cross between two crisphead cultivars, *L. sativa* cv. Emperor x *L. sativa* cv. El Dorado; evaluations were carried out at each of two parental maturities in one planting and at one intermediate maturity in a second planting in each of 2 years for a total of six evaluations. A genetic map was developed using 449 polymorphic SNP markers; it comprises 807 cM in 20 linkage groups that covered 51 % of the nine lettuce chromosomes. Composite interval mapping revealed a total of 36 significant QTLs for eight out of the ten traits evaluated. Significant QTLs were distributed in 11 linkage groups on seven of the chromosomes and accounted for up to 83 % of the phenotypic variation observed. The three largest QTLs for rib discoloration, which accounted individually for 7–21 % of the variation, were clustered with stem length, two with ribbiness and one with head firmness. Three major clusters of QTLs revealed pleiotropic effects or tight linkage between tipburn incidence and severity, head type, stem length, head firmness and ribbiness. One QTL, *qTPB5.2*, was detected in multiple trials and described 38–70 % of the variation in tipburn incidence. *qTPB5.2* is, therefore, a useful candidate gene for breeding for tipburn resistance using marker-assisted selection.

Introduction

Quantitative variation in phenotype results from the combined action of environmental factors and a few or many genetic factors, each having small or large effects on the overall phenotype (Kearsey and Pooni 1996; Mackay et al. 2009). Among environmental factors, heat stress is a critical one for plant growth and development, particularly in cool-season vegetables such as lettuce. Lettuce grows best under cool temperature conditions in the range of 7–24 °C (Lorenz and Maynard 1988). Growing the crop above this range decreases quality and yield and commonly results in economically important physiological disorders such as tipburn, rib discoloration, premature bolting, and ribbiness. While rib discoloration is directly associated with episodes of heat stress during head development, premature bolting and ribbiness are favored by high temperature in long-day growing conditions. As a long-day plant, seed stalk elongation is initiated by day lengths longer than 13 h and accelerated by high temperatures (Rappaport and Wittwer 1956). In the Southern Quebec region (Canada), the photoperiod is longer than 13 h between March and September, which coincides with the period of lettuce production. Combined with the warm and humid conditions, the Quebec summers provide appropriate conditions for the induction of tipburn, rib discoloration, premature bolting and ribbiness.

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Tipburn is a complex and unpredictable disorder which frequently, but not always, occurs in all lettuce types under warm growing conditions. This disorder has been associated with a localized calcium deficiency in young developing tissues (Barta and Tibbitts 2000). Calcium is a nutrient that is poorly mobilized and mainly translocated in the transpiration stream. Consequently, factors that promote tipburn include: (1) rapid growth due to high temperature, high light intensity and duration, and high fertilization levels as well as (2) reduced transpiration due to enclosure of the leaves during heading, low air movement, high relative humidity during daytime, or irregular water supply due to drought or water logging (Collier and Tibbitts 1982). Since no management practices can control most of the factors that induce tipburn, breeding for tipburn resistance remains the most appropriate long-term strategy to limit the problem.

Internal rib cracking is a physiological disorder that appears as breaks in the inner part of the lower petiole and is observed after irrigation of mature lettuce in dry soil (Marlatt 1974). The occurrence of internal rib cracking and other disorders such as tipburn are likely to increase with rising temperatures and more frequent drought and wet cycles resulting from climate change. More information is required to facilitate the development of new heat-tolerant varieties of lettuce to mitigate the adverse effects of climate change.

Variations in susceptibilities to rib discoloration, tipburn, premature bolting and ribbiness among cultivars and breeding lines have been reported in lettuce (Jenkins 1962; Nagata and Stratton 1994). However, little is known about the genetic determination of these disorders. Many genes may be involved in mechanisms for tolerance to these disorders. Quantitative trait loci (QTL) analysis provides a means to determine the genetic basis of these disorders as well as to identify desired chromosome segments for use in marker-assisted selection (MAS; Howarth 2005). Multiple QTL have been shown to be involved in heat tolerance in wheat (Yang et al. 2002) and maize (Jorgensen and Nguyen 1995).

Lettuce is a diploid ($2n = 18$) species in the Compositae family with an estimated genome size of 2.7 Gb (The Lettuce Genome Sequencing Consortium, unpublished). It has a consensus integrated genetic map that includes over 2,700 markers in nine chromosomal linkage groups (LGs) and spans 1,505 cM (Landry et al. 1987; Kesseli et al. 1994; Truco et al. 2007; McHale et al. 2009; http://cgpdb.ucdavis.edu/database/genome_viewer/viewer/) as well as an ultra-dense, transcript-based map comprised of 13,943 markers (Truco et al. 2013). QTL analyses have previously been conducted in lettuce for root architecture (Johnson et al. 2000), seed and seedling traits (Argyris et al. 2005; Hayashi et al. 2008; Schwember and Bradford 2010), post-harvest shelf life (Zhang et al. 2007), and resistance to downy mildew (Jeuken and Lindhout 2002). These analyses were performed using recombinant inbred line (RIL) populations derived from crosses between cultivated lettuce *L. sativa* and the wild, non-heading species *L. serriola*, *L. saligna*, or a F_2 population between *L. saligna* also non-heading, and a cultivated *L. sativa* butterhead type.

There had been no previous reports of QTL analyses of tipburn, rib discoloration, premature bolting, ribbiness, or internal rib cracking in lettuce. One of the difficulties in studying rib discoloration is that the disorder has only been reported in crisphead lettuce and therefore has to be studied using RILs derived from two parents both of crisphead type. Also, the pleiotropic effects of plant architecture necessitate the analysis of these traits using a population with minimal segregation for plant architecture and time to maturity.

The objective of this study was to determine the genetic basis of heat stress-induced physiological disorders in lettuce. To do this, a population of 152 F_7 RILs was developed from an intra-specific cross of two crisphead cultivars, *L. sativa* cv. Emperor x *L. sativa* cv. El Dorado. Seven physiological disorders and three agronomic traits were evaluated in a total of six field trials. We identified 36 significant QTLs determining eight out of the ten traits evaluated.

Materials and methods

Plant material

A population consisting of 152 F_7 RILs was derived by single-seed descent from a *L. sativa* cv. Emperor x *L. sativa* cv. El Dorado cross (Jenni et al. 2008). The cultivar 'Emperor' originated from a single plant selection of the cultivar 'Empire'; it is adapted to warm growing conditions and has a good tolerance to rib discoloration, premature bolting and ribbiness (Ryder and McCreight 1996). The cultivar 'El Dorado' (USDA 1993) is a Salinas type crisphead lettuce that is adapted to mineral soils and the cool coastal areas of California; it is susceptible to rib discoloration, premature bolting and ribbiness when grown in organic soils and the warm, humid, and long-day conditions of the Quebec summers.

This RIL population was planted twice each year in 2008 and 2009 at the Agriculture and Agri-Food Canada Sainte-Clotilde Research Sub-Station to ensure that lettuce heads were harvested during the warmest period of the summer, i.e., between mid-July and mid-August. Each experiment was arranged in a randomized complete block design with three blocks and was surrounded by guard rows. Each block consisted of 30 plants of each parental line and F_7 RIL and was planted within a day. Transplant dates were May 26, 27, 29 for Planting 1 and June 26, 27, 30 for Planting 2 in 2008 and May 25, 26, 27 for Planting 1 and June 30 and July 2, 3 for Planting 2 in 2009. Plants were 22–25 days old at transplanting. All plots were overhead-irrigated immediately after transplanting. Preplant N was applied at a rate of 80 kg ha^{-1} in accordance with commercial recommendations (Centre de référence en agriculture et agroalimentaire du Québec 2003). Potassium and phosphorous were applied in response to soil tests and recommendations. Pest and disease control was carried out according to standard procedures (Ontario Ministry of Agriculture, Food and Rural Affairs 2004).

Phenotyping

Since rib discoloration and tipburn symptoms develop when the plant reaches acceptable commercial firmness and cv. El Dorado reaches maturity 5–6 days earlier than cv. Emperor, evaluations were performed in two ways. In the first planting, evaluation took place twice, at the optimal maturity of each of the parental lines (M1 for maturity of El Dorado and M2 for maturity of Emperor). In the second planting, evaluation was performed at a maturity intermediate between the two parents (M1_M2). A total of five plants per plot were randomly harvested for each evaluation. Each of the three blocks was evaluated within a day. In 2008, lettuce heads were evaluated during 8 to 11 July (Planting 1 M1), 14 to 17 July (Planting 1 M2), and 11 to 13 August (Planting 2 M1_M2). In 2009, trials were scored during 8 to 10 July (Planting 1 M1), 13 to 15 July 2009 (Planting 1 M2), and 10 to 13 August (Planting 2 M1_M2). These six evaluations are referred to hereafter as trials. Each head was examined for the following traits: incidence and severity of tipburn, incidence and severity of rib discoloration, stem length, head firmness, conic head incidence, lettuce type, internal rib cracking, and ribbiness (Table 1).

Genotyping, map construction and QTL analysis

DNA from parental lines and the 152 RILs were genotyped for 768 SNPs (OPA4 and OPA5: http://compgenomics.ucdavis.edu/compositae_SNP.php) using Illumina Golden Gate SNP assays. Polymorphic markers were used to construct a genetic map using Joinmap v 4.0 with default settings and Kosambi mapping function (Van Ooijen 2006). QTL analysis was conducted using WinQTL Cartographer (Basten et al. 2002) using Composite Interval Mapping. Significance thresholds at $P < 0.05$ were calculated for each trait

Table 1 Measured traits on the *L. sativa* cv. Emperor x *L. sativa* cv. El Dorado RIL population and parents

Abbreviation	Traits	Unit	Rating scale
TBINC	Tipburn incidence	(%)	Percent plants showing tipburn symptoms
TBSEV	Tipburn severity	(1–5)	1 = severe symptoms, the aggregate area of necrotic spots representing a rectangle exceeding 25 mm in length by 12 mm in width; 2 = moderate symptoms, the aggregate area of necrotic spots representing a rectangle of 25 mm in length by 6–12 mm in width; 3 = light symptoms, heads are still marketable, more than 2 spots or the aggregate area of necrotic spots represents less than a rectangle of 25 mm in length by 6 mm in width; 4 = very light symptoms, very slight spots on 1 or 2 locations; 5 = no symptoms
RDINC	Rib discoloration incidence	(%)	Percent plants showing rib discoloration symptoms
RDSEV	Rib discoloration severity	(1–5)	1.0 = extreme occurrence of long streaks along main and secondary veins, secondary bacterial rots may be present (more than 8.00 cm^2); 2.0 = moderate levels of lesions of various sizes (about 1 cm^2); 3.0 = two or three small lesions or streaks, the lettuce head is still marketable (about 0.09 cm^2); 4.0 = two small spots or one fine streak, light in color (about 0.04 cm^2), 5.0 = no symptoms
STL	Stem length	(cm)	Distance from the base of the head to the shoot tip
FIRM	Head firmness	(1–5)	1 = soft, easily compressed or spongy; 2 = fairly firm, neither soft nor firm, good head formation; 3 = firm, compact, but may yield slightly to moderate pressure, commercially acceptable; 4 = hard, compact and solid; and 5 = extra-hard, over-mature, may have cracked mid-ribs (Kader et al. 1973)
CONINC	Conic head incidence	(%)	Percent heads with a conic shape and a spiraled cap leaf
TYPE	Lettuce type	(1–4)	1 = Empire type meaning light-green leaves, deeply serrated leaves, very crisp, heads often conical in shape, butt whitish with thin and flat ribs, 2 = more Empire than Salinas type, 3 = more Salinas than Empire type, 4 = Salinas type meaning dull-green leaves with softer texture, wavy leaf margins, round head shape, green butt with large ribs (adapted from Ryder 1986)
IRCINC	Internal rib cracking	(%)	Percent plants showing V-shape breaks on the inner side of the main rib (not present on the outside)
RIB	Ribbiness	(1–5)	1 = very ribby; 2 = ribby, 3 = slightly ribby; 4 = mostly flat ribs; 5 = flat ribs

by permutation analysis with 1,000 permutations (Churchill and Doerge 1994).

All traits were analyzed using mean values for each RIL. Normality and homogeneity of variance was verified for each trait using PROC UNIVARIATE of SAS (SAS Institute 2008) and no data transformation was required. The correlation among traits was analyzed using XLSTAT software (Addinsoft 2010). A Student's *t* test was performed for each variable to assess the significance of the difference between the two parents, Emperor and El Dorado.

Results

Trait variation

Planting dates and maturity affected the expression of the physiological disorders in the mapping population. As expected, stems became longer and heads firmer with advancing maturity. When conditions were conducive to the expression of tipburn and rib discoloration, incidences were consistently higher and symptoms more severe when heads were harvested at later maturity (Table 2; Fig. 1). With the exception of tipburn, the performance of the two parents for the measured traits showed the same trends across maturities, plantings and years. El Dorado had higher incidences of rib discoloration than Emperor ($P = 0.01$–0.0001 in 4 out of 6 trials) and more severe symptoms ($P = 0.05$–0.001 in 4 out of 6 trials) as well as longer stems ($P = 0.01$–0.001 in 5 out of 6 trials), firmer heads ($P = 0.05$–0.0001 in 5 out of 6 trials), and was more prone to ribbiness ($P = 0.05$ and 0.001 in 2 trials) and to internal rib cracking ($P < 0.05$ in 1 out of 6 trials). Conic head shape was not observed in the parents in 2008 and was only present at low (1–13 %) incidence in Emperor in 2009 with no significant difference between the parents. Tipburn was present in both parents in all trials but trends were not consistent and depended on year, planting and maturity. In 2008, more Emperor plants exhibited tipburn than El Dorado plants at the earlier maturity in the first planting ($P < 0.001$), but the two parents were not significantly different at the later maturity or the intermediate maturity in the second planting. In 2009, tipburn was absent or rarely present in the parents in the first planting and there was no significant difference between the parents. However, in the second planting El Dorado had a higher incidence of tipburn compared to Emperor ($P < 0.05$) (Table 2; Fig. 1).

Tipburn, rib discoloration, stem length, head firmness, lettuce type and ribbiness all segregated widely among the 152 RILs (Fig. 1). Transgressive segregation was observed for tipburn incidence; RILs with higher and lower incidences than either parent were observed at both maturities in planting 1 and only in one direction in planting 2 in 2008, and in both directions in planting 2 but only in one direction in planting 1 in 2009. For the incidence of rib discoloration, transgressive segregation in one parental direction occurred in all trials except in the second planting in 2008. For stem length, transgressive phenotypes were observed in one parental direction in all trials except in the second planting of 2008 when they surpassed both parents. For head firmness, transgressive segregation was observed in one direction in planting 1 maturity 2 in 2008, and in both directions in planting 1 maturity 1 and planting 2 in 2009. Finally, transgressive phenotypes were observed for ribbiness in both directions in the two trials in which the trait was measured (Fig. 1).

Correlations among traits

Incidence and severity were highly correlated ($P < 0.0001$) for both rib discoloration and tipburn in each environment-by-maturity combination (Table 3). Therefore, either incidence or severity would be an appropriate measurement of the manifestation of each of these two physiological disorders. However, incidences of tipburn and rib discoloration were not correlated with each other. Incidence of rib discoloration was highly positively correlated with firmer heads (r from 0.32 to 0.63, $P < 0.0001$) and longer stems (r from 0.41 to 0.64, $P < 0.0001$) in each of the environment-by-maturity combinations. Tipburn incidence was not correlated with stem length and only moderately correlated with head firmness in the first planting of 2008 ($r = 0.16$, $P < 0.05$ for M1 and $r = 0.22$, $P < 0.01$ for M2). High incidence of tipburn was clearly associated with the Empire (Emperor) head type; correlation coefficients ranged from -0.39 to -0.75 ($P < 0.0001$) depending on planting and maturity (Table 1). In contrast, rib discoloration was more associated with the Salinas (El Dorado) head type, with correlation coefficients ranging from 0.17 ($P < 0.05$) to 0.33 ($P < 0.0001$) for the second planting of 2008 and all planting-by-maturity combinations in 2009 (Table 1). Ribbiness was evaluated only in 2009 in two different plantings. High incidences of tipburn and rib discoloration were associated with a high degree of ribbiness (i.e., low ribbiness index; Table 1) only in the second planting, a trial during which heat stress was particularly severe. Incidences of neither tipburn nor rib discoloration were correlated with the incidence of internal rib cracking.

Lettuce heads of the Salinas type were associated with longer stems, particularly in the second plantings of 2008 ($r = 0.35$, $P < 0.0001$) and 2009 ($r = 0.38$, $P < 0.0001$) when most of the growing season occurred during long days and warm growing conditions. Ribby heads (i.e., with low ribbiness index) were also associated with long stems ($r = -56$, $P < 0.0001$ in 2008 and $r = -0.41$, $P < 0.0001$ in 2009) but not with head type. Internal rib

Table 2 Mean and range values for measured traits in RIL population and parents

Planting	Maturity	2008						2009					
		Parents		RILs				Parents		RILs			
		Emperor	Eldorado	Min	Max	Mean	±SE	Emperor	Eldorado	Min	Max	Mean	±SE
Tipburn incidence (TBINC) (%)													
P1	M1	56.7	13.3***	0.0	100.0	43.6	2.90	0.0	0.0 ns	0.0	80.0	6.0	0.99
P1	M2	70.0	86.7 ns	0.0	100.0	68.1	2.55	0.0	5.7 ns	0.0	100.0	25.4	2.37
P2	M1_M2	3.3	26.7 ns	0.0	100.0	41.7	2.82	10.0	34.7*	0.0	100.0	47.9	3.03
Tipburn severity (TBSEV) (1–5)													
P1	M1	3.6	4.5 ns	1.0	5.0	3.5	0.11	5.0	5.0 ns	2.4	5.0	4.9	0.02
P1	M2	2.6	2.0 ns	1.0	5.0	2.5	0.11	5.0	4.9 ns	1.2	5.0	4.3	0.08
P2	M1_M2	4.9	4.1 ns	1.0	5.0	3.5	0.11	4.8	4.2 ns	1.0	5.0	3.4	0.11
Rib discoloration incidence (RDINC) (%)													
P1	M1	0.0	100.0****	0.0	100.0	32.7	2.15	1.0	75.2**	0.0	86.7	11.5	1.43
P1	M2	86.7	100.0 ns	33.3	100.0	88.9	1.26	4.8	87.6****	0.0	100.0	34.5	2.26
P2	M1_M2	60.0	100.0 ns	6.7	100.0	84.5	1.55	0.0	86.7**	0.0	100.0	29.9	2.19
Rib discoloration severity (RDSEV) (1–5)													
P1	M1	5.0	1.3**	1.3	5.0	4.2	0.06	5.0	3.5**	3.0	5.0	4.8	0.03
P1	M2	2.8	1.0**	1.0	4.2	1.8	0.06	4.9	2.6***	1.5	5.0	4.2	0.06
P2	M1_M2	3.6	1.0 ns	1.0	4.8	2.1	0.07	5.0	2.4*	2.2	5.0	4.4	0.06
Stem length (STL) (cm)													
P1	M1	3.9	6.5 ns	3.2	7.2	4.8	0.06	2.3	4.9***	2.1	5.7	3.6	0.05
P1	M2	3.7	7.9***	3.4	13.3	5.9	0.13	3.0	6.4***	3.0	8.3	4.7	0.08
P2	M1_M2	6.9	14.8**	5.1	27.9	11.9	0.36	2.8	6.3***	2.8	7.9	4.6	0.08
Head firmness (FIRM) (1–5)													
P1	M1	3.1	4.0*	2.0	4.2	3.3	0.04	2.4	3.8**	1.2	4.1	2.8	0.06
P1	M2	3.7	4.5**	2.4	4.5	3.8	0.03	3.3	4.2*	2.3	4.4	3.6	0.04
P2	M1_M2	3.8	4.0 ns	2.7	4.4	3.7	0.03	2.6	3.9****	1.7	4.3	3.3	0.04
Conic head incidence (CONINC) (%)													
P1	M1	0.0	0.0	0.0	80.0	4.3	0.95	13.3	0.0 ns	0.0	66.7	6.7	1.03
P1	M2	0.0	0.0	0.0	66.7	3.5	0.79	2.5	0.0 ns	0.0	40.0	1.4	0.41
P2	M1_M2	0.0	0.0	0.0	33.3	1.2	0.43	1.3	0.0 ns	0.0	66.7	1.8	0.65
Lettuce type (TYPE) (1–4)													
P1	M1	1.0	4.0	1.0	4.0	2.7	0.10	1.0	4.0	1.0	4.0	2.6	0.11
P1	M2	1.0	4.0	1.0	4.0	2.6	0.10	1.0	4.0	1.0	4.0	2.5	0.11
P2	M1_M2	1.0	4.0	1.0	4.0	2.7	0.09	1.0	4.0	1.0	4.0	2.3	0.10
Internal rib cracking (IRCINC) (%)													
P1	M1	0.0	6.7 ns	0.0	20.0	1.0	0.24	0.8	1.7 ns	0.0	26.7	1.9	0.34
P1	M2	0.0	3.3 ns	0.0	6.7	0.2	0.10	0.8	4.2*	0.0	60.0	2.2	0.50
P2	M1_M2	0.0	0.0	0.0	8.3	0.3	0.11	0.0	1.3 ns	0.0	13.3	0.6	0.16
Ribbiness (RIB) (1–5)													
P1	M1	–	–	–	–	–	–	–	–	–	–	–	–
P1	M2	–	–	–	–	–	–	3.1	2.0***	1.2	4.5	2.7	0.06
P2	M1_M2	–	–	–	–	–	–	3.3	1.9*	1.0	4.0	2.4	0.06

–, data not recorded or with no variation; ns, not significant

Significance at *, **, ***, **** $P < 0.05$, 0.01, 0.001 and 0.0001, respectively

cracking was not associated with any of the other traits recorded with the exception of high ribbiness in the second planting of 2009 ($r = -0.18$, $P < 0.05$), both disorders being likely associated with rapid growth of the ribs. Except in one trial in which average stem length was extremely long (Planting 2 2008, average length 11.9 cm,

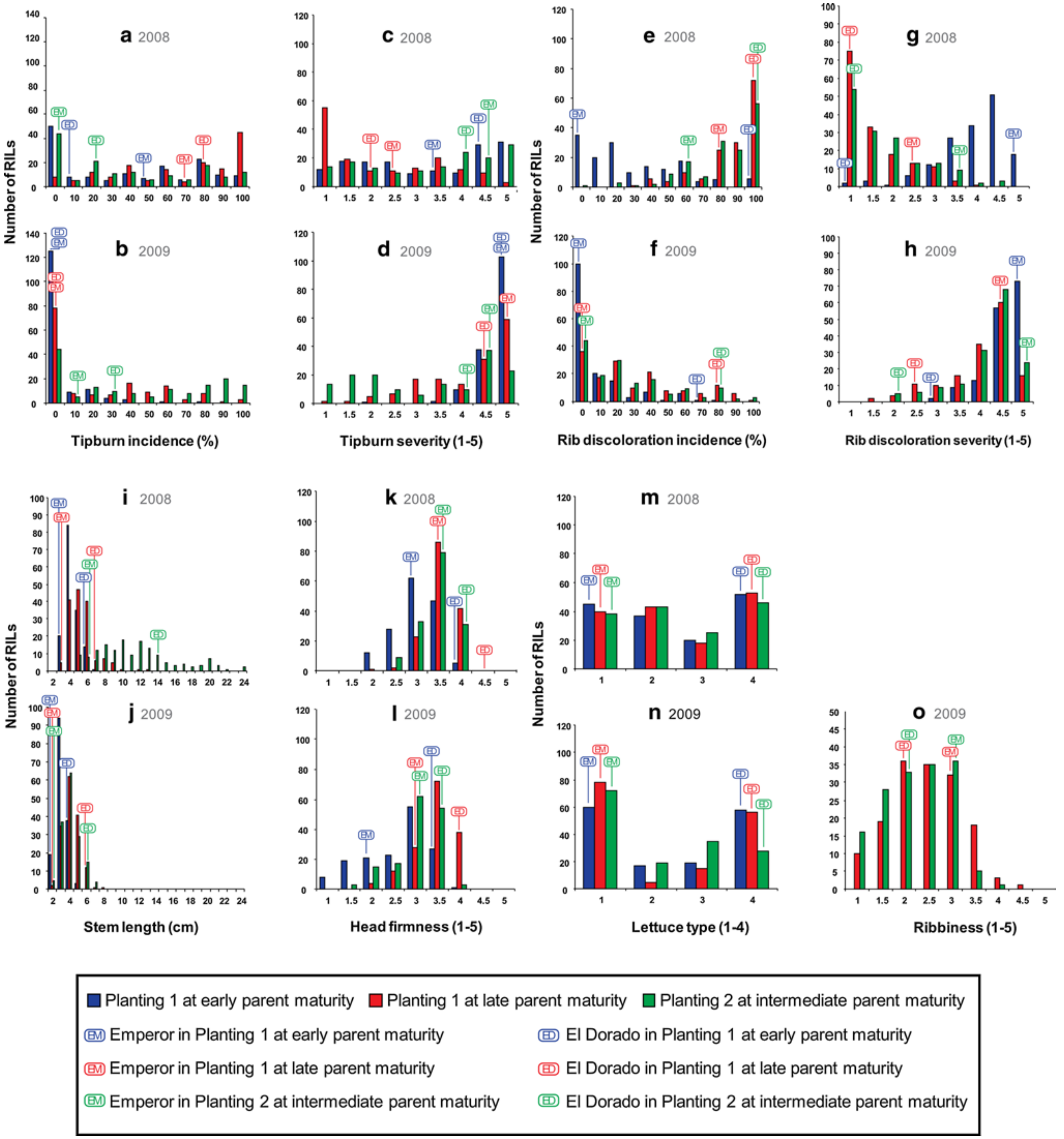

Fig. 1 Frequency distributions of selected traits in the RIL mapping population. **a** Tipburn incidence in 2008 and **b** in 2009, **c** tipburn severity in 2008 and **d** in 2009, **e** rib discoloration incidence in 2008 and **f** in 2009, **g** rib discoloration severity in 2008 and **h** in 2009, **i** stem length in 2008 and **j** in 2009, **k** head firmness in 2008 and **l** in 2009, **m** lettuce type in 2008 and **n** in 2009; and **o** ribbiness in 2009. Data were collected at two maturities (M1 = maturity of the early parent, El Dorado; M2 = maturity of the late parent, Emperor) for the first planting (P1) and one maturity (M1_M2 = maturity intermediate between the two parents) for the second planting (P2) in 2008 and 2009

Table 2), stem length and head firmness were highly and positively correlated (*r* from 0.16 to 0.36, $P < 0.05$ to $P < 0.0001$).

High incidence of conic heads was significantly correlated with low incidence of rib discoloration and shorter stems in both maturities of the first planting in 2009, high

Table 3 Pearson's correlation coefficients of trait means of parents and RIL mapping population ($n = 154$) collected from lettuce heads at two maturities (M1 = maturity of the early parent Eldorado, M2 = maturity of the late parent Emperor) for the first planting (P1) and one maturity (M1_M2 = maturity intermediate between the two parents) for the second planting (P2) in 2008 and 2009

Traits	Year, planting and maturity	TBINC	TBSEV	RDINC	RDSEV	STL	FIRM	CONINC	TYPE	IRCINC
TBSEV	2008P1M1	−0.99****								
TBSEV	2008P1M2	−0.99****								
TBSEV	2008P2M1_M2	−0.99****								
TBSEV	2009P1M1	−0.95****								
TBSEV	2009P1M2	−0.98****								
TBSEV	2009P2M1_M2	−0.98****								
RDINC	2008P1M1	0.11	−0.10							
RDINC	2008P1M2	0.09	−0.09							
RDINC	2008P2M1_M2	−0.06	0.08							
RDINC	2009P1M1	0.13	−0.19*							
RDINC	2009P1M2	−0.10	0.10							
RDINC	2009P2M1_M2	−0.02	0.03							
RDSEV	2008P1M1	−0.12	0.12	−0.97****						
RDSEV	2008P1M2	−0.12	0.13	−0.95****						
RDSEV	2008P2M1_M2	0.10	−0.10	−0.94****						
RDSEV	2009P1M1	−0.13	0.18*	−0.97****						
RDSEV	2009P1M2	0.07	−0.07	−0.96****						
RDSEV	2009P2M1_M2	0.02	−0.03	−0.97****						
STL	2008P1M1	−0.11	0.12	0.48****	−0.50****					
STL	2008P1M2	−0.02	0.02	0.41****	−0.48****					
STL	2008P2M1_M2	−0.11	0.11	0.53****	−0.60****					
STL	2009P1M1	−0.02	0.02	0.46****	−0.44****					
STL	2009P1M2	−0.01	0.00	0.56****	−0.53****					
STL	2009P2M1_M2	−0.01	−0.01	0.64****	−0.60****					
FIRM	2008P1M1	0.16*	−0.13	0.63****	−0.61****	0.29***				
FIRM	2008P1M2	0.22**	−0.21*	0.55****	−0.60****	0.22**				
FIRM	2008P2M1_M2	0.01	0.01	0.32****	−0.33****	−0.03				
FIRM	2009P1M1	0.03	−0.04	0.40****	−0.36****	0.16*				
FIRM	2009P1M2	−0.11	0.09	0.40****	−0.36****	0.22**				
FIRM	2009P2M1_M2	0.03	−0.05	0.45****	−0.40****	0.36****				
CONINC	2008P1M1	0.34****	−0.35****	−0.04	0.03	0.14	−0.14			
CONINC	2008P1M2	0.21**	−0.21**	−0.04	0.08	−0.08	−0.24**			
CONINC	2008P2M1_M2	−0.02	0.03	0.07	−0.07	0.14	−0.11			
CONINC	2009P1M1	0.19*	−0.17*	−0.17*	0.15	−0.17*	−0.46****			
CONINC	2009P1M2	0.11	−0.07	−0.20*	0.17*	−0.16*	−0.34****			
CONINC	2009P2M1_M2	0.02	−0.01	−0.02	0.05	−0.01	−0.20*			
TYPE	2008P1M1	−0.61****	0.59****	−0.13	0.11	0.19*	−0.41****	0.02		
TYPE	2008P1M2	−0.69****	0.70****	−0.04	0.04	0.19*	−0.35****	−0.15		
TYPE	2008P2M1_M2	−0.50****	0.50****	0.17*	−0.17*	0.26***	−0.33****	0.23**		
TYPE	2009P1M1	−0.39****	0.32****	0.19*	−0.18*	0.35****	−0.04	−0.24**		
TYPE	2009P1M2	−0.67****	0.62****	0.17*	−0.16*	0.20*	−0.09	−0.14		
TYPE	2009P2M1_M2	−0.75****	0.72****	0.33****	−0.33****	0.38****	0.06	0.04		
IRCINC	2008P1M1	0.09	−0.10	0.00	0.01	0.04	−0.08	0.00	0.02	
IRCINC	2008P1M2	−0.01	0.03	0.09	−0.06	0.10	0.13	−0.07	0.08	
IRCINC	2008P2M1_M2	0.10	−0.07	0.07	−0.02	−0.08	0.02	−0.01	0.03	
IRCINC	2009P1M1	−0.02	0.02	−0.03	0.02	0.13	−0.13	0.12	0.04	

Table 3 continued

Traits	Year, planting and maturity	TBINC	TBSEV	RDINC	RDSEV	STL	FIRM	CONINC	TYPE	IRCINC
IRCINC	2009P1M2	0.00	0.03	−0.06	0.05	0.00	−0.02	−0.02	0.03	
IRCINC	2009P2M1_M2	0.06	−0.09	0.07	−0.03	0.15	−0.01	0.04	0.05	
RIB[a]	2009P1M2	−0.07	0.07	−0.14	0.15	−0.56****	0.16*	−0.01	−0.04	−0.16
RIB	2009P2M1_M2	−0.21**	0.22**	−0.17*	0.19*	−0.41****	0.05	0.12	0.04	−0.18*

TBINC tipburn incidence (%), *TBSEV* tipburn severity (1 to 5, 1 = severe symptoms, 5 = no symptoms), *RDINC* rib discoloration incidence (%), *RDSEV* rib discoloration severity (1 to 5, 1 = severe symptoms, 5 = no symptoms), *STL* stem length (cm), *FIRM* head firmness (1 to 5, 1 = soft, 5 = extra-hard), *CONINC* conic head incidence (%), *TYPE* lettuce type (1 to 4, 1 = Empire type, 4 = Salinas type), *IRCINC* internal rib cracking incidence (%), *RIB* ribbiness (1 to 5, 1 = very ribby, 5 = flat ribs)

Significant at *, **, ***, **** $P < 0.05$, 0.01, 0.001 and 0.0001, respectively

[a] Ribbiness was evaluated only for the two plantings in 2009

incidence of tipburn in both maturities of the first planting in 2008 and first maturity of first planting in 2009, and softer heads in all plantings and maturities in 2009. Under high temperature conditions, the presence of a cap leaf on top of a conically shaped head may protect the head from heating up and reduce rib discoloration, but it may also reduce transpiration and calcium movement in the transpiration stream, thereby promoting tipburn (Barta and Tibbitts 1986).

Map construction and QTL analysis

Of the 768 SNP markers assayed, 449 were polymorphic between Emperor and El Dorado. Polymorphic markers were scored in the 152 RILs and a genetic map developed. This genetic map and genotypic information for all the polymorphic markers can be accessed at http://cgpdb.ucdavis.edu/supplemental_data/. The map covered 807 cM in 20 LGs compared to 1,505 cM in the ultrahigh-density map of the nine lettuce chromosomes (Truco et al. 2013). Linkage groups were assigned to chromosomes by aligning them to previous lettuce maps (http://cgpdb.ucdavis.edu/GeneticMapViewer/display/). Distances in each of the two maps were concordant (Fig. 2). Chromosomes 5, 6 and 9 were represented by two LGs and chromosomes 1, 3, 4, 8 by three. Map fragmentation may be the result of the presence of chromosomal regions that are identical by descent due to the close genetic relatedness of the two crisphead parents. Overall, the 20 LGs in this map covered 51 % of the lettuce ultrahigh-density map (Truco et al. 2013), with coverage ranging from 8 % for chromosome 3 to 94 % for chromosome 2 (Fig. 2).

Composite interval mapping revealed 36 significant QTLs for eight out of the ten traits evaluated in up to four plantings and two harvest maturities (Table 4). These eight traits included four physiological disorders (tipburn, rib discoloration, premature bolting and ribbiness) and two agronomic traits (head firmness and head type). Individual QTLs accounted for 1–83 % of the phenotypic variation and were distributed in 11 LGs on 7 chromosomes.

Fig. 2 Genetic linkage map showing distribution of QTLs for traits ▶ associated with physiological disorders and maturity. QTLs for each trait are color coded: tipburn incidence and severity in dark blue; rib discoloration incidence and severity in red; stem length in olive; head firmness in dark green; lettuce head type in maroon and ribbiness in fuchsia. QTL names have three-letter abbreviations followed by the number of the linkage group where it was detected and a numeral to distinguish multiple QTLs for the trait in the same linkage group. To aid identification of QTLs in this figure, we added to the name the maturity (M1 = maturity of the early parent, El Dorado; M2 = maturity of the late parent, Emperor first planting; M1_M2 = maturity intermediate between the two parents second planting) and the trial year: 2008 (08; *solid bars*) and 2009 (09; *hatched bars*). Linkage group numbers are shown on top of the groups, and map distance (cM) is shown on the left margin of the figure. The ultrahigh-density map (Truco et al. 2013) is depicted as reference for coverage to the left of each of the linkage groups. Common markers flanking the intervals are shown

Five significant QTLs for tipburn incidence and severity were identified in LGs 2, 5a, 5b, 8a and 8c (Table 4). QTL *qTPB5.2* was detected on LG 5b in all five trials in which the disorder was well expressed and exhibited more than 25 % incidence (Table 2). This QTL contributed from 38 to 70 % of the variance in tipburn incidence and 29–70 % in severity. The allele from El Dorado decreased tipburn incidence and severity. Two other QTLs for tipburn, *qTPB2.1* on LG 2 and *qTPB5.1* on LG 5a (Table 4), were significant in two and one trial(s), respectively. *qTPB2.1* contributed 4–6 % of the variance in incidence and 6–9 % of the variance of severity, whereas *qTPB5.1* contributed to 5 and 6 % of the variance in incidence and severity of the trait, respectively. At these two QTLs, the Emperor allele decreased tipburn incidence and severity. Two additional QTLs for tipburn incidence and severity, *qTPB8.2* on LG 8a and *qTPB8.1* on LG 8a, respectively, contributed less than 4 % of the variance and were detected in a single trial. Altogether these five QTLs accounted for up to 70 and 73 % of the phenotypic variance for tipburn incidence and severity, respectively, depending on the trial.

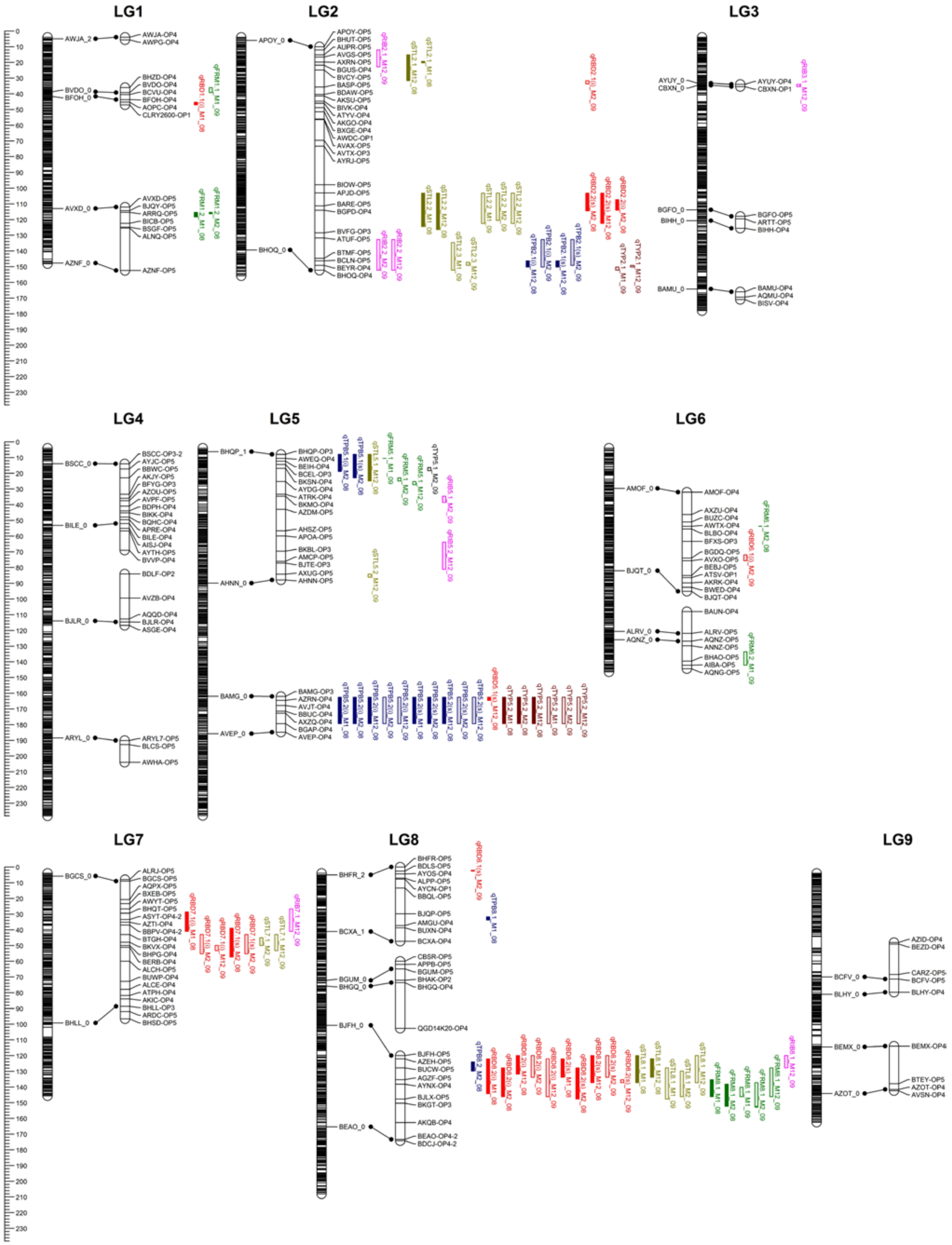
LG1
LG2
LG3
LG4
LG5
LG6
LG7
LG8
LG9
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
AWJA_2
AWJA-OP4
AWPG-OP4
BVDO_0
BFOH_0
BHZD-OP4
BVDO-OP4
BCVU-OP4
BFOH-OP4
AOPC-OP4
CLRY2600-OP1
qFRM1.1_M1_09
qRBD1.1(i)_M1_08
AVXD_0
AVXD-OP5
BJQY-OP5
ARRQ-OP5
BICB-OP5
BSGF-OP5
ALNQ-OP5
qFRM1.2_M1_08
qFRM1.2_M2_08
AZNF_0
AZNF-OP5
APOY_0
APOY-OP5
BHUT-OP5
AUPR-OP5
AVGS-OP5
AXRN-OP5
BGUS-OP4
BVCY-OP5
BASP-OP5
BDAW-OP5
AKSU-OP5
BIVK-OP4
ATYV-OP4
AKGO-OP4
BXGE-OP4
AWDC-OP1
AVAX-OP5
AVTX-OP3
AYRJ-OP5
BIOW-OP5
APJD-OP5
BARE-OP5
BGPD-OP4
BVFG-OP3
ATUF-OP5
BTMF-OP5
BCLN-OP5
BEYR-OP4
BHOQ-OP4
BHOQ_0
qRIB2.1_M12_09
qSTL2.1_M12_08
qSTL2.1_M1_08
qRBD2.1(i)_M2_09
qSTL2.2_M1_08
qSTL2.2_M12_08
qSTL2.2_M1_09
qSTL2.2_M2_09
qSTL2.2_M12_09
qRBD2.2(s)_M2_08
qRBD2.2(s)_M12_08
qRBD2.2(i)_M2_08
qRIB2.2_M2_09
qRIB2.2_M12_09
qSTL2.3_M1_09
qSTL2.3_M12_09
qTPB2.1(i)_M12_08
qTPB2.1(i)_M2_09
qTPB2.1(s)_M12_08
qTPB2.1(s)_M2_09
qTYP2.1_M1_09
qTYP2.1_M12_09
AYUY_0
CBXN_0
AYUY-OP4
CBXN-OP1
qRIB3.1_M12_09
BGFO_0
BIHH_0
BGFO-OP5
ARTT-OP5
BIHH-OP4
BAMU_0
BAMU-OP4
AQMU-OP4
BISV-OP4
BSCC_0
BSCC-OP3-2
AYJC-OP5
BBWC-OP5
AKJY-OP5
BFYG-OP3
AZOU-OP5
AVPF-OP5
BDPH-OP4
BIKK-OP4
BQHC-OP4
APRE-OP4
BILE-OP4
AISJ-OP4
AYTH-OP5
BVVP-OP4
BILE_0
BDLF-OP2
AVZB-OP4
AQQD-OP4
BJLR-OP4
ASGE-OP4
BJLR_0
ARYL_0
ARYL7-OP5
BLCS-OP5
AWHA-OP5
BHQP_1
BHQP-OP3
AWEQ-OP4
BEIH-OP4
BCEL-OP3
BKSN-OP4
AYDG-OP4
ATRK-OP4
BKMO-OP4
AZDM-OP5
AHSZ-OP5
APOA-OP5
BKBL-OP3
AMCP-OP5
BJTE-OP3
AXUG-OP5
AHNN-OP5
AHNN_0
qTPB5.1(i)_M2_08
qTPB5.1(s)_M2_08
qSTL5.1_M12_08
qFRM5.1_M1_09
qFRM5.1_M2_09
qFRM5.1_M12_09
qTYP5.1_M2_09
qRIB5.1_M2_09
qRIB5.2_M12_09
qSTL5.2_M12_09
BAMG_0
AVEP_0
BAMG-OP3
AZRN-OP4
AVJT-OP4
BBUC-OP4
AXZQ-OP4
BGAP-OP4
AVEP-OP4
qTPB5.2(i)_M1_08
qTPB5.2(i)_M2_08
qTPB5.2(i)_M12_08
qTPB5.2(i)_M2_09
qTPB5.2(i)_M12_09
qTPB5.2(s)_M1_08
qTPB5.2(s)_M2_08
qTPB5.2(s)_M12_08
qTPB5.2(s)_M2_09
qTPB5.2(s)_M12_09
qRBD5.1(s)_M12_08
qTYP5.2_M1_08
qTYP5.2_M2_08
qTYP5.2_M12_08
qTYP5.2_M1_09
qTYP5.2_M2_09
qTYP5.2_M12_09
AMOF_0
AMOF-OP4
AXZU-OP4
BUZC-OP4
AWTX-OP4
BLBO-OP4
BFXS-OP3
BGDQ-OP5
AVXO-OP5
BEBJ-OP5
ATSV-OP1
AKRK-OP4
BWED-OP4
BJQT-OP4
BJQT_0
qFRM6.1_M2_08
qRBD6.1(i)_M2_09
BAUN-OP4
ALRV_0
AQNZ_0
ALRV-OP5
AQNZ-OP5
ANNZ-OP5
BHAO-OP5
AIBA-OP5
AQNG-OP5
qFRM6.2_M1_09
BGCS_0
ALRJ-OP5
BGCS-OP5
AQPX-OP5
BXEB-OP5
AWYT-OP5
BHQT-OP5
ASYT-OP4-2
AZTI-OP4
BBPV-OP4-2
BTGH-OP4
BKVX-OP4
BHPG-OP4
BERB-OP4
ALCH-OP5
BUWP-OP4
ALCE-OP4
ATPH-OP4
AKIC-OP4
BHLL-OP3
ARDC-OP5
BHSD-OP5
BHLL_0
qRBD7.1(i)_M1_08
qRBD7.1(i)_M2_09
qRBD7.1(i)_M12_09
qRBD7.1(s)_M2_08
qRBD7.1(s)_M2_09
qSTL7.1_M2_09
qSTL7.1_M12_09
qRIB7.1_M12_09
BHFR_2
BHFR-OP5
BDLS-OP5
AYOS-OP4
ALPP-OP5
AYCN-OP1
BBQL-OP5
BJQP-OP5
AMGU-OP4
BUXN-OP4
BCXA-OP4
BCXA_1
CBSR-OP5
APPB-OP5
BGUM-OP5
BHAK-OP2
BHGQ-OP4
BGUM_0
BHGQ_0
QGD14K20-OP4
BJFH_0
BJFH-OP5
AZEH-OP5
BUCW-OP5
AGZF-OP5
AYNX-OP4
BJLX-OP5
BKGT-OP3
AKQB-OP4
BEAO-OP4-2
BDCJ-OP4-2
BEAO_0
qRBD8.1(s)_M2_09
qTPB8.1_M1_08
qTPB8.2_M2_08
qRBD8.2(i)_M1_08
qRBD8.2(i)_M2_08
qRBD8.2(i)_M12_08
qRBD8.2(i)_M2_09
qRBD8.2(i)_M12_09
qRBD8.2(s)_M1_08
qRBD8.2(s)_M2_08
qRBD8.2(s)_M12_08
qRBD8.2(s)_M2_09
qRBD8.2(s)_M12_09
qSTL8.1_M1_08
qSTL8.1_M12_08
qSTL8.1_M1_09
qSTL8.1_M2_09
qSTL8.1_M12_09
qFRM8.1_M1_08
qFRM8.1_M2_08
qFRM8.1_M1_09
qFRM8.1_M2_09
qFRM8.1_M12_09
qRIB8.1_M12_09
AZID-OP4
BEZD-OP4
CARZ-OP5
BCFV-OP5
BLHY-OP4
BCFV_0
BLHY_0
BEMX_0
BEMX-OP4
BTEY-OP5
AZOT-OP4
AVSN-OP4
AZOT_0

Table 4 QTLs detected for tipburn, physiological disorders associated with head stress and head maturity in a RIL population evaluated during two plantings (P1 and P2) at maturity of the early parent El Dorado (M1), at maturity of the late parent Emperor (M2) and at maturity intermediate between the two parents (M1_M2) in 2008 and 2009

Trait	Year	Planting	Maturity	QTL[a]	Linkage group	Closest marker	Significant QTL interval (cM)	LOD	R^2 [b]	Parental allele[c]
Tip burn incidence (%)	2008	P1	M1	*qTPB5.2*	*5b*	AVJT-OP4	0–17	43.2	68.1	Emperor
	2008	P1	M2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	30.4	58.1	Emperor
	2008	P2	M1_2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	23.4	44.2	Emperor
	2009	P1	M2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	23	38.4	Emperor
	2009	P2	M1_2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	44.6	70.0	Emperor
	2008	P2	M1_2	*qTPB2.1*	*2*	BCLN-OP5	136–140	3.5	4.5	El Dorado
	2009	P1	M2	*qTPB2.1*	*2*	BCLN-OP5	123–140	4.6	5.8	El Dorado
	2008	P1	M2	*qTPB5.1*	*5a*	BEIH-OP4	0–11	4.7	5.2	El Dorado
	2008	P1	M2	*qTPB8.2*	*8c*	AZEH-OP5	4–10	3.4	3.9	El Dorado
Tip burn severity (1–5 scale)	2008	P1	M1	*qTPB5.2*	*5b*	AVJT-OP4	0–17	42.5	70.3	El Dorado
	2008	P1	M2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	33.3	59.1	El Dorado
	2008	P2	M1_2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	23.3	41.7	El Dorado
	2009	P1	M2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	17	29.0	El Dorado
	2009	P2	M1_2	*qTPB5.2*	*5b*	AVJT-OP4	0–17	41	65.0	El Dorado
	2008	P2	M1_2	*qTPB2.1*	*2*	BCLN-OP5	136–140	4.4	5.6	Emperor
	2009	P1	M2	*qTPB2.1*	*2*	BTMF-OP5	123–139	4.5	8.5	Emperor
	2008	P1	M2	*qTPB5.1*	*5a*	BCEL-OP3	0–15	5.7	5.8	Emperor
	2008	P1	M1	*qTPB8.1*	*8a*	BJQP-OP5	32–34	3	2.7	El Dorado
Rib discoloration incidence (%)	2008	P1	M1	*qRBD8.1*	*8c*	BUCW-OP5	2–24	10.6	23.6	El Dorado
	2008	P1	M2	*qRBD8.1*	*8c*	AYNX-OP4	8–26	6.5	18.2	El Dorado
	2008	P2	M1_2	*qRBD8.1*	*8c*	BUCW-OP5	0–12	6.3	12.7	El Dorado
	2009	P1	M2	*qRBD8.1*	*8c*	AZEH-OP5	0–14	6	16.0	El Dorado
	2009	P2	M1_2	*qRBD8.1*	*8c*	BUCW-OP5	2–26	6.7	15.7	El Dorado
	2008	P1	M1	*qRBD7.1*	*7*	BBPV-OP4-2	21–33	4.3	8.7	El Dorado
	2009	P1	M2	*qRBD7.1*	*7*	BKVX-OP4	35–47	3.7	8.2	El Dorado
	2009	P2	M1_2	*qRBD7.1*	*7*	BHPG-OP4	42–45	4.3	8.9	El Dorado
	2008	P1	M2	*qRBD2.2*	*2*	BARE-OP5	97–104	4.9	11.7	El Dorado
	2009	P1	M2	*qRBD2.1*	*2*	BASP-OP5	22–24	2.9	6.9	El Dorado
	2009	P1	M2	*qRBD6.1*	*6a*	AVXO-OP5	40–44	3.2	6.7	El Dorado
	2008	P1	M1	*qRBD1.1*	*1b*	AOPC-OP4	9–11	3.7	6.5	Emperor
Rib discoloration severity (1–5 scale)	2008	P1	M1	*qRBD8.1*	*8c*	AZEH-OP5	2–14	8	18.8	Emperor
	2008	P1	M2	*qRBD8.1*	*8c*	AYNX-OP4	8–28	8.5	20.5	Emperor
	2008	P2	M1_2	*qRBD8.1*	*8c*	AZEH-OP5	0–17	9.3	20.5	Emperor
	2009	P1	M2	*qRBD8.1*	*8c*	AZEH-OP5	0–14	5.4	13.2	Emperor
	2009	P2	M1_2	*qRBD8.1*	*8c*	AGZF-OP5	15–17	5.8	13.8	Emperor
	2008	P1	M2	*qRBD7.1*	*7*	BERB-OP4	31–49	4.7	9.3	Emperor
	2009	P1	M2	*qRBD7.1*	*7*	BKVX-OP4	35–47	4.1	8.2	Emperor
	2008	P1	M2	*qRBD2.2*	*2*	BARE-OP5	93–105	6.2	14.4	Emperor
	2008	P2	M1_2	*qRBD2.2*	*2*	BGPD-OP4	93–113	4.7	9.1	Emperor
	2009	P1	M2	*qRBD8.1*	*8a*	BDLS-OP5	2–3	3.5	7.1	Emperor
	2008	P2	M1_2	*qRBD5.1*	*5b*	AZRN-OP4	0–2	3.2	6.0	Emperor
Stem length (cm)	2008	P1	M1	*qSTL2.2*	*2*	BARE-OP5	93–115	6.2	14.1	El Dorado
	2008	P2	M1_2	*qSTL2.2*	*2*	BARE-OP5	93–117	7.1	14.3	El Dorado
	2009	P1	M1	*qSTL2.2*	*2*	BGPD-OP4	93–113	4.6	7.9	El Dorado
	2009	P1	M2	*qSTL2.2*	*2*	BGPD-OP4	93–111	5.3	11.2	El Dorado
	2009	P2	M1_2	*qSTL2.2*	*2*	BARE-OP5	93–113	7	14.4	El Dorado

Table 4 continued

Trait	Year	Planting	Maturity	QTL[a]	Linkage group	Closest marker	Significant QTL interval (cM)	LOD	R^2 [b]	Parental allele[c]
	2008	P1	M1	*qSTL8.1*	*8c*	AGZF-OP5	0–17	5.8	11.3	El Dorado
	2008	P2	M1_2	*qSTL8.1*	*8c*	AZEH-OP5	2–14	4.4	8.6	El Dorado
	2009	P1	M1	*qSTL8.1*	*8c*	AYNX-OP4	8–28	5.5	11.8	El Dorado
	2009	P1	M2	*qSTL8.1*	*8c*	AGZF-OP5	10–26	3.8	7.1	El Dorado
	2009	P2	M1_2	*qSTL8.1*	*8c*	BUCW-OP5	0–17	5.7	11.3	El Dorado
	2009	P1	M1	*qSTL:2.3*	*2*	BHOQ-OP4	125–142	7.4	14.2	El Dorado
	2009	P2	M1_2	*qSTL2.3*	*2*	BEYR-OP4	137–139	3.2	5.2	El Dorado
	2008	P1	M1	*qSTL2.1*	*2*	BGUS-OP4	9–10	3.2	6.1	El Dorado
	2008	P2	M1_2	*qSTL2.1*	*2*	AXRN-OP5	5–22	4.6	7.8	El Dorado
	2009	P1	M2	*qSTL7.1*	*7*	BKVX-OP4	37–42	3.8	7.8	El Dorado
	2009	P2	M1_2	*qSTL7.1*	*7*	BKVX-OP4	35–45	4.3	7.1	El Dorado
	2008	P2	M1_2	*qSTL5.1*	*5a*	BEIH-OP4	0–17	6.5	11.2	Emperor
	2009	P2	M1_2	*qSTL5.2*	*5a*	AXUG-OP5	76–78	3.6	6.5	El Dorado
Firmness (1–5 scale)	2008	P1	M1	*qFRM8.1*	*8c*	AYNX-OP4	15–26	6.2	16.6	El Dorado
	2008	P1	M2	*qFRM8.1*	*8c*	AYNX-OP4	18–32	4.3	10.0	El Dorado
	2009	P1	M1	*qFRM8.1*	*8c*	AYNX-OP4	20–26	3.4	8.2	El Dorado
	2009	P1	M2	*qFRM8.1*	*8c*	AYNX-OP4	17–33	5.3	13.3	El Dorado
	2009	P2	M1_2	*qFRM8.1*	*8c*	AYNX-OP4	8–26	6	14.7	El Dorado
	2009	P1	M1	*qRFM5.1*	*5a*	AWEQ-OP4	2	3	6.2	El Dorado
	2009	P1	M2	*qRFM5.1*	*5a*	BKSN-OP4	15–17	3.3	9.2	El Dorado
	2009	P2	M1_2	*qRFM5.1*	*5a*	AYDG-OP4	17–19	3.1	6.4	El Dorado
	2009	P1	M1	*qRFM6.2*	*6b*	BHAO-OP5	26–34	3.4	9.4	El Dorado
	2009	P1	M1	*qFRM1.1*	*1b*	BHZD-OP4	0–3	4.1	9.3	Emperor
	2008	P1	M1	*qRFM1.2*	*1c*	ARRQ-OP5	3–6	3.9	8.2	Emperor
	2008	P1	M2	*qRFM1.2*	*1c*	ARRQ-OP5	3–4	3.5	7.8	Emperor
	2008	P1	M2	*qFRM6.1*	*6a*	AWTX-OP4	22	3.3	6.9	El Dorado
Lettuce Type (1–4 scale)	2008	P1	M1	*qTYP5.2*	*5b*	AVJT-OP4	0–17	22.5	47.0	El Dorado
	2008	P1	M2	*qTYP5.2*	*5b*	AVJT-OP4	0–17	34.3	61.9	El Dorado
	2008	P2	M1_2	*qTYP5.2*	*5b*	AZRN-OP4	0–17	20.3	42.2	El Dorado
	2009	P1	M1	*qTYP5.2*	*5b*	AVJT-OP4	0–17	43.8	41.1	El Dorado
	2009	P1	M2	*qTYP5.2*	*5b*	AVJT-OP4	0–17	61.9	83.2	El Dorado
	2009	P2	M1_2	*qTYP5.2*	*5b*	AVJT-OP4	0–17	38.5	67.3	El Dorado
	2009	P1	M1	*qTYP2.1*	*2*	BHOQ-OP4	140–142	3.2	1.9	El Dorado
	2009	P2	M1_2	*qTYP2.1*	*2*	BHOQ-OP4	139–140	2.89	2.3	El Dorado
	2009	P1	M2	*qTYP5.1*	*5a*	BCEL-OP3	8–10	3.1	1.0	Emperor
Ribbiness (1–5 scale)	2009	P1	M2	*qRIB2.2*	*2*	BHOQ-OP4	123–142	15.3	31.7	Emperor
	2009	P2	M1_2	*qRIB2.2*	*2*	BTMF-OP5	123–142	5.7	14.3	Emperor
	2009	P2	M1_2	*qRIB7.1*	*7*	BHQT-OP5	19–33	8.5	18.0	Emperor
	2009	P2	M1_2	*qRIB2.1*	*2*	BHUT-OP5	2–13	8.3	15.7	El Dorado
	2009	P2	M1_2	*qRIB5.2*	*5a*	BJTE-OP3	56–73	3.6	7.5	Emperor
	2009	P2	M1_2	*qRIB8.1*	*8c*	BJFH-OP5	0–8	3.5	5.8	Emperor
	2009	P1	M2	*qRIB5.1*	*5a*	BKMO-OP4	27–31	3.2	5.7	Emperor
	2009	P2	M1_2	*qRIB3.1*	*3a*	CBXN-OP1	0–1	3	4.9	Emperor

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[a] QTL acronyms have a three-letter designation followed by the linkage group where the QTL was detected and a number indexing the QTL. QTLs for the same trait with partial or complete overlapping positions in the genetic map were given the same acronym

[b] Percentage of the phenotypic variance explained by this QTL

[c] The parental allele from Emperor or El Dorado causing an increase in the trait value

To estimate the additive effects of QTLs *qTPB5.2*, *qTPB2.1* and *qTPB5.1* on tipburn tolerance, we calculated the change in average tipburn incidence in the population by analyzing RILS with either parental allele conferring high or low incidence at the marker closest to the QTL. The average tipburn incidence of the RILs was based on the six evaluations and valued 23.3 % for Emperor and 27.8 % for El Dorado. RILs with the El Dorado allele at marker AVTJ-OP4 (*qTPB5.2*) had 15 % lower average tip burn incidence. Emperor alleles at markers BCLN-OP5 (*qTPB2.1*) and BEIH-OP4 (*qTPB5.1*) further decreased tipburn incidence to below 10 %. Average tipburn incidence increased from 39 % to 68 % when the marker from the other parent was present at these three loci.

Eight QTLs for rib discoloration were identified on LGs 1b, 2, 5b, 6a, 7, 8a and 8c. The three largest QTLs were detected on LGs 2, 7 and 8c, with the allele from Emperor decreasing both incidence and severity of rib discoloration at all three loci. The largest, *qRBD8.1*, was detected on LG 8c in five trials in which an average of 30–89 % incidence of rib discoloration was observed and accounted for 13–24 % and 13–21 % of the variation in incidence and severity, respectively. The next largest QTL, *qRBD7.1* on LG 7, accounted for 8–9 % of the variance in incidence (three trials) and severity (two trials) of rib discoloration. The third largest, *qRBD2.2* on LG 2, accounted for 12 % of the variance in the incidence in one trial and 9–14 % of the variation in the severity in two trials. Five minor QTLs for rib discoloration were detected on LGs 1b, 2, 5b, 6a and 8a in only one trial in each case and individually contributed 6–7 % of the variation of incidence or severity (Table 4). With the exception of the QTL at LG 1b, the allele from Emperor decreased rib discoloration incidence or severity at these minor QTLs (Table 4). In total, the eight QTLs accounted for up to 39 and 44 % of the incidence and severity of rib discoloration, respectively, depending on the trial.

Seven QTLs for stem length were identified in LGs 2, 5a, 7 and 8c. This trait was the manifestation of premature bolting triggered under the long-day and warm-temperature growing conditions of these field experiments. Two QTLs, *qSTL2.2* on LG2 and *qSTL8.1* on LG 8c, were detected in five of the six trials and accounted for 8–14 % and 7–12 % of the variation in stem length, respectively. Three other QTLs, *qSTL2.3* and *qSTL2.1* on LG 2 and *qSTL7.1* on LG 7, were detected in two trials and explained 5–14 %, 6–8 % and 7–8 % of the variation, respectively. The other two QTLs were detected on LG 5a in only one trial and individually contributed 11 % (*qSTL5.1*) and 7 % (*qSTL5.2*) to the variation in stem length. With the exception of one QTL, *qSTL5.1*, the El Dorado allele was associated with increased stem length for all of the stem length QTLs. In aggregate, these seven QTLs accounted for up to 45 % of the variation for stem length.

Six QTLs for head firmness were identified in six LGs (1b, 1c, 5a, 6a, 6b and 8c). For four of the six, the allele from El Dorado increased head firmness: *qFRM8.1* on LG 8c was detected in five trials and contributed 8–16 % of the variation; *qFRM5.1* on LG5 was detected in three trials and contributed 6–9 %; the last two located separately on chromosome 6, *qFRM6.1* and *qFRM6.2*, were detected in one trial and contributed 7 and 9 % of the variation, respectively. The Emperor allele was responsible for increased head firmness at two other QTLs located on chromosome 1; *qFRM1.1* explained 9 % of the variance in a single trial and *qFRM1.2* 8 % of the variation in two trials. These six QTLs explained up to 33 % of the observed variation in head firmness.

Three QTLs for head type were detected on LGs 2, 5a and 5b. *qTYP5.2* was significant in all six trials and explained from 41 to 83 % of the variation. The other two QTLs were minor, explaining 2 % or less of the variance (Table 4).

Ribbiness was only evaluated in the two trials in 2009. One QTL, *qRIB2.2* on LG 2, was detected in both Plantings 1 and 2. In Planting 1, two QTLs were detected; *qRIB2.2* contributed 32 % and *qRIB5.1* on LG 5a contributed 6 % of the variance. In Planting 2, *qRIB2.2* contributed 14 % of the variation in ribbiness and five other QTLs were additionally detected: *qRIB7.1 on LG 7* and *qRIB2.1 on LG 2* explained 18 and 16 % of the variance, respectively, and three other QTLs individually explained 5–8 %. With the exception of *qRIB2.1*, the allele from Emperor reduced ribbiness at all of the *RIB* QTLs.

QTLs for multiple traits often mapped to the same chromosomal regions (Fig. 2; Table 4). Two clusters were identified on LG 2, one with QTLs for stem length, tipburn incidence and severity, head type, and ribbiness and the other with QTLs for stem length and incidence and severity of rib discoloration. QTLs for tipburn incidence and severity and lettuce type also overlapped in LG 5b. In addition, QTLs for tipburn, stem length, incidence and severity of rib discoloration, head firmness and ribbiness were co-located at the end of LG 8c.

Discussion

We identified a total of 36 significant QTLs determining of four economically important physiological disorders, tipburn, rib discoloration, premature bolting and ribbiness, and two agronomic traits, head firmness and head type. The map generated by analyzing RILs derived from our intraspecific crisphead x crisphead cross and used for the QTL analysis covered 51 % of the most recent and most dense lettuce genetic map (Truco et al. 2013). It is possible that there are additional QTLs in other genomic regions for which no polymorphic markers were available. However, crisphead cultivars have a narrow genetic base and the lack of polymorphic markers probably reflects regions that are identical by descent between the two parental cultivars; consequently, the chances of identifying additional QTLs that determine differences in the traits analyzed between these two cultivars are low. Therefore, most of the major QTLs determining these eight traits were likely identified in this study.

We identified five QTLs for tipburn, a trait that has thus far proven difficult to improve through breeding. The largest tipburn QTL, *qTPB5.2* on LG 5b, was detected using data from five out of six trials and contributed 38–70 % to the variation in incidence and 29–70 % to the variation in severity of tipburn. QTLs for tipburn overlapped with QTLs for lettuce head type on LGs 2, 5a and 5b reflecting the high correlation between tipburn and head type (Table 3). The region encompassing 0–9 cM on LG 5a contained overlapping QTLs for tipburn (*qTPB5.1*) and head firmness (*qFRM5.1*), with the Emperor haplotype responsible for decreasing both tipburn incidence and head firmness. LG 2 contained overlapping QTLs for tipburn, stem length, head type and ribbiness, with the Emperor haplotype decreasing incidence and severity of tipburn, stem length, and ribbiness. The co-location of QTLs determining plant architecture and tipburn is consistent with the pleiotropic effects of plant architecture on calcium movement and consequently tipburn.

Our QTL mapping results provide evidence for allele dispersion; when multiple QTLs control a trait, alleles of positive and negative effect tend to be dispersed among genotypes (Xu 2010). Although there were no significant differences in incidence and severity of tipburn between the two parents in four out of six trials, significant QTLs were still detected in the RIL data. The transgressive phenotypes observed in the RIL population arose from combinations of alleles of similar effect from different parents. Combinations of beneficial alleles from both parents resulted in a reduction in tipburn incidence; RILs with the El Dorado allele at marker AVJT-OP4 and the Emperor allele at markers BCLN-OP5 and BEIH-OP4 had average tipburn incidence of 6 % compared to 39 % for the population as a whole (Table 4).

The rib discoloration phenotype has been strongly associated with traits indicative of advanced maturity, i.e., when the head becomes firm and the stem starts to elongate (Jenni et al. 2008). In the present study, rib discoloration incidence and severity were highly correlated with stem length and head firmness and, in one case, ribbiness (Table 3). Of the three largest QTLs for rib discoloration, the largest, *qRBD8.1*, co-located on LG 8c with QTLs for stem length, head firmness and ribbiness, the second largest, *qRBD7.1*, co-located on LG 7 with QTLs for stem length and ribbiness, and the third, *qRBD2.2*, co-located on LG 2 with a QTL for stem length. In all three cases, the QTLs showed the same gene action with the allele from Emperor reducing rib discoloration incidence and severity, stem length, ribbiness and head firmness. Selection for improved resistance to any one of rib discoloration, bolting, or ribbiness should therefore result in improvement of all three traits.

Head firmness results from young leaves accumulating under the layer of leaves covering the growing point during the heading process. Conditions for successful head formation include the development of large individual leaves, a slow rate of stem elongation, short petioles and a high rate of leaf production (Bensink 1961). We identified six QTLs for head firmness segregating in this crisphead x crisphead cross. Bassett (1975) previously reported that a minimum of three and probably four or more genes contributed to heading differences between Minetto, a crisphead type, and Gallega, a non-heading Latin type with an open rosette.

Stem elongation is tightly correlated with bolting and flowering time. Analyses of several crosses between *L. sativa* and *L. serriola*, which flowered in ~36 and ~56 days, respectively, identified six genes control flowering time in lettuce (Ryder and Milligan 2005). We identified seven QTLs for stem length on four LGs; however, their relationship to previously reported genes for flowering time is currently unknown because the latter have not been mapped.

Stem length and head firmness are important traits that influence agronomic maturity of crisphead lettuce. Optimal maturity occurs at the end of the vegetative phase when the head is firm, compact and solid, but before the reproductive phase when the stem inside the head begins to elongate rapidly (Kader et al. 1973). Under optimal temperature regimes, head firmness normally increases with stem length as maturity progresses. Under heat stress which promotes premature bolting, the stem will rapidly elongate and the head may become fluffy. With the exception of one trial, in which stems grew rapidly, stem length and head firmness were highly correlated (Tables 2, 3). The overlapping of QTLs for stem length and head firmness on LG 8c (0–30 cM) revealed in multiple trials was consistent with the high correlation between these two traits. However, at least nine other QTLs associated with these traits were not located in the same LG indicating that independent mechanistic processes underlie the heading and the stem elongation phenotypes.

In summary, we identified QTLs for economically important physiological disorders and developmental phenotypes associated with heat stress in lettuce. One of the most important findings in this study was the detection of three major clusters of QTLs revealing pleiotropic effects or tight linkage between tipburn incidence and severity, head type and stem length, and head firmness and ribbiness.

One QTL contributed up to 70 % of the variance of tipburn incidence indicating that this QTL is a good candidate for fine mapping and for developing informative markers for MAS. The number and contribution of QTLs depended on the environment and maturity at which the phenotypic data was collected. Genotype-by-environment interactions that were not repeatable over years have been previously documented for tipburn using a wide range of lettuce types and varieties (Jenni and Hayes 2010); therefore, breeding for tipburn tolerance should be based on mean performance and stability over multiple environments. Consequently, QTL-by-environment interactions should be tested to evaluate whether the allele at *qTPB5.2* conferring resistance to tipburn will be useful in multiple genetic backgrounds and across multiple environments and locations.

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