Theor Appl Genet (2012) 124:1487–1502
DOI 10.1007/s00122-012-1803-0

ORIGINAL PAPER

Mapping QTL, epistasis and genotype × environment interaction of antioxidant activity, chlorophyll content and head formation in domesticated lettuce (*Lactuca sativa*)

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Received: 10 June 2011 / Accepted: 16 January 2012 / Published online: 11 February 2012

Abstract Fruits and vegetables are rich sources of antioxidants in human diets and their intake is associated with chronic disease prevention. Lettuce (*Lactuca sativa* L.) is a common vegetable in diets worldwide, but its nutritional content is relatively low. To elucidate the genetic basis of antioxidant content in lettuce, we measured the oxygen radical absorbance capacity (ORAC) and chlorophyll (Chl) content as a proxy of β-carotene in an F_8 recombinant inbred line (RIL) in multiple production cycles at two different production sites. Plants were phenotyped at the open-leaf stage to measure genetic potential (GP) or at market maturity (MM) to measure the influence of head architecture ('head' or 'open'). Main effect quantitative trait loci (QTL) were identified at MM (three Chl and one ORAC QTL) and GP (two ORAC QTL). No main effect QTL for Chl was detected at GP, but epistatic interaction was identified in one pair of marker intervals for each trait at GP. Interactions with environment were also detected for both main and epistatic effects (two for main effect, and one for epistatic effect). Main effect QTL for plant architecture and nutritional traits at MM colocated to a single genomic region. Chlorophyll contents and ORAC values at MM were significantly higher and Chl *a* to Chl *b* ratios were lower in 'open' types compared to 'head' types. The nutritional traits assessed for GP showed a significant association with plant architecture suggesting pleiotropic effects or closely linked genes. Taken together, the antioxidant and chlorophyll content of lettuce is controlled by complex mechanisms and participating alleles change depending on growth stage and production environment.

Communicated by M. Havey.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-012-1803-0) contains supplementary material, which is available to authorized users.

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Introduction

Fruits and vegetables are major contributors of nutrients and vitamins and a rich source of compounds possessing antioxidative and free-radical scavenging activity that may have health benefits. These phytochemicals protect cell components from oxidation by reactive oxygen species generated in response to a wide range of abiotic and biotic stresses (Penuelas and Munne-Bosch 2005; Pourcel et al. 2007; Rice-Evans et al. 1997; Smirnoff et al. 2001). In humans, epidemiological evidence support an association between diets high in antioxidants and a reduced risk of chronic and degenerative diseases such as cancer, diabetes, cardiovascular, and neurological diseases (Crowe et al. 2011; Liu 2004; Tapiero et al. 2004). Worldwide, the consumption of fruits and vegetables are at levels far less than the recommended rates (Agudo et al. 2002; Blanck et al. 2008; Hall et al. 2009; Kanungsukkasem et al. 2009; Peltzer and Pengpid 2010). Dietary intervention which encourages a more healthful diet is effective, but adherence to dietary guidelines typically lapses within a short period of time (Guenther et al. 2006; Johnston et al. 2000). As an

alternative to changing an individual's diet, an attractive strategy is to enrich the nutritional content of foods that already constitute a significant portion of a typical diet. Lettuce, for example, has a very high per capita consumption in the USA, but is relatively low in most essential minerals and vitamins and other phytonutrients such as antioxidants, partly because of its low phenolic content (Song et al. 2010). The nutritional quality in plants can be enhanced through conventional breeding or transgenic approaches (Giuliano et al. 2008; Kinney 2006). Traditional breeding approaches are arguably more attractive than transgenic approaches because of resistance by a portion of the public to accept minimally processed transgenic foods such as fruits and vegetables (Canavari and Nayga 2009; Costa-Font and Gil 2009; Dannenberg 2009; Legge and Durant 2010). Further, because regulatory mechanisms in biosynthetic pathways may maintain homeostasis in transgenic plants, the end product may be less predictable and/or accumulate at concentrations less than anticipated (Fraser et al. 2009; Giugliano 2000). Many health benefits are thought to arise from the synergistic effects of complex mixtures of phytochemicals instead of specific molecules (Cooper 2004; Liu 2004) and therefore conventional breeding will likely affect a greater number of genes in favorable biosynthetic pathways than a biotech approach. Marker-assisted breeding can improve efficiency and offers a practical strategy to develop nutrient-dense lettuce and thereby increase the dietary intake of beneficial compounds, but at the same time constitutes a challenging task due to the complex nature of this trait.

Diets rich in fruits and vegetables are generally regarded as antioxidant rich, but wide variation exists in phytochemical profiles and content among the different fruits and vegetables (Cao et al. 1996; Hassimotto et al. 2005; Wu et al. 2004). Even within a species, the antioxidant content varies in response to the environmental conditions under which the crop is produced (García-Macías et al. 2007; Oh et al. 2009b; Romani et al. 2002; Torres et al. 2006). This variation is due to the fact that phytochemicals, including polyphenols, vitamins, ascorbic acid, carotenoids, and tocopherols, are encoded by a large number of genes and have shared and/or unique biosynthesis pathways whose regulation is at least partially environmentally regulated (Hirschberg 2001; Lichtenthaler 2007; Vogt 2010). Thus, a potentially large environmental component may make it difficult to improve those nutritional components that are genetically complex.

Lettuce is the second most popular fresh-market vegetable in the USA (USDA 2010a) and is an important crop species in Asteraceae. Its genetic and phenotypic diversity is being characterized by the Compositae Genome Project (CGP; http://compgenomics.ucdavis.edu/) and its complete genome sequence is being assembled and annotated by a consortium of seed companies, the CGP and BGI (Kozik et al. 2011). Of the four lettuce types typically consumed in the USA, iceberg (crisphead) lettuce is the most popular type and comprises over 60% of the lettuce market (USDA 2010a). Over a 2-day period, over 40% of adults surveyed reported having consumed iceberg lettuce at least once (Johnston et al. 2000). Lettuce, however, is one of the least nutritional vegetables compared to fruits and other vegetables (Cao et al. 1996; Hassimotto et al. 2005; Wu et al. 2004). Depending on type (butterhead, romaine, iceberg, red or green leaf), lettuce contains 20–80% of the antioxidant content of red cabbage and 30–160% of that of spinach (USDA 2010b). Iceberg lettuce shows the least amount of antioxidant activity of the four main lettuce types (Cao et al. 1996; Llorach et al. 2008; Mou 2005; Wu et al. 2004). However, because of its high per capita consumption, lettuce is the fourth highest contributor of antioxidants of all vegetables consumed in the USA (Song et al. 2010). Quantitative trait loci (QTL) have been associated with increased antioxidants in carrot (Santos and Simon 2002), tomato (Rousseaux et al. 2005), rapeseed (Marwede et al. 2005), chickpea (Abbo et al. 2005), apple (Davey et al. 2006), maize (Chander et al. 2008; Wong et al. 2003, 2004), durum wheat (Patil et al. 2008), and oat (Jackson et al. 2008). Significant genotype × environment interaction (GEI) was reported for antioxidant content in tomatoes (Rousseaux et al. 2005), tocopherol in oat (Jackson et al. 2008) and rapeseed (Marwede et al. 2005), and carotenoids in durum wheat (Patil et al. 2008). Epistasis, the interaction between two alleles, is often expected for complex traits and was found to be a significant component influencing nutritional content in a number of crops (Gutierrez-Gonzalez et al. 2009; Hu et al. 2004; Monteros et al. 2008). Although difficult to measure, the ability to detect epistasis can improve QTL detection and accuracy (Yi and Xu 2002).

Given the broad range of variation in antioxidant content across lettuce types reported (Cao et al. 1996; Llorach et al. 2008; Mou 2005; Wu et al. 2004), a potentially large environmental influence and the possibility of epistasis, elucidating the genetic basis of antioxidant content in lettuce is required to understand and improve this trait. In this study, a lettuce recombinant inbred line (RIL) population was grown in two different production sites in three consecutive years. Total antioxidant content and chlorophyll (as a proxy of β-carotene) were measured for each RIL family at each production site and production cycle. A QTL analysis was performed to identify genomic regions responsible for the chlorophyll and antioxidant traits and estimate the genetic parameters, including additive, epistatic effects of QTL, and genotype × environment interaction. The effects of plant architecture on these traits were also determined.

Materials and methods

Plant materials and cultural conditions

An F_8 RIL population consisting of 169 families was developed by single seed descent of an F_2 population (Hayashi et al. 2008). The parents of the RIL were two commercial cultivars, 'Diplomat' (an iceberg lettuce type) and 'Margarita' (a butter lettuce type), hereafter referred to as the D × M RIL population. Both parents were heading types.

To determine the chlorophyll traits and total antioxidant content under commercial production conditions, the RIL population was planted at the University of Arizona, Yuma Agriculture Center in Yuma, AZ (YAC production site). For each family, 20–30 plants were grown in each plot under standard cultural conditions (Kerns et al. 1999) and three representative plants were harvested for evaluation. The first YAC crop production was planted on 9 November 2006 and harvested for evaluation on 5 March 2007; the second crop was planted on 10 January 2008 and harvested 21 April 2008, and the third was planted 11 December 2008 and harvested on 23 March 2009; all evaluations were performed when the RIL population was considered to be at market maturity (MM). These will be referred to as "winter 07", "spring 08", and "winter 09" production cycles, respectively. Although both parents were heading types, the RIL families segregated for heading or non-heading, open loose-leaf types.

To determine the genetic potential (GP) of each family without the potentially confounding influence of plant architecture, the RIL population was assessed before head formation began and an increasing number of leaves, or cupping of newly formed leaves would shade the existing leaves nearby. The population used for the GP assessments were planted in a glasshouse located at California State Polytechnic University, Pomona (Pomona, CA), hereafter referred to as the CPP production site. Three to four plants per family were grown in 3.8-L pots containing Premier Pro-Mix peat-based growing medium (Premier Tech, Quebec, Canada), fertilized weekly with a 20N–20P–20K soluble fertilizer (Peter's Professional 20–20–20 GP, Scotts, Marysville, Ohio) at 300 mg/L and watered as needed to prevent stress. The D × M RIL population for the first GP evaluation was planted 5 June 2006 and leaves were harvested for evaluation on 3 July 2006; the second evaluation was planted on 25 June 2007 and harvested on 20 July 2007; the third was planted on 14 December 2007 and harvested on 18 March 2008; the fourth was planted on 15 July 2008 and harvested on 28 August 2008. These will be referred to as "summer 06", "summer 07", "spring 08", and "summer 08" production cycles, respectively.

Chlorophyll and antioxidant assays

At the YAC production field, whole plants were harvested at crown level for the market maturity (MM) assessment. Because there is a general inverse relationship of nutritional content (β-carotene, vitamin C, and phenolics) with head formation in lettuce (Cano and Arnao 2005; Hohl et al. 2001; Mou and Ryder 2004), individual leaves were harvested at an early growth stage from the plants grown at the CPP glasshouse and represent the genetic potential (GP) of each line. For all production cycles and sites, the harvested plants or leaves were kept on ice and transported to walk-in coolers or refrigerators and held at 4°C until processed; all processing was completed within 72 h of harvest. For the MM evaluations, a 7-mm cork borer was used to obtain cross sections through the entire plant while avoiding the midrib. Random discs were then pooled to obtain a 125-mg fresh weight sample to represent an individual plant, or the whole head property (MM). For the GP assessments, multiple leaf discs were taken from individual leaves using a cork borer, again avoiding the midrib. Random discs were pooled to comprise a 125-mg fresh weight sample.

The leaf tissue was homogenized in 500 µl of 80% acetone using a ball mill (Retsch MM301) for 2 min at maximum frequency and centrifuged at 2,250*g* for 10 min. The supernatant was transferred to a new tube and stored at −80°C until assayed for chlorophyll and antioxidant content. We measured chlorophyll content as a proxy of β-carotene, as Mou (2005) reported that β-carotene and lutein concentrations were highly correlated with chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total chlorophyll (tChl) content. Chlorophyll content was determined by diluting an aliquot of the acetone-extracted samples into 80% acetone, mixed, and added to a 96-well microplate, and the absorbance at 649 and 665 nm were obtained using a BioTek μQuant Microplate Spectrophotometer (Bio-Tek Instruments, Winooski, VT). The concentration of Chl *a*, Chl *b*, and total tChl were calculated according to extinction coefficient given in the following equations (Strain et al. 1971).

$$Chl\,a\,(\mu g/ml) = 11.63\,(A_{665}) - 2.39\,(A_{649})$$

$$Chl\,b\,(\mu g/ml) = 20.11\,(A_{649}) - 5.18\,(A_{665})$$

$$tChl\,(\mu g/ml) = 6.45\,(A_{665}) + 17.72\,(A_{649})$$

where A_{665} is absorbance at 665 nm and A_{649} is the absorbance at 649 nm. The concentration value reported is an average of three biological replications, each consisting of three technical replications and expressed as the chlorophyll content per gram fresh weight (µg/g FW). The ratio of Chl *a* to Chl *b* is given as Chl *a/b*. Chlorophyll content

was not determined for the summer 06 harvest of the CPP crop.

The antioxidant content of each RIL was determined by using an oxygen radical absorbance capacity (ORAC) assay (Ou et al. 2001) with minor modifications to adapt the assay to a 96-well microplate format. Fluorescein (227 nM) was prepared in 75 mM sodium phosphate (Na_2HPO_4 and NaH_2PO_4) buffer (pH7.4) and 2 µl of acetone extract was added. The mixture was dispensed into a 96-well microplate and AAPH (2,2′-azobis (2-amidinopropane), 332.8 mM) was added. The final volume of the assay was 300 µl with a final fluorescein and AAPH concentration of 155 µM and 104 mM, respectively. The samples and standards were mixed using a multichannel pipette and the plate was immediately transferred to a Carey Eclipse Fluorescence spectrophotometer (Varian, Palo Alto, CA). The decrease in fluorescence of fluorescein was determined by reading each well at an excitation of 535 nm and emission of 595 nm at 1.3 min intervals for approximately 120 min. Samples in which the fluorescent readings had not dropped to baseline levels were subsequently re-assayed with the read time extended to 240 min. The ORAC value was determined as the area under the curve (AUC) and calculated by eq. (1) according to Prior et al. (2005).

$$AUC = CT \times \left\{ 0.5 + \sum_{i=2}^{n} (fi/f1) \right\}$$

where CT is the cycle time in minutes (interval between readings), n is the total number of readings, and fi is fluorescence at the ith reading. The standard curve was determined by polynomial regression of AUC obtained from a serial dilution series of trolox (6.25–200 µM) included on each plate. The trolox equivalent (TE) for each sample was calculated by regression based on the standard curve and expressed as TE per gram fresh weight of sampled leaf tissue (µmol TE/g FW). The phenotypic value of each RIL was determined from three biological replications, each consisting of three technical replications, and the averaged value was used in subsequent statistical analyses and mapping.

Plant architecture

Plant architecture of each RIL at MM was determined by photographing individual plants of each RIL family at the YAC production field. The population was photographed on the same date as the chlorophyll and ORAC sampling for the spring 08 and winter 09 evaluations. The photographs were subsequently used to categorize the plant architecture of each family. Families in which plants had open or loosely arranged leaves were classified as 'open' and families with plants that formed a tight head were classified as a 'head' type.

Statistical analysis

To determine associations among chlorophyll traits and ORAC values, Pearson's correlation coefficients were computed using the CORR procedure of the Statistical Analysis Software (SAS) version 9.2 (SAS Institute Inc., Cary, NC). Normality of the phenotypic distributions was tested by the Shapiro–Wilk's W statistics using the UNIVARIATE procedure in SAS. Analysis of variance (ANOVA) was performed using the GLM procedure in SAS to test effects by family, production cycle and their interaction. In cases where the trait distribution of the population did not fit a normal distribution, the association between a marker and phenotype was confirmed with the non-parametric Kruskal–Wallis test using the NPAR1WAY procedure of SAS. The linkage map comprised 134 amplified fragment length polymorphism (AFLP) markers and a seed coat color (*w*) locus as previously described (Hayashi et al. 2008). An additional four cleaved amplified polymorphic sequence (CAPS) markers were developed from sequence differences found between parents and combined with the AFLP-based linkage map using mapping software CARTHAGENE (de Givry et al. 2005). In the grouping step, a logarithm of odds (LOD) score of 3 was used as the threshold for determining if a pair of markers was linked. A recombination fraction of 0.25 was used as the threshold for dropping markers. Kosambi's mapping function was used for map distance. The 'buildfw' command was used to create the framework map with settings of 3 for the adding threshold and the keep threshold. Flanking markers were iteratively added by the 'flips' command, with window size of four and threshold of three, and testing against the remaining loci. QTL analysis was performed with QTLNetwork-2.2 (Yang et al. 2007) using mixed-model composite interval mapping with a10-cM window size and 1-cM walking speed. A 10-cM filtration window was applied to determine whether two adjacent test statistic peaks indicated a single QTL. The data for main effects were combined across all years to increase statistical power. Putative QTLs were detected by applying an F test along the whole genome (1D scan) to detect intervals in which a region exceeded a critical threshold. Following the 1D genome scan to detect QTL, a 2D genome scan was used to detect epistasis between the QTL and marker-interval interactions. To establish critical threshold F values at the 5% level of probability for the 1D and 2D scans, 1,000 permutations were performed on each trait in the combined data from different production cycles to control the genome-wise type I error (Doerge and Churchill 1996). All detected QTL and epistatic loci were

fitted by a full-QTL model to estimate the main effect QTL and epistasis. After obtaining locations of the putative QTL and epistatic effects, all genetic effects were estimated by employing a Bayesian method via Gibb sampling (Wang et al. 1994) using a burn-in of 20,000 cycles, chain length of 200,000 and a thinning interval of 10 cycles. The relative contribution of a genetic component (q^2) was calculated as the proportion of the phenotypic variation explained by the corresponding component.

Results

Chlorophyll and ORAC phenotypes

Chlorophyll phenotypes (Chl *a*, Chl *b*, tChl, and Chl *a/b*) were determined in three production cycles at two production sites, YAC when plants were at the market maturity stage, and at CPP when the plants were at the open-leaf stage to assess genetic potential. At both production sites and for all traits, broad phenotypic variation and a number of transgressive segregants were observed (Figs. 1, 2). For the MM assessment, the distribution of each chlorophyll phenotype did not fit a normal distribution in any production cycle ($P \leq 0.0023$) and were skewed toward lower chlorophyll content. This was especially evident in the winter 07 production where, for example, the Chl *a* content was less than 60 μg/g FW in about 56% of the RIL families (Fig. 1). No consistent trend of the phenotypic values of Chl was observed between the parents across production cycles. That is, 'Diplomat' was larger than 'Margarita' in spring 08, while the reverse was true in winter 09.

The chlorophyll content of the GP phenotype averaged across all production cycles was 3.3 (Chl *a*), 3.2 (Chl *b*), and 3.3 (tChl) times greater than the phenotype average for the MM population (Fig. 2), most likely because the GP samples were assessed from leaves fully exposed to the sun. In the GP assessment, with the exception of Chl *a/b* in spring 08 ($P < 0.0001$) and summer 08 ($P = 0.0153$), each chlorophyll phenotype fit a normal distribution as determined by the Shapiro–Wilk's W statistics with the probability ranging from $P = 0.0776$–0.6496. 'Margarita' consistently showed greater chlorophyll values compared to 'Diplomat' across the production cycles. Within a

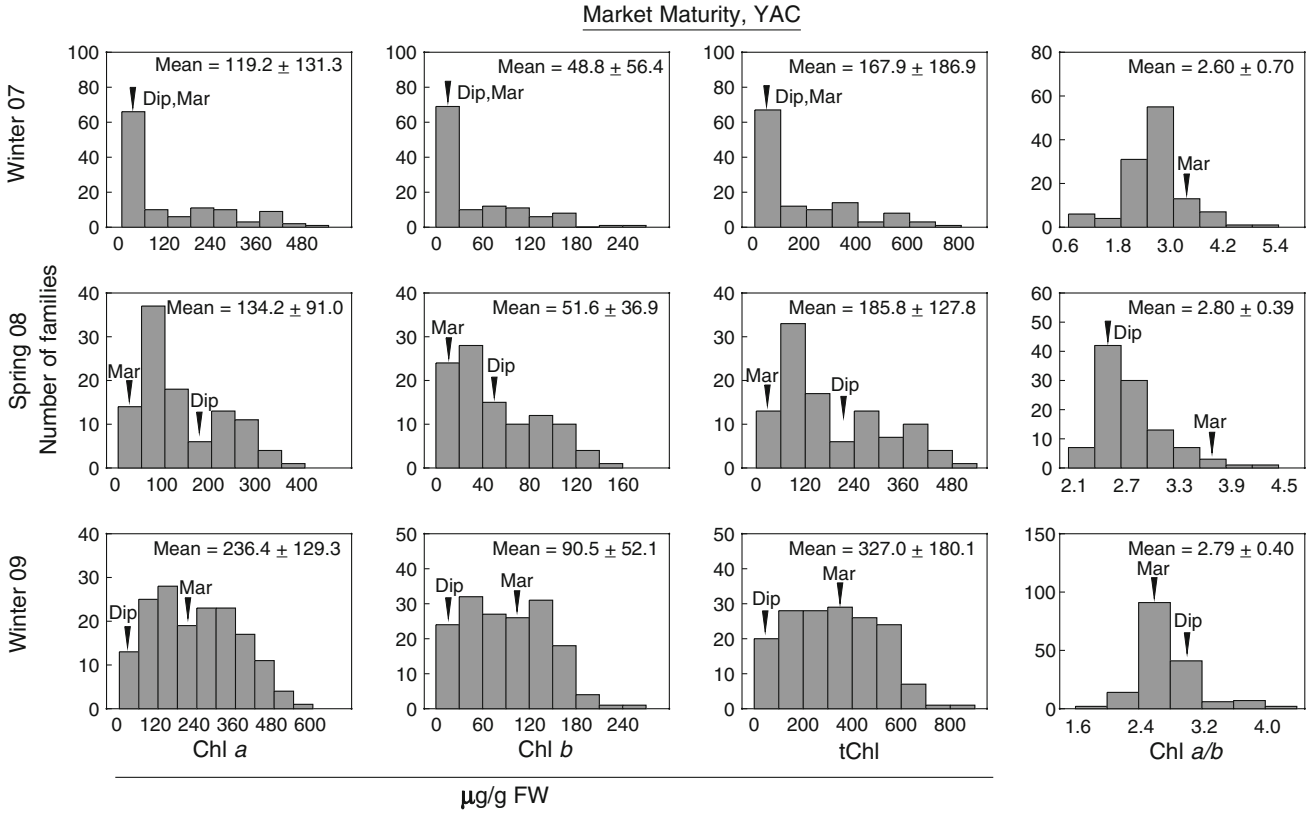

Fig. 1 Frequency distributions of chlorophyll components (Chl *a*, Chl *b*, and total Chl (tChl); μg/g FW) and chlorophyll ratio (Chl *a/b*) of the lettuce D × M RIL population harvested at YAC and evaluated at market maturity in three production cycles. *Arrowheads* indicate the phenotypic value of the parents, *Dip*, 'Diplomat', and *Mar*, 'Margarita'. Values presented indicate the mean ± standard deviation of the RIL population. The chlorophyll values for 'Diplomat' were at the lower limit of detection which precluded an accurate estimation of Chl *a/b* ratios for the winter 07 production cycle

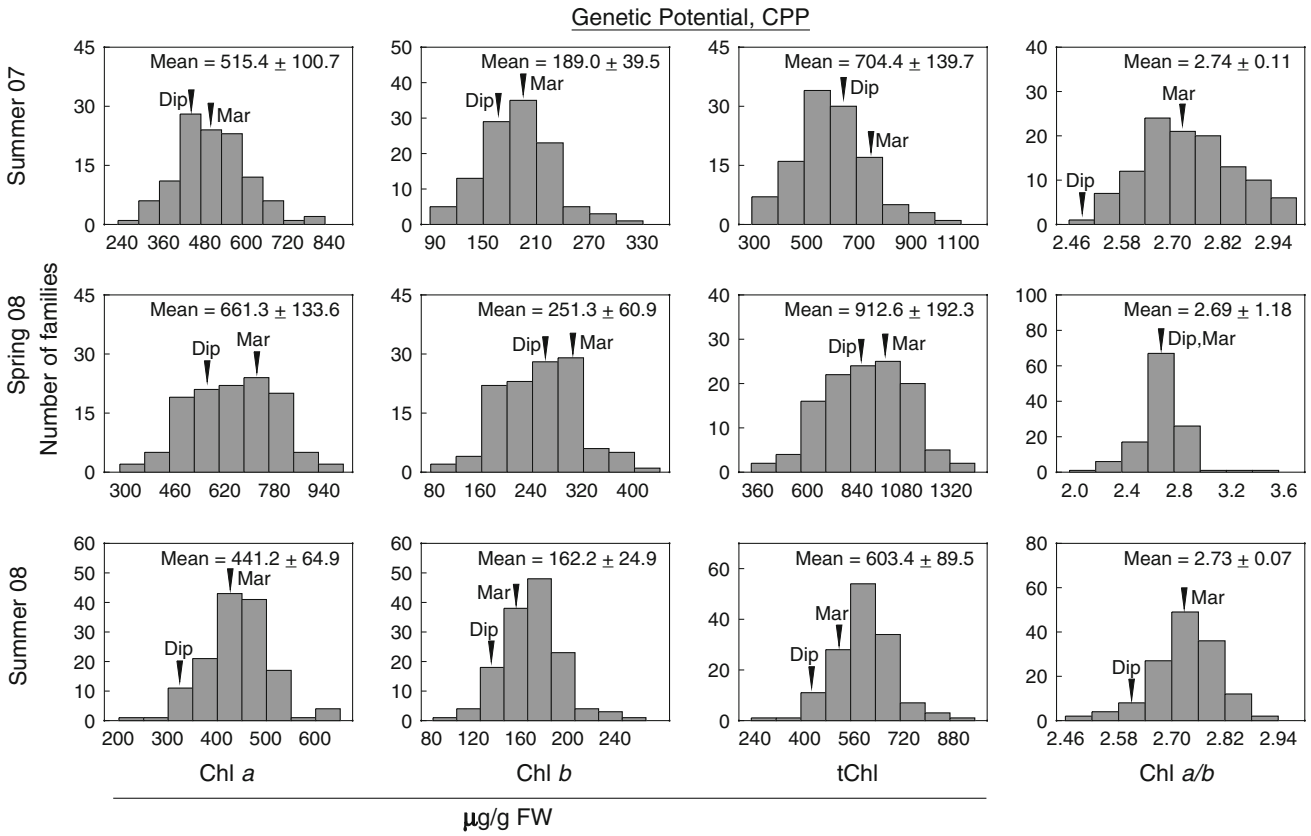

Fig. 2 Frequency distributions of chlorophyll components (Chl *a*, Chl *b*, and total Chl (tChl); µg/g FW) and chlorophyll ratio (Chl *a/b*) of the lettuce D × M RIL population grown in a glasshouse at Cal Poly Pomona and evaluated at the open-leaf stage to assess genetic potential in three production cycles. *Arrowheads* indicate the phenotypic value of the parents; *Dip*, 'Diplomat' and *Mar*, 'Margarita'. Values presented indicate the mean ± standard deviation of the RIL population

production site significant differences among families and production cycles, and significant interactions between family and production cycles were detected by ANOVA for each chlorophyll component (Chl *a*, Chl *b*, and tChl; $P < 0.0001$; data not presented). Chlorophyll contents were about two times higher in winter 09 compared to winter 07 at MM (Fig. 1) and 1.5 times higher in spring 08 compared to summer 08 at the GP assessment (Fig. 2). Thus, chlorophyll contents varied greatly among production cycles confirming a large environmental influence on chlorophyll content.

At MM, significant differences were detected for Chl a/b among families ($P < 0.0001$) and among production cycles ($P = 0.0002$) with the standard deviation of production cycles ranging from 0.39 to 0.70 (Fig. 1). The Chl *a/b* ratios in the GP assessment also differed among families ($P < 0.0001$) and production cycles ($P = 0.0003$) with the standard deviation of production cycles ranging from 0.07 to 1.18 (Fig. 2). The interactions between family and production cycles were also significant for both production sites ($P < 0.0001$). The chlorophyll values of 'Diplomat' in the winter 07 production cycle assessed at MM were at the lowest level of detection of the assay so the Chl *a/b* ratios could not be accurately estimated.

Total antioxidants, assessed with the ORAC assay, were quantified at MM for each RIL family for three production cycles at YAC and four production cycles at CPP to determine the GP. The ORAC distribution exhibited broad phenotypic variation both in the MM and GP assessments and a number of transgressive segregants were observed (Fig. 3). None of the ORAC data fit a normal distribution except for the summer 07 and summer 08 populations at CPP ($P = 0.061$ and 0.39, respectively). The ORAC value averaged across all production cycles was lower in GP compared to MM ($P < 0.0001$). The RIL population mean ORAC (µmol TE/g FW) values assessed at MM ranged from 21.0 ± 15.0 in winter 09 to 73.6 ± 40.8 in winter 07. The population mean ORAC values of the RIL population assessed for GP ranged from 21.7 ± 6.0 (summer 08) to 43.2 ± 14.1 (summer 07) with relatively smaller standard deviations compared to MM with the exception of the summer 06 population. 'Margarita' had larger ORAC values than 'Diplomat' in five out of six MM and GP evaluations for which both parental values were obtained.

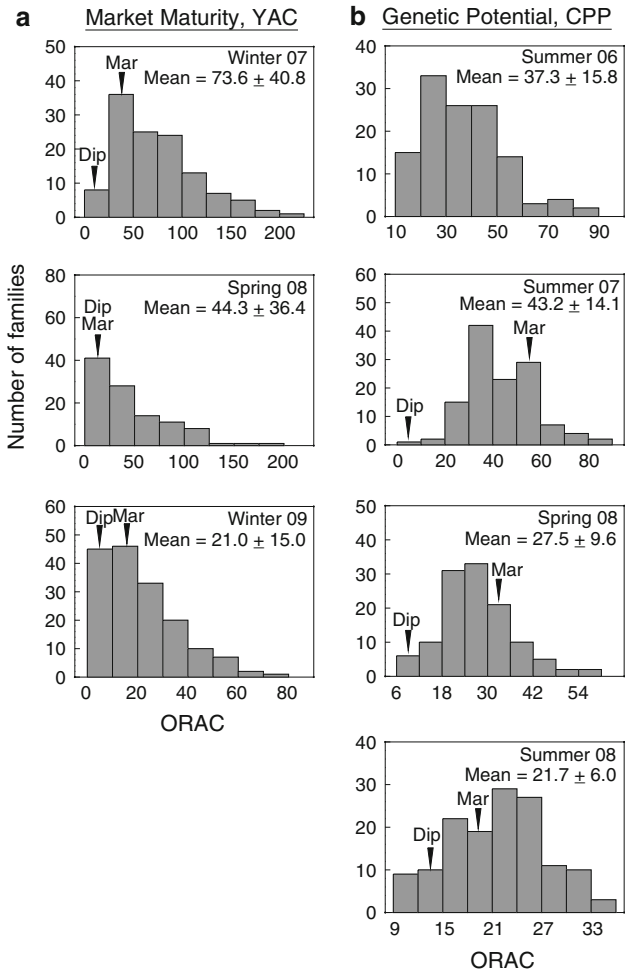

Fig. 3 Frequency distributions of ORAC (μmol TE/g FW) of the lettuce D × M RIL population harvested at two different production sites, YAC (**a**) or CPP (**b**) and assessed at market maturity or at the open-leaf stage, respectively. The CPP experiments were designed to assess genetic potential. *Arrowheads* indicate the phenotypic value of the parents, Dip, 'Diplomat', and Mar, 'Margarita'. Values presented indicate the mean ± standard deviation of the RIL population. Parental data were not available for the summer 06 CPP production crop

Within each production site (YAC or CPP), significant differences were observed among families and production cycles ($P < 0.0001$). These results confirm that total antioxidant content is not only controlled by genetic and environment effects, but genotype × environment interactions are also important.

Correlations among tChl content, ORAC values, assessment stage (MM or GP) and production cycles were examined (Table 1). Within the MM assessment, tChl correlations were highly significant between production cycles. Similarly, within the GP assessment, all tChl correlations were highly significant between production cycles, but the Pearson correlation *r* values were lower than those observed for the MM assessment. When comparing tChl correlations between the MM and GP assessments, five of nine (56%) pairwise comparisons were significant. Similar trends for correlations among ORAC values were observed. Specifically, highly significant correlations were observed between production cycles for ORAC comparisons within MM or within GP, with the GP assessment displaying somewhat lower *r* values than those for MM. Comparisons of ORAC between the MM and GP assessment yielded 92% (11 out of 12) significant correlations. Within a production cycle, the tChl content was highly correlated with ORAC values for the MM assessment, with *r* values ranging from 0.72 to 0.85 (Table 1, ID number 1 vs. 7, 2 vs. 8, 3 vs. 9). However, when comparing tChl with ORAC within the GP assessments, only a relatively weak positive and negative correlation was observed in the spring 2008 and summer 2008 production cycles, respectively (Table 1, ID number 5 vs. 12, 6 vs. 13).

Chlorophyll traits (Chl *a*, Chl *b*, tChl, and Chl *a*/*b*) for the MM and GP assessments and production cycles were examined in a separate correlation analysis (Supplementary Table 1). Within a given assessment (MM or GP) and production cycle, highly significant positive correlations were observed in all pairwise comparisons with the exception of Chl *a*/*b*. When Chl *a*/*b* was compared with other chlorophyll components within a production cycle, 67% (6/9) of the total correlations were significant, while 78% (7/9) of the GP comparisons were significant. Although high *r* values and levels of significance were observed for comparisons made within the GP or MM assessment across production cycles, there was either no correlation or a relatively weak, but significant correlation, between MM and GP for a given chlorophyll trait. Thus, for chlorophyll traits, GP was not a consistent or strong predictor of chlorophyll content at MM.

QTL analysis

To identify QTL associated with ORAC, Chl *a*, Chl *b*, and tChl, the D × M RIL population was assessed at market maturity and genetic potential at YAC and CPP, respectively. With the exception of ORAC in the Winter 07 production cycle, all traits were detected in each production cycle in approximately the same location on linkage group 1 (data not presented). No chlorophyll trait QTL were detected in the genetic potential evaluation at the CPP production site. Subsequently, to increase statistical power, the data for all production cycles within a location were pooled for the main effect QTL. The D × M RIL assessed at MM revealed a total of four QTL associated with chlorophyll and ORAC (Table 2). The QTL for each chlorophyll component (Chl *a*, Chl *b*, and tChl) colocated to the same genomic region on linkage group 1 (LG1), while no QTL was detected for Chl *a*/*b* QTL (Table 2;

Table 1 Pearson correlation coefficients of total chlorophyll (tChl) and ORAC values in the lettuce 'Diplomat' × 'Margarita' RIL population field grown in Yuma, AZ, and assessed at market maturity (MM), or glasshouse grown and assessed for genetic potential (GP)

Trait	Assessment	Production cycle	ID	1	2		3		4	5		6		7	
tChl	MM	Winter 2007	1	1.00	0.60	***	0.59	***	0.17	0.30	**	0.25	**	0.74	***
		Spring 2008	2		1.00		0.57	***	0.19	0.31	**	0.12		0.54	***
		Winter 2009	3				1.00		0.13	0.24	*	0.23	**	0.49	***
	GP	Summer 2007	4						1.00	0.44	***	0.39	***	0.19	
		Spring 2008	5							1.00		0.42	***	0.23	*
		Summer 2008	6									1.00		0.20	*
ORAC	MM	Winter 2007	7											1.00	
		Spring 2008	8												
		Winter 2009	9												
	GP	Summer 2006	10												
		Summer 2007	11												
		Spring 2008	12												
		Summer 2008	13												

Trait	Assessment	Production cycle	ID	8		9		10		11		12		13	
tChl	MM	Winter 2007	1	0.53	***	0.70	***	0.21	*	0.19		0.24	*	0.31	**
		Spring 2008	2	0.85	***	0.55	***	0.30	**	0.07		0.31	**	0.35	**
		Winter 2009	3	0.43	***	0.72	***	0.15		0.26	**	0.29	**	0.36	***
	GP	Summer 2007	4	0.19		0.23	*	0.14		0.02		−0.04		0.20	*
		Spring 2008	5	0.20		0.26	**	0.07		−0.01		−0.29	**	0.08	
		Summer 2008	6	0.04		0.23	**	−0.01		0.11		0.00		0.20	*
ORAC	MM	Winter 2007	7	0.51	***	0.61	***	0.30	**	0.22	*	0.28	**	0.40	***
		Spring 2008	8	1.00		0.55	***	0.38	**	0.21		0.48	***	0.41	***
		Winter 2009	9			1.00		0.29	**	0.22	*	0.43	***	0.42	***
	GP	Summer 2006	10					1.00		0.28	**	0.31	**	0.34	**
		Summer 2007	11							1.00		0.44	***	0.56	***
		Spring 2008	12									1.00		0.43	***
		Summer 2008	13											1.00	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

Supplementary Fig. 1). For each QTL (*Chla$_M$1.1*, *Chlb$_M$1.1*, *tChl$_M$1.1*), the 'Diplomat' allele served to increase the chlorophyll content, and the variation explained by each of these QTL was 21.7, 22.3, and 22.0%, respectively. One QTL associated with ORAC values, *ORAC$_M$1.1*, was identified on the same genomic region in which the chlorophyll QTL were found (Table 2; Supplementary Fig. 1). The non-parametric Kruskal–Wallis test was conducted to confirm associations between these traits and flanking DNA markers of the QTL identified in the MM assessment. A 'Diplomat' allele served to increase the observed ORAC values with 17.5% of the variation explained by the QTL. A significant QTL × environment (production cycle) interaction was detected for the *ORAC$_M$1.1* QTL in the winter 09 production cycle (Table 2), but was not observed in any other production cycle. A significant ($P = 0.0341$) additive × environment interaction was detected for ORAC content and accounted for 1.1% of the total phenotypic variation, which was relatively small compared to that of the corresponding main-effect QTL.

For the GP assessment, two QTL were associated with ORAC values, one on LG 2 and another on LG 11 and epistasis was detected between these two loci (Table 2; Supplementary Fig. 1). Both parents contributed alleles which served to increase ORAC values, each with a similar magnitude of additive effect. An additive × environment (production cycle) interaction was detected in two production cycles (summer 06 and summer 07) for *ORAC$_G$2.1*. In addition, four additive × additive interactions were detected for chlorophyll and ORAC value in the RIL population assessed for GP (Table 3). Although no main effect QTL were identified for chlorophyll traits, three additive × additive interactions were identified for

Table 2 Estimates of additive and additive × environment interaction of quantitative trait loci associated with chlorophyll traits, ORAC, and plant architecture in the D × M RIL population field grown in Yuma, AZ, and assessed at market maturity (MM), or grown in a greenhouse at Cal Poly Pomona and assessed at the open-leaf stage for genetic potential (GP)

QTL[a]	LG[b]	Flanking marker	cM[c]	Range	$\underline{A}$[d]	q_a^2 [e]	AE[d] (q_{ae}^2 [e])				*P*-KW[f]
							1	2	3	4	
MM											
Chla$_M$1.1	1	*E33M61D432–E33M59D245*	65.6	60.6–71.5	64.0	21.7					7.19E−5
Chlb$_M$1.1	1	*E33M61D432–E33M59D245*	65.6	60.6–71.5	26.2	22.3					1.17E−4
tChl$_M$1.1	1	*E33M61D43–E33M59D245*	65.6	60.6–75.5	90.1	22.0					6.92E−5
ORAC$_M$1.1	1	*E33M61D432–E33M59D245*	62.6	57.6–67.6	16.8	17.5			−6.0 (1.1)		1.22E−2
Lpa1.1	1	*E33M59D245–QGF13P18*	69.6	64.6–76.5	−0.25	26.1					5.95E−4
GP											
ORAC$_G$2.1	2	*E45M61M453–E33M54M549*	150.8	145.4–156.8	3.2	4.3	2.5 (0.9)	2.8 (1.1)			1.29E−01
ORAC$_G$11.1	11	*E45M48M323–E35M59M443*	14.8	12.8–14.8	−3.2	4.3					2.15E−02

[a] QTL with main effect for market maturity (*MM*) and genetic potential (GP). The data for main effect QTL were combined across all production cycles for the MM and GP assessment. Locus nomenclature is designated as: (trait) (linkage group) (numerical order on linkage group) with 'M' indicating association established during the market maturity assessment and 'G' indicating association established during the genetic potential assessment. *Lpa*, *Lactuca* plant architecture

[b] Linkage group number

[c] Position of the QTL on the linkage group

[d] Effect of genetic source; *A*, additive effect of the QTL; *AE*, additive × environment interaction effect for MM at the Yuma production cycle, where environment 1 = winter 07, 2 = spring 08, 3 = winter 09; and for GP at the greenhouse production cycle, where environment 1 = summer 06, 2 = summer 07, 3 = spring 08, 4 = summer 08. Positive values indicate that the 'Diplomat' allele increases the phenotype, whereas negative values indicate an allelic effect from the 'Margarita' parent. Chlorophyll units are expressed as the chlorophyll content per gram fresh weight (μg/g FW); ORAC units are expressed as TE per gram fresh weight of sampled leaf tissue (μmol TE/g FW)

[e] Heritability of the QTL effect or percentage of variation that is explained by the component of the corresponding genetic source (a) additive effect and (ae) additive × environment (production cycle) interaction, respectively, in the total phenotypic variance. Percent variation is given only when the QTL effect was significant

[f] *P* value (Kruskal–Wallis test)

chlorophyll traits, each with the same pair of marker intervals and each with a similar magnitude of contribution to the total phenotypic variance (q^2 values, 5.1–5.5%, Table 3). For the ORAC value, significant additive × additive interactions were detected between the main-effect QTL identified for the same trait presented in Table 2 (summer 06 $P = 0.018$; summer 07 $P = 0.0098$). The effect of the epistatic interaction on the ORAC value was relatively small compared to that of the main-effect QTL.

QTL association with plant architecture

The plant architecture of each family was classified into two categories based on their capacity to form a head. The RIL population field-grown at YAC in spring 2008 and winter 2009 was phenotyped; those forming heads were classified as 'head' types while those with a loose-leaf type were classified as 'open'. Families showing an intermediate appearance precluded classification into either one of these categories and subsequently those intermediate genotypes were excluded from mapping plant architecture QTL and the analysis of variance between architecture and chlorophyll/ORAC phenotypes. Head formation appeared to be influenced by the environment, as a total of 17 families in the spring 08 and 37 families in the winter 09 production cycles were classified as intermediate. The number of families planted and phenotyped was approximately equal in both production cycles. There were 39 and 49 heading families and 55 and 71 open families in the spring 08 and winter 09 production cycles, respectively. Ten families changed classification between production cycles; three families were classified as 'head' in 08 and 'open' in 09, while seven families that were classified as 'open' in 08 were classified as 'head' in 09. When averaged in each category (open or head), the Chl *a*, Chl *b*, and tChl content, and ORAC values were greater in families with open types than in head types in both production cycles ($P < 0.0001$, Fig. 4). The Chl *a/b* ratio was higher in head types than open types, indicating relatively greater Chl *b* production (or reduced Chl *a* degradation) in open type lettuce (2.95 vs. 2.70 in spring 08, $P = 0.0018$; 3.00 vs. 2.64 in winter

Table 3 Estimates of epistasis (AA) and epistasis × environment interaction (AAE) identified in the D × M RIL population grown in a glasshouse at CPP and assessed at the open-leaf stage for genetic potential of chlorophyll and antioxidant content

Trait	QTL_i^a	LG_i^b	Flanking marker	cM_i^c	$Range_i$	QTL_j^a	LG_j^b	Flanking marker	cM_j^c	$Range_j$	AA^d	q_{aa}^{2e}	AAE^d (q_{aae}^{2e})		
													1	2	3	4
Chl *a*		2	*E45M59M381–CLA1*	57.7	54.2–61.7		8	*E35M59D377–E45M48D67*	36.5	30.8–38.5	34.3	5.1	n.a.			
Chl *b*		2	*E45M59M381-CLA1*	57.7	54.2–61.7		8	*E35M59D377–E45M48D67*	36.5	31.8–37.5	47.6	5.5	n.a.		8.4 (0.9)	
tChl		2	*E45M59M381–CLA1*	57.7	54.2–61.7		8	*E35M59D377–E45M48D67*	36.5	31.8–37.5	49.0	5.3	n.a.			
ORAC	$ORAC_G2.1$	2	*E45M61M453–E45M61M379*	150.8	145.4–156.8	$ORAC_G11.1$	11	*E45M48M323–E35M59M443*	14.8	12.8–14.8	1.9	1.5				

[a] QTL with main effect of locus i or j

[b,c,e] See Table 2 for footnotes

[d] Effect of genetic source; AA, additive × additive interaction; AAE, epistasis × environment interaction for production cycle, where environment 1 = summer 06, 2 = summer 07, 3 = spring 08, 4 = summer 08. n.a., not assayed. Positive values indicate the 'Diplomat' allele increases the phenotype. Chlorophyll units are expressed as the chlorophyll content per gram fresh weight (μg/g FW); ORAC units are expressed as TE per gram fresh weight of sampled leaf tissue (μmol TE/g FW)

[e] Heritability of the QTL effect or percentage of variation that is explained by the component of the corresponding genetic source (aa) additive × additive interaction and (aae) epistasis × environment interaction, respectively, in the total phenotypic variance. Percent variation is given only when the QTL effect was significant

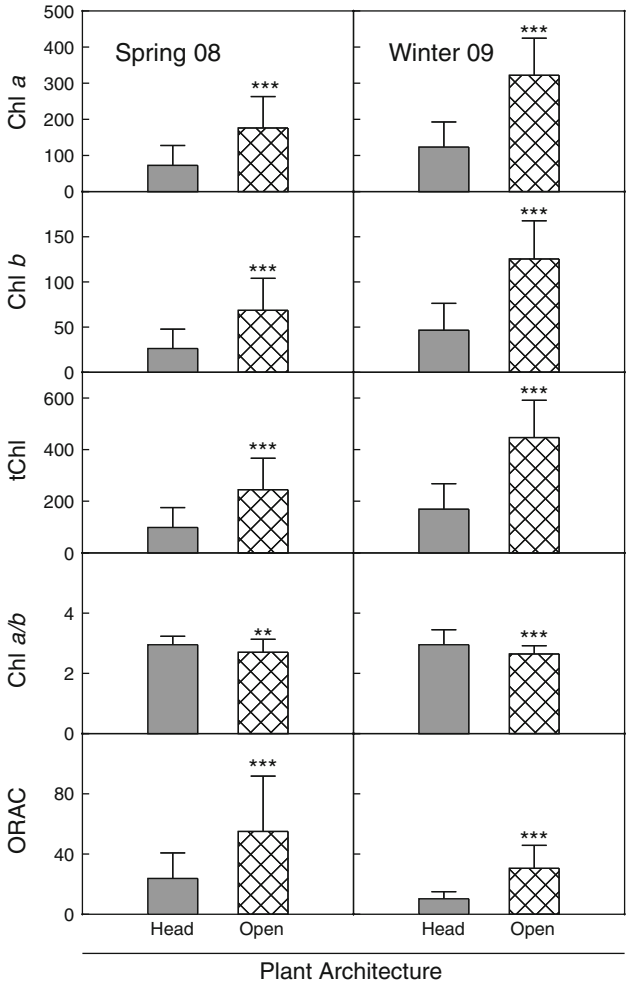

Fig. 4 Chlorophyll (μg/g FW) and ORAC (μmol TE/g FW) content as a function of plant architecture ('head', *solid bars* or '*open', hatched bars*) of the lettuce D × M RIL population grown at YAC and assessed at market maturity. The RIL population was grown in two seasons, spring 08 and winter 09. ***$P < 0.0001$; **$P < 0.01$

09, $P < 0.0001$). A single locus for plant architecture accounted for approximately 26% of the phenotypic variation of the trait and co-located with QTL associated with ORAC values and chlorophyll traits at MM on LG1 (Table 2; Supplementary Fig. 1).

Imposing the plant architecture classification determined on the population grown at YAC and phenotyped at MM, we next sought to determine if the chlorophyll content and ORAC value differed in the plants grown at CPP and assessed at the GP stage. The ORAC and chlorophyll content was determined on the GP plants while all plants were at the rosette stage, and the values were averaged across all RIL families of those classified as head types and compared to those classified as open types. Differences in content detected between open and head plant architecture types would suggest linkage with genes controlling plant architecture. In the three CPP GP production cycles

Table 4 Trait values for Chl *a*, Chl *b*, tChl, Chl *a*/*b*, and ORAC assessed at the open-leaf stage for genetic potential

Production site and cycle	Trait[a]	Spring 2008 classification		*P*		Winter 2009 classification		*P*	
		Head	Open			Head	Open		
CPP summer 2007	Chl *a*	481.6 ± 91.0	531.9 ± 80.4	0.0276	*	515.3 ± 114.8	528.2 ± 78.3	0.5477	
	Chl *b*	178.8 ± 35.1	194.5 ± 32.5	0.0795		190.1 ± 43.7	192.3 ± 31.5	0.7930	
	tChl	660.4 ± 125.6	726.3 ± 112.3	0.0370	*	705.4 ± 158.0	720.5 ± 109.3	0.6112	
	Chl *a*/*b*	2.71 ± 0.11	2.75 ± 0.10	0.2447		2.72 ± 0.12	2.76 ± 0.11	0.1232	
	ORAC	41.4 ± 14.7	45.0 ± 13.4	0.3205		36.9 ± 13.6	47.3 ± 12.2	0.0003	**
CPP spring 2008	Chl *a*	626.5 ± 130.4	698.9 ± 115.8	0.0190	*	634.6 ± 122.7	693.0 ± 113.6	0.0204	*
	Chl *b*	238.3 ± 62.3	268.2 ± 56.9	0.0445	*	242.7 ± 55.8	261.9 ± 54.5	0.1010	
	tChl	864.9 ± 190.7	967.1 ± 169.6	0.0234	*	877.3 ± 176.7	954.9 ± 165.4	0.0330	*
	Chl *a*/*b*	2.69 ± 0.20	2.66 ± 0.19	0.5151		2.66 ± 0.13	2.70 ± 0.19	0.3524	
	ORAC	22.7 ± 8.6	28.9 ± 9.2	0.0059	**	21.2 ± 6.7	31.2 ± 8.2	<0.0001	***
CPP summer 2008	Chl *a*	435.2 ± 72.9	461.3 ± 54.5	0.0824		424.0 ± 62.7	446.8 ± 57.9	0.0624	
	Chl *b*	161.8 ± 27.6	169.0 ± 21.5	0.2130		157.3 ± 23.8	163.4 ± 22.1	0.1924	
	tChl	597.1 ± 100.3	630.3 ± 75.5	0.1080		581.3 ± 86.1	610.1 ± 79.8	0.0861	
	Chl *a*/*b*	2.70 ± 0.07	2.74 ± 0.08	0.0208	*	2.71 ± 0.09	2.75 ± 0.06	0.0133	*
	ORAC	18.9 ± 6.6	23.8 ± 5.8	0.0010	**	18.8 ± 6.4	23.9 ± 5.2	<0.0001	***

Each RIL family was assigned as a heading- or open-leaf type based on the phenotype observed at market maturity when grown under field conditions at the Yuma, AZ, location in spring 2008 or winter 2009. Values represent the average for all RIL families in that category ± SD

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

[a] Chlorophyll units are expressed as the chlorophyll content per gram fresh weight (μg/g FW); ORAC units are expressed as TE per gram fresh weight of sampled leaf tissue (μmol TE/g FW)

examined, the values for the chlorophyll and ORAC traits were lower in families that would develop heads than those which remain open at market maturity despite the fact that evaluations were performed at the open-leaf stage. Using the spring 2008 classification, 8 out of 15 significant differences were detected, whereas in the winter 09 classification, 6 out of 15 were significant (Table 4). In the 2008 classification, Chl *a*, tChl, and ORAC were significantly different in two of the three CPP production sites, while in the 2009 classification only ORAC was significantly different in all three production years (Table 4). These results strongly suggest linkage between genes that control plant architecture, chlorophyll content, and ORAC value, or alternatively a single pleiotropic gene. Thus, these data suggest that chlorophyll and antioxidant content in the D × M RIL population is not a simple function of shading effects.

Discussion

Multiple factors affect the antioxidant and chlorophyll content of lettuce, as indicated by the numerous QTL, epistasis, and genotype × environment interaction detected in this study (Tables 2, 3). The genetic architecture controlling chlorophyll and antioxidant traits differed according to the developmental phase of the plant. Whereas additive effects were the main factor affecting chlorophyll content and ORAC values in the RIL population evaluated at MM, no main-effect QTL were associated chlorophyll traits in the GP assessment; instead, epistasis was the primary genetic factor. Epistasis × environment interactions were also detected in the GP assessment for Chl *b*, indicating the complex nature of this trait. Individual QTL associated with either chlorophyll content or ORAC value accounted for up to 22% of the variation associated with these traits in the D × M RIL population harvested at MM. Our results clearly indicated a significant environmental effect on nutritional content and head formation. In turn, QTL associated with chlorophyll content and ORAC value (but not Chl *a*/*b* ratios) colocated to a QTL linked to head formation on LG 1 (Table 2). The chlorophyll content was about threefold higher at the GP stage than MM, presumably because of the head effect on leaf shading. Evaluating the RIL population prior to head formation removed this confounding effect and revealed epistatic interactions with relatively smaller effects on chlorophyll content and ORAC value than the main additive effect QTL. The GP evaluations identified epistasis that accounted for up to 5.5 and 1.5% of the total variation associated with chlorophyll content and ORAC value, respectively (Table 3). Understanding the genetic basis and stability of

these QTL conditioning these traits is the first step in improving the nutritional content of lettuce through marker-assisted breeding.

We assessed antioxidant activity using the ORAC assay, which measures the capacity of an antioxidant sample to quench free radicals (Cao and Prior 1998). Numerous plant compounds impart anti-oxidative activity, including well-known oxidants such as vitamins, α-tocopherol, ascorbic acid, and β-carotene, as well as lesser-known compounds including carotenoids, polyphenols, and flavonoids (especially proanthocyanidins) (Demmig-Adams and Adams 2002). The well-known compounds may constitute only a small portion of the total antioxidant activity of dietary plants and is highly species specific (Paganga et al. 1999; Prior and Cao 2000). These observations are further supported by the wide variety of antioxidant-contributing compounds that have been reported for lettuce (DuPont et al. 2000; Llorach et al. 2004; Nicolle et al. 2004; Romani et al. 2002). These include carotenoids, vitamins E and C, lutein and xanthophyll (Mou and Ryder 2004; Nicolle et al. 2004), and polyphenols including neochlorogenic acid, caffeoyltartaric acid, crytochlorogenic acid, chlorogenic acid, quercitin conjugates, and chicoric acid (DuPont et al. 2000; Llorach et al. 2004; Nicolle et al. 2004; Romani et al. 2002). Of these different antioxidants, dicaffeoyl tartaric acid accounted for about 55% of the total antioxidant content in the lettuce cultivars tested (Nicolle et al. 2004). Mou (2005) reported very high correlations between chlorophyll (Chl *a*, Chl *b*, and tChl) and β-carotene in lettuce. Because chlorophyll assays are simpler than β-carotene assays, chlorophyll could be used as a proxy for improving β-carotene content in lettuce. This study showed that genetic effect for GP in chlorophyll content and ORAC value was relatively smaller, as no main effect QTL for chlorophyll and two QTL with minor effect for ORAC value were found. However, the genetic components including main effect QTL (only for ORAC) and epistatic QTL for both trait detected in the GP assessments suggest that relatively smaller number of genes are likely to be involved in the phenotypic variation in nutritional content observed in this population.

In the D × M RIL population assessed at MM, total chlorophyll content of plants assessed at MM was highly correlated with ORAC values and the $ORAC_M1.1$ locus colocated to the same genomic interval as $Chla_M1.1$, $Chlb_M1.1$, and $tChl_M1.1$ (Table 2). This association is likely due to the fact that chlorophyll and carotenoids are intricately intertwined as the core antennae of photosystems I and II (Fromme et al. 2003), and the C40 isoprenoid backbone for carotenoids synthesis occurs in plastids. In lettuce, direct positive correlations between phenolic content and antioxidant content and between chlorophyll content and β-carotene at market maturity were reported (Kang and Saltveit 2002; Mou 2005). Given these observations, it might be expected that RIL families with high chlorophyll content also have high ORAC values in the D × M RIL population. With the Pearson correlation coefficient ranging from 0.72 to 0.85, a strong positive correlation was observed between tChl and ORAC within any production cycle for the MM assessment (Table 1). When the RIL population was assessed at GP (open-leaf stage), the strong relationship between tChl and ORAC disappeared, indicating that there was not a direct correspondence between chlorophyll and ORAC at the open-leaf stage. Interestingly, the population mean for the chlorophyll components (chl *a*, chl *b*, tchl) averaged across all production cycles were about 3.3-fold higher than the population averages for the population assessed at MM. At the same time, the ORAC values of the GP assessments were about 70% of the MM values, again suggesting an uncoupling of antioxidants and chlorophyll at the different growth stages. No main-effect QTL were detected from any chlorophyll component, but epistasis was indicated between LG 2 and 8 and a single additive × environment interaction was detected. This indicates that head formation obscures other QTL, which might further improve the nutritional content of lettuce.

A portion of the phytochemical content in lettuce is under genetic control, as variation among leaf and head cultivars is reported for quercetin and kaempferol (Bilyk and Sapers 2002), carotenoids and lutein (Mou 2005), and anthocyanins (Kleinhenz et al. 2003; Ryder 1999; Simonne et al. 2002). Loci for anthocyanins and leaf greenness have been reported (Robinson 1983; Waycott et al. 1999), but we are not aware of QTL or other DNA-based markers associated with nutritional content having been reported for lettuce. 'Diplomat' alleles which serve to increase nutritional content were identified in the MM assessments. This observation could be in part explained by the fact that the head formation appears to be more strongly influenced by 'Margarita'. At the same time, this implies that iceberg lettuce is still capable of contributing alleles favorable to improving nutritional content, despite the fact that iceberg lettuce has a lower nutritional content than leaf or romaine lettuce cultivars (Cao et al. 1996; Llorach et al. 2008; Mou 2005; Wu et al. 2004). The *E33M61D432–E33M59D245* flanking QTL markers were associated with antioxidant and chloropyll content, although the percent variation accounted for by this QTL varied across traits. A further indication that both chlorophyll and antioxidant contents were affected by environmental factors was indicated by the significant AE (main effect QTL × environment interaction) detected on $ORAC_M1.1$ (Table 2) and $ORAC_G2.1$ (Table 3), as well as an AAE and environment interaction with epistatic loci identified between LG2 and LG8 for chlorophyll *b* (Table 3). Seasonal variation of carotenoid

and lutein content has been reported in lettuce (Mou 2005; Rouchaud et al. 1984).

In response to diurnal and seasonal light environment changes, plants actively regulate Chl *a* and Chl *b* to control the amount of absorbed light energy. To accomplish this, chlorophyll synthesis in plants is tightly controlled by gene expression, feedback inhibition, and protein stability in response to the demand of photosystems and environmental conditions (Matsumoto et al. 2004; Vothknecht et al. 1998; Yamasato et al. 2005). Chlorophyll *a* is converted to chlorophyll *b* during the last step of chlorophyll synthesis, and is reconverted to chlorophyll *a* as the initial step in chlorophyll *b* degradation (Hörtensteiner 2006; Kusaba et al. 2007; Tanaka et al. 1998). When Arabidopsis plants are transferred from a high-light to a low-light environment, the light harvesting complex of photosystem II degrades, and the chlorophyll *a* to chlorophyll *b* ratio increases (Tanaka and Tanaka 2005; Yang et al. 2001). Although the population means of chlorophyll content were 3.3× higher in the MM than the GP assessments, the Chl *a*/Chl *b* ratios were essentially identical. No QTL for Chl *a*/Chl *b* ratios were detected in the D × M RIL population assessed at both MM and GP with data from multiple production cycles combined. However, when each production cycle was analyzed separately, one QTL for the trait assessed at the GP stage was identified on LG1 at the same position of the QTL identified for chlorophyll content and ORAC value in MM (data not shown). A significant but small QTL effect in one production cycle may be minimized when combining data sets, which may explain this observation. An assessment of commercial cultivars suitable for the coastal growing region of California indicated that Chl *a*/*b* ratios of crisphead and butterhead cultivars were about 20% higher in fall grown than summer grown (Mou 2005). Further, Chl *a*/*b* ratios were lower in crisphead than butterhead cultivars in both the summer and fall production cycles (Mou 2005). In agreement with Mou (2005), the iceberg cultivar (Diplomat) consistently had lower Chl *a*/*b* ratios than the butterhead cultivar (Margarita) for the GP assessment, but no consistent trend between the parental genotypes was observed at MM (Figs. 1, 2). The genetic materials and cultivars suitable for commercial production in the low desert lettuce production area are different from those of the coastal regions of California (Ryder 1986) and, increasingly, environmental adaptability will be a key breeding objective (Mikel 2007). Although the chlorophyll data among these studies are consistent but limited, it suggests that the chlorophyll modulation system of the cultivars developed for these distinctly different production areas may be different and reflect an adaptation which may have been unconsciously selected for by breeders.

Individual QTLs associated with ORAC accounted for up to 18% of the phenotypic variation for ORAC assessed at MM (Table 2). A QTL associated with head formation was also detected on linkage group 1 and accounted for 26% of the phenotypic variation across the two production cycles it was detected (Table 3). Although both parents were heading types, the allele contributed by 'Margarita' had a larger effect than the 'Diplomat' allele. The head formation QTL colocated with ORAC, Chl *a*, Chl *b*, and tChl QTL, and clearly influenced these latter traits. For the MM assessment grown in the YAC fields, RIL families with closed heads had 43.1% (spring 08) and 33.4% (winter 09) of the ORAC value of the RIL families with a loose-leaf phenotype (Fig. 4). Because the influence of the head architecture was removed by assessing at an early stage in the GP assessment, distinct QTL profiles were detected. Further, assessments of the RIL population at the CPP production site when the plant leaves were fully open (before head formation) revealed that those RIL families which would eventually form heads in the field had only 68–92% of the ORAC value of those families that had a loose-leaf architecture habit when mature (Table 4), strongly suggesting linkage or pleiotropy. This confirms both the complex nature of the accumulation of antioxidant and the influence of head architecture presumably through light activation of genes contributing to antioxidant content. Within a head-type lettuce plant, the phytochemical content varies, with the outer leaves having higher phenolic content and lipophilic antioxidant activity than inner leaves. Genes encoding many of these compounds in lettuce are regarded as generally being light regulated (Ebisawa et al. 2008; Kleinhenz et al. 2003; Oh et al. 2009a; Park et al. 2007), but direct evidence exists only for a few genes in these biosynthetic and regulatory pathways. Experimental evidence from Arabidopsis suggests that this supposition may be an oversimplification, as several genes of the phenylpropanoid pathway do not respond to light (Hemm et al. 2004). To date, no regulatory genes controlling carotenoid biosynthesis have been isolated (Toledo-Ortiz et al. 2010), but identifying which genes are light responsive will provide a strategy by which to improve the nutritional content of lettuce either through marker-assisted breeding or biotechnology approaches.

Recent advances in the field of nutragenetics/nutragenomics may help assess the relationship between fruit and vegetable intake and risk of chronic diseases. For example, genetic variants in the chromosome 9p21 region have been associated with cardiovascular disease and myocardial infarction (McPherson et al. 2007; Samani et al. 2007). Individuals with two copies of the rs2383206 risk allele and low consumption of fruits and vegetables were reported to have a 1.6- to 2.0-fold increased risk for myocardial infarctions (Do et al. 2011). As specific gene–diet interactions become known, it is increasingly likely that the role of specific foods and phytochemical components can be

identified and clarified and subsequently targeted for improvement in plant breeding programs. Lettuce displays a large variation in several nutritional components that are potentially beneficial to human health and can be exploited to improve its nutritional density. For example, Mou (2005) reported a 70-fold range in variation in β-carotene of commercial lettuce cultivars and a range of up to 28-fold in ORAC content was observed in our study (Fig. 2). To that end, our laboratory is currently assessing the expression and regulation of genes encoding compounds in lettuce with the goal of increasing the concentration of compounds that may be beneficial to human health.

Acknowledgments This work was supported by the California State University Agricultural Research Institute grant number 10-4-182 and the National Science Foundation grant number 0820451.

References

Abbo S, Molina C, Jungmann R, Grusak MA, Berkovitch Z, Reifen R, Kahl G, Winter P, Reifen R (2005) Quantitative trait loci governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). Theor Appl Genet 111:185–195. doi:10.1007/s00122-005-1930-y

Agudo A, Slimani N, Ocke MC, Naska A, Miller AB, Kroke A, Bamia C, Karalis D, Vineis P, Palli D, Bueno-de-Mesquita HB, Peeters PH, Engeset D, Hjartaker A, Navarro C, Martinez Garcia C, Wallstrom P, Zhang JX, Welch AA, Spencer E, Stripp C, Overvad K, Clavel-Chapelon F, Casagrande C, Riboli E (2002) Consumption of vegetables, fruit and other plant foods in the European prospective investigation into cancer and nutrition (EPIC) cohorts from 10 European countries. Public Health Nutr 5:1179–1196. doi:10.1079/PHN2002398

Bilyk A, Sapers GM (2002) Distribution of quercetin and kaempferol in lettuce, kale, chive, garlic chive, leek, horseradish, red radish, and red cabbage tissues. J Agric Food Chem 33:226–228. doi:10.1021/jf00062a017

Blanck HM, Gillespie C, Kimmons JE, Seymour JD, Serdula MK (2008) Trends in fruit and vegetable consumption among U.S. men and women, 1994–2005. Prev Chronic Dis 5. http://www.cdc.gov/pcd/issues/2008/apr/07_0049.htm. Accessed 27 May 2010

Canavari M, Nayga RM (2009) On consumers' willingness to purchase nutritionally enhanced genetically modified food. Appl Econ 41:125–137

Cano A, Arnao MB (2005) Hydrophilic and lipophilic antioxidant activity in different leaves of three lettuce varieties. Int J Food Prop 8:521–528. doi:10.1080/10942910500269584

Cao G, Prior RL (1998) Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem 44:1309–1315

Cao G, Sofic E, Prior RL (1996) Antioxidant capacity of tea and common vegetables. J Agric Food Chem 44:3426–3431. doi:10.1021/jf9602535

Chander S, Guo Y, Yang X, Zhang J, Lu X, Yan J, Song T, Rocheford T, Li J (2008) Using molecular markers to identify two major loci controlling carotenoid contents in maize grain. Theor Appl Genet 116:223–233. doi:10.1007/s00122-007-0661-7

Cooper DA (2004) Carotenoids in health and disease: recent scientific evaluations, research recommendations and the consumer. J Nutr 134:221S–224S

Costa-Font M, Gil JM (2009) Structural equation modelling of consumer acceptance of genetically modified (GM) food in the Mediterranean Europe: a cross country study. Food Qual Prefer 20:399–409. doi:10.1016/j.foodqual.2009.02.011

Crowe FL, Roddam AW, Key TJ, Appleby PN, Overvad K, Jakobsen MU, Tjønneland A, Hansen L, Heiner B, et al. and European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart Study Collaborators (2011) Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study Eur Heart J doi:10.1093/eurheartj/ehq465

Dannenberg A (2009) The dispersion and development of consumer preferences for genetically modified food—a meta-analysis. Ecol Econ 68:2182–2192. doi:10.1016/j.ecolecon.2009.03.008

Davey MW, Kenis K, Keulemans J (2006) Genetic control of fruit vitamin C contents. Plant Physiol 142:343–351. doi:10.1104/pp.106.083279

de Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) CARHTAGENE: multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21:1703–1704. doi:10.1093/bioinformatics/bti222

Demmig-Adams B, Adams WW III (2002) Antioxidants in photosynthesis and human nutrition. Science 298:2149–2153. doi:10.1126/science.1078002

Do R, Xie C, Zhang X, Männistö S, Harald K, Islam S, Bailey SD, Rangarajan S, McQueen MJ, Diaz R et al (2011) The effect of chromosome 9p21 variants on cardiovascular disease may be modified by dietary intake: evidence from a case/control and a prospective study. PLoS Med 9(10):e1001106. doi:10.1371/journal.pmed.1001106e

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294

DuPont MS, Mondin Z, Williamson G, Price KR (2000) Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. J Agric Food Chem 48:3957–3964. doi:10.1021/jf0002387

Ebisawa M, Shoji K, Kato M, Shimomura K, Goto F, Yoshihara T (2008) Supplementary ultraviolet radiation B together with blue light at night increased quercetin content and flavonol synthase gene expression in leaf lettuce (*Lactuca sativa* L.). Environ Contol Biol 46:1–11

Fraser PD, Enfissi EM, Bramley PM (2009) Genetic engineering of carotenoid formation in tomato fruit and the potential application of systems and synthetic biology approaches. Arch Biochem Biophys 483:196–204. doi:10.1016/j.abb.2008.10.009

Fromme P, Melkozernov A, Jordan P, Krauss N (2003) Structure and function of photosystem I: interaction with its soluble electron carriers and external antenna systems. FEBS Lett 555:40–44. doi:10.1016/s0014-5793(03)01124-4

García-Macías P, Ordidge M, Vysini E, Waroonphan S, Battey NH, Gordon MH, Hadley P, John P, Lovegrove JA, Wagstaffe A (2007) Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency. J Agric Food Chem 55:10168–10172. doi:10.1021/jf071570m

Giugliano D (2000) Dietary antioxidants for cardiovascular prevention. Nutr Metab Cardiovasc Dis 10:38–44

Giuliano G, Tavazza R, Diretto G, Beyer P, Taylor MA (2008) Metabolic engineering of carotenoid biosynthesis in plants. Trends Biotechnol 26:139–145. doi:10.1016/j.tibtech.2007.12.003

Guenther PM, Dodd KW, Reedy J, Krebs-Smith SM (2006) Most Americans eat much less than recommended amounts of fruits and vegetables. J Am Diet Assoc 106:1371–1379. doi:10.1016/j.jada.2006.06.002

Gutierrez-Gonzalez J, Wu X, Zhang J, Lee J-D, Ellersieck M, Shannon J, Yu O, Nguyen H, Sleper D (2009) Genetic control of soybean seed isoflavone content: importance of statistical model and epistasis in complex traits. Theor Appl Genet 119:1069–1083. doi:10.1007/s00122-009-1109-z

Hall JN, Moore S, Harper SB, Lynch JW (2009) Global variability in fruit and vegetable consumption. Am J Prev Med 36:402–409. doi:10.1016/j.amepre.2009.01.029

Hassimotto NMA, Genovese MI, Lajolo FM (2005) Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. J Agric Food Chem 53:2928–2935. doi:10.1021/jf047894h

Hayashi E, Aoyama N, Still DW (2008) Quantitative trait loci associated with lettuce seed germination under different temperature and light environments. Genome 51:928–947. doi:10.1139/G08-077

Hemm MR, Rider SD, Ogas J, Murry DJ, Chapple C (2004) Light induces phenylpropanoid metabolism in *Arabidopsis* roots. Plant J 38:765–778. doi:10.1111/j.1365-313X.2004.02089.x

Hirschberg J (2001) Carotenoid biosynthesis in flowering plants. Curr Opin Plant Biol 4:210–218. doi:10.1016/S1369-5266(00)00163-1

Hohl U, Neubert B, Pforte H, Schonhof I, Böhm H (2001) Flavonoid concentrations in the inner leaves of head lettuce genotypes. Eur Food Res Technol 213:205–211. doi:10.1007/s002170100361

Hörtensteiner S (2006) Chlorophyll degradation during senescence. Annu Rev Plant Biol 57:55–77. doi:10.1146/annurev.arplant.57.032905.105212

Hu Z-L, Li P, Zhou M-Q, Zhang Z-H, Wang L-X, Zhu L-H, Zhu Y-G (2004) Mapping of quantitative trait loci (QTLs) for rice protein and fat content using doubled haploid lines. Euphytica 135:47–54. doi:10.1023/b:euph.0000009539.38916.32

Jackson EW, Wise M, Bonman JM, Obert DE, Hu G, Peterson DM (2008) QTLs affecting α-tocotrienol, α-tocopherol, and total tocopherol concentrations detected in the Ogle/TAM O-301 oat mapping population. Crop Sci 48:2141–2152. doi:10.2135/cropsci2007.11.0658

Johnston CS, Taylor CA, Hampl JS (2000) More Americans are eating "5 a day" but intakes of dark green and cruciferous vegetables remain low. J Nutr 130:3063–3067

Kang HM, Saltveit ME (2002) Antioxidant capacity of lettuce leaf tissue increases after wounding. J Agric Food Chem 50:7536–7541. doi:10.1021/jf020721c

Kanungsukkasem U, Ng N, Van Minh H, Razzaque A, Ashraf A, Juvekar S, Ahmed SM, Bich TH (2009) Fruit and vegetable consumption in rural adults population in INDEPTH HDSS sites in Asia. Glob Health Action. 2. doi: 10.3402/gha.v2i0.1988

Kerns D, Matheron ME, Palumbo JC, Sanchez CA, Still DW, Tickes BR, Umeda K, Wilcox MA (1999) Guidelines for head lettuce production in Arizona. University of Arizona, Tucson

Kinney AJ (2006) Metabolic engineering in plants for human health and nutrition. Curr Opin Biotechnol 17:130–138. doi:10.1016/j.copbio.2006.02.006

Kleinhenz MD, Gazula A, Scheerens JC, French DG (2003) Variety, shading, and growth stage effects on pigment concentrations in lettuce grown under contrasting temperature regimens. HortTechnology 13:677–683

Kozik A, Truco M-J, Yang B, Matvienko M, Lavelle D, Hill T, Ashrafi H, Chin-Wo SR, Xu H, Stoffel K, Van Deynze A, Xu X, Huang S, Michelmore R (2011) The Genetic Map And Genome Sequencing of Lettuce, Plant & Animal Genomes XIX Conference. Scherago International, San Diego

Kusaba M, Ito H, Morita R, Iida S, Sato Y, Fujimoto M, Kawasaki S, Tanaka R, Hirochika H, Nishimura M, Tanaka A (2007) Rice non-yellow coloring1 is involved in light-harvesting complex II and grana degradation during leaf senescence. Plant Cell 19:1362–1375. doi:10.1105/tpc.106.042911

Legge JSJ, Durant RF (2010) Public opinion, risk assessment, and biotechnology: lessons from attitudes toward genetically modified foods in the European Union. Rev Policy Res 27:59–76. doi:10.1111/j.1541-1338.2009.00427.x

Lichtenthaler HK (2007) Biosynthesis, accumulation and emission of carotenoids, α-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. Photosynth Res 92:163–179. doi:10.1007/s11120-007-9204-y

Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 134:3479S–3485S

Llorach R, Tomas-Barberan FA, Ferreres F (2004) Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. J Agric Food Chem 52:5109–5116

Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, Gil MI, Ferreres F (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 108:1028–1038. doi:10.1016/j.foodchem.2007.11.032

Marwede V, Gul MK, Becker HC, EcKe W (2005) Mapping of QTL controlling tocopherol content in winter oilseed rape. Plant Breed 124:20–26. doi:10.1111/j.1439-0523.2005.tb01870.x

Matsumoto F, Obayashi T, Sasaki-Sekimoto Y, Ohta H, Takamiya K-i, Masuda T (2004) Gene expression profiling of the tetrapyrrole metabolic pathway in Arabidopsis with a mini-array system. Plant Physiol 135:2379–2391. doi:10.1104/pp.104.042408

McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybjaerg-Hansen A, Folsom AR, Boerwinkle E, Hobbs HH, Cohen JC (2007) A common allele on chromosome 9 associated with coronary heart disease. Science 316:1488–1491. doi:10.1126/science.1142447

Mikel MA (2007) Genealogy of contemporary North American lettuce. HortScience 42:489–493

Monteros MJ, Burton JW, Boerma HR (2008) Molecular mapping and confirmation of QTLs associated with oleic acid content in N00-3350 soybean. Crop Sci 48:2223–2234. doi:10.2135/cropsci2008.05.0287

Mou B (2005) Genetic variation of beta-carotene and lutein contents in lettuce. J Amer Soc Hort Sci 130:870–876

Mou B, Ryder EJ (2004) Relationship between the nutritional value and the head structure of lettuce. Acta Hort (ISHS) 637:361–367

Nicolle C, Carnat A, Fraisse D, Lamaison J-L, Rock E, Michel H, Amouroux P, Remesy C (2004) Chracterisation and variation of antioxidant micronutrients in lettuce (*Lactuca sativa* folium). J Sci Food Agric 84:2061–2069. doi:10.1002/jsfa.1916

Oh M–M, Carey EE, Rajashekar CB (2009a) Environmental stresses induce health-promoting phytochemicals in lettuce. Plant Physiol Biochem 47:578–583. doi:10.1016/j.plaphy.2009.02.008

Oh M–M, Trick HN, Rajashekar CB (2009b) Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. J Plant Physiol 166:180–191. doi:10.1016/j.jplph.2008.04.015

Ou B, Hampsch-Woodill M, Prior RL (2001) Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J Agric Food Chem 49:4619–4626. doi:10.1021/jf010586o

Paganga G, Miller N, Rice-Evans CA (1999) The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radic Res 30:153–162

Park JS, Choung MG, Kim JB, Hahn BS, Bae SC, Roh KH, Kim YH, Cheon CI, Sung MK, Cho KJ (2007) Genes up-regulated during red coloration in UV-B irradiated lettuce leaves. Plant Cell Rep 26:507–516. doi:10.1007/s00299-006-0255-x

Patil R, Oak M, Tamhankar S, Sourdille P, Rao V (2008) Mapping and validation of a major QTL for yellow pigment content on 7AL in durum wheat (*Triticum turgidum* L. ssp. *durum*). Mol Breed 21:485–496. doi:10.1007/s11032-007-9147-1

Peltzer K, Pengpid S (2010) Fruit and vegetables consumption and associated factors among in-school adolescents in seven African countries. Int J Public Health 55:669–678. doi:10.1007/s00038-010-0194-8

Penuelas J, Munne-Bosch S (2005) Isoprenoids: an evolutionary pool for photoprotection. Trends Plant Sci 10:166–169. doi:10.1016/j.tplants.2005.02.005

Pourcel L, Routaboul JM, Cheynier V, Lepiniec L, Debeaujon I (2007) Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends Plant Sci 12:29–36. doi:10.1016/j.tplants.2006.11.006

Prior RL, Cao G (2000) Antioxidant phytochemicals in fruits and vegetables: diet and health implications. HortScience 35:588–592

Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53:4290–4302. doi:10.1021/jf0502698

Rice-Evans C, Miller N, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2:152–159. doi:10.1016/s1360-1385(97)01018-2

Robinson RW (1983) The genes of lettuce and closely related species. In: Janick J (ed) Plant breeding reviews. Timber Press, Portland, pp 267–293

Romani A, Pinelli P, Galardi C, Sani G, Cimato A, Heimler D (2002) Polyphenols in greenhouse and open-air-grown lettuce. Food Chem 79:337–342. doi:10.1016/s0308-8146(02)00170-x

Rouchaud J, Moons C, Meyer JA (1984) Effects of pesticide treatments on the carotenoid pigments of lettuce. J Agric Food Chem 32:1241–1245

Rousseaux M, Jones C, Adams D, Chetelat R, Bennett A, Powell A (2005) QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. Theor Appl Genet 111:1396–1408. doi:10.1007/s00122-005-0071-7

Ryder EJ (1986) Lettuce breeding. In: Bassett M (ed) Breeding vegetable crops. AVI Publishing Co., Westport, pp 433–474

Ryder EJ (1999) Lettuce, endive, and chicory. Cabi Publishing, New York

Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P et al (2007) Genomewide association analysis of coronary artery disease. N Engl J Med 357:443–457

Santos CA, Simon PW (2002) QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. Mol Genet Genomics 268:122–129. doi:10.1007/s00438-002-0735-9

Simonne A, Simonne E, Eitenmiller R, Coker CH (2002) Bitterness and composition of lettuce varieties grown in the southeastern United States. HortTechnology 12:721–726

Smirnoff N, Conklin PL, Loewus FA (2001) Biosynthesis of ascorbic acid in plantas: a renaissance. Annu Rev Plant Physiol Plant Mol Biol 52:437–467. doi:10.1146/annurev.arplant.52.1.437

Song W, Derito CM, Liu MK, He X, Dong M, Liu RH (2010) Cellular antioxidant activity of common vegetables. J Agric Food Chem 58:6621–6629. doi:10.1021/jf9035832

Strain H, Cope B, Svec W (1971) Analytical procedures for the isolation, identification, estimation and investigation of the chlorophylls. Methods Enzymol 23:452–476

Tanaka R, Tanaka A (2005) Effects of chlorophyllide *a* oxygenase overexpression on light acclimation in *Arabidopsis thaliana*. Photosynthesis Res 85:327–340. doi:10.1007/s11120-005-6807-z

Tanaka A, Ito H, Tanaka R, Tanaka NK, Yoshida K, Okada K (1998) Chlorophyll *a* oxygenase (*CAO*) is involved in chlorophyll *b* formation from chlorophyll *a*. Proc Natl Acad Sci USA 95:12719–12723

Tapiero H, Townsend DM, Tew KD (2004) The role of carotenoids in the prevention of human pathologies. Biomed Pharmacother 58:100–110. doi:10.1016/j.biopha.2003.12.006

Toledo-Ortiz G, Huq E, Rodriguez-Concepcion M (2010) Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. Proc Natl Acad Sci USA 107:11626–11631. doi:10.1073/pnas.0914428107

Torres CA, Andrews PK, Davies NM (2006) Physiological and biochemical responses of fruit exocarp of tomato (*Lycopersicon esculentum* Mill.) mutants to natural photo-oxidative conditions. J Exp Bot 57:1933–1947. doi:10.1093/jxb/erj136

USDA (2010a) Vegetables and melons yearbook data/#89011. Vegetables and melons yearbook data/#89011. Economic Research Service. http://usda.mannlib.cornell.edu/usda/ers/89011/89011.pdf. Accessed 27 May 2010

USDA (2010b) United States Department of Agriculture, Agricultural Research Service. 2010. Oxygen radical absorbance capacity (ORAC) of selected foods, Release 2. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/orac. Accessed 9 December 2011

Vogt T (2010) Phenylpropanoid biosynthesis. Mol Plant 3:2–20. doi:10.1093/mp/ssp106

Vothknecht UC, Kannangara CG, von Wettstein D (1998) Barley glutamyl $tRNA^{Glu}$ reductase: mutations affecting haem inhibition and enzyme activity. Phytochemistry 47:513–519. doi:10.1016/s0031-9422(97)00538-4

Wang CS, Rutledge JJ, Gianola D (1994) Bayesian analysis of mixed linear models via Gibbs sampling with an application to litter size in Iberian pigs. Genet Sel Evol 26:1–25. doi:10.1186/1297-9686-26-2-91

Waycott W, Fort SB, Ryder EJ, Michelmore RW (1999) Mapping morphological genes relative to molecular markers in lettuce (*Lactuca sativa* L.). Heredity 82:245–251. doi:10.1038/sj.hdy.6884730

Wong JC, Lambert RJ, Tadmor Y, Rocheford TR (2003) QTL associated with accumulation of tocopherols in maize. Crop Sci 43:2257–2266

Wong JC, Lambert RJ, Wurtzel ET, Rocheford TR (2004) QTL and candidate genes phytoene synthase and ζ-carotene desaturase associated with the accumulation of carotenoids in maize. Theor Appl Genet 108:349–359. doi:10.1007/s00122-003-1436-4

Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2004) Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J Agric Food Chem 52:4026–4037. doi:10.1021/jf049696w

Yamasato A, Nagata N, Tanaka R, Tanaka A (2005) The N-terminal domain of chlorophyllide *a* oxygenase confers protein instability in response to chlorophyll *b* accumulation in Arabidopsis. Plant Cell 17:1585–1597. doi:10.1105/tpc.105.031518

Yang D-H, Andersson B, Aro E-M, Ohad I (2001) The redox state of the plastoquinone pool controls the level of the light-harvesting chlorophyll *a/b* binding protein complex II (LHC II) during photoacclimation. Photosynthesis Res 68:163–174. doi:10.1023/A:1011849919438

Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:1527–1536. doi:10.1093/bioinformatics/btm143

Yi N, Xu S (2002) Mapping quantitative trait loci with epistatic effects. Genet Res 79:185–198