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Quantitative trait loci (QTL) analysis for antioxidant and agronomically important traits in tomato (*Lycopersicon esculentum*)

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Abstract: Tomato is one of the most widely produced and consumed vegetable crops worldwide. Plant breeders have usually focused on improvement of horticulturally important traits such as yield, fruit size, shape and colour. With increased attention on human health, however, plant breeders also consider the improvement of health-related traits of fruits and vegetables such as antioxidant characters. In the present study, genes controlling both health-related and horticulturally important traits were mapped in the tomato genome using 152 *Lycopersicon hirsutum* BC_2F_2 individuals. For this aim, all plants were phenotypically and genotypically characterised and a total of 75 QTLs were identified for all traits. Of the 75 QTLs, 28 were identified for 5 antioxidant traits including total water soluble antioxidant capacity, vitamin C, total phenolics, total flavonoids, and lycopene contents, and 47 QTLs were identified for 8 agronomic traits including fruit weight, external and internal fruit colour, fruit firmness, fruit shape, stem scar size, locule number, and wall thickness. Markers linked with these QTLs can be used in marker assisted selection (MAS) for improvement of elite tomato lines.

Key words: Tomato, quantitative trait loci, linkage map, antioxidant, agronomic traits, marker assisted selection

Domates (*Lycopersicon esculentum*)'te antioksidant ve agronomik olarak önemli karakterler için kantitatif karakter lokus analizleri

Özet: Domates dünyada üretimi ve tüketimi en çok yapılan sebzelerden biridir. Bundan dolayı birçok bitki ıslahçısı bugüne kadar domateste ürün verimliliği, meyve büyüklüğü, şekli ve rengi gibi tarımsal açıdan önem teşkil eden karakterlerin geliştirilmesi için çaba sarfetmişlerdir. İnsan sağlığına verilen önemin artmasıyla beraber, bitki ıslahçıları artık meyve ve sebzelerde antioksidant karakterleri gibi sağlıkla ilişkili karakterlerin de geliştirilmesini dikkate almaya başlammışlardır. Yapılan bu çalışmada, 152 bireyden oluşan BC_2F_2 *Lycopersicon hirsutum* populasyonu kullanılarak, hem sağlık açısından hem de tarımsal açıdan önem teşkil eden karakterler domates genomunda haritalanmıştır. Bu amaç doğrultusunda, populasyondaki bütün bireyler fenotipik ve genotipik olarak karakterize edildikten sonra analiz edilen bütün karakterler için toplamda 75 QTL (genetik lokus) belirlenmiştir. Bu 75 QTL içerisinden, suda çözünen toplam antioksidant aktivitesi, C vitamini, toplam fenolik, toplam flavonoid ve likopen miktarını da içerisine alan beş

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antioksidant karakteri için 28 adet, tarımsal açıdan önem teşkil eden dış ve iç meyve rengi, meyve ağırlığı, sertliği, şekli, gövde izi, lokul sayısı ve perikarp kalınlığı gibi sekiz karakter için ise toplamda 47 QTL belirlenmiştir. Sonuç olarak belirlenen bu QTL'lerle ilişkili olan markörler, markör dayalı seçilim tekniği (MAS) kullanılmak suretiyle birinci sınıf kültür domates hatları geliştirilmesinde kullanılabilir.

Anahtar sözcükler: Domates, nicel özellik lokusu, bağlantı haritası, antioksidant, tarımsal açıdan önemli karakterler, markör dayalı seçilim

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely produced and consumed vegetable crops worldwide. Cultivated tomato was derived from its wild ancestors via long and intensive domestication and breeding that aimed to improve crop and fruit characteristics. For such improvement, plant breeders usually use elite germplasm, which has low genotypic variation but increases the possibility of regaining the elite line's phenotype during the breeding process (Tanksley and Nelson 1996). Modern plant breeders have avoided using wild species to develop new cultivars because of unfavourable genetic linkages that may hamper recovery of the elite phenotype (Tanksley and Nelson 1996; Monforte and Tanksley 2000). However, many quantitative trait locus (QTL) studies have revealed that exotic germplasm has genetic potential for improvement of agronomically important traits of elite lines even if the germplasm itself has an undesirable phenotype (Eshed and Zamir 1995; Bernacchi et al. 1998; Doğanlar et al. 2002; Frary et al. 2004; Rousseaux et al. 2005). Thus, it can be said that the appearance of a wild species does not always reflect its genetic potential.

Fruit quality, yield, fruit size, shape, and colour are the most agronomically important quantitatively inherited traits because they attract the attention of both farmers and consumers. Therefore, many plant breeders have focused on improvement of these agronomic traits throughout agricultural history (Tanksley and McCouch 1997). However, breeders largely ignored the health-related traits of fruit and vegetables because of their inability to be visualised in plant phenotype. With the development of new and appropriate biochemical and trait mapping techniques, it is now possible for plant breeders to consider improvement of health-related traits such as increased antioxidant content.

Antioxidants are any substances that can delay or inhibit oxidation reactions caused by free radicals. Therefore, antioxidants protect organisms against the deleterious effects of oxidative stress such as increased incidence of cardiovascular diseases, several cancers, and neurological disorders in animals and leaf senescence, chlorophyll destruction, and decreased photosynthesis rate in plants (Yao et al. 2004; Podsedek 2007). There are many reports about the positive impact of antioxidants on human health (Madhavi et al. 1996; Bramley 2000; Arab and Steck 2000; Roberfroid 2000; Yao et al. 2004; Rodrigez et al. 2006; Podsedek 2007).

In the present study, QTL analysis was performed for multiple agronomic and health-related traits. QTLs were identified for some selective nutritional quality traits such as total water soluble antioxidant capacity, vitamin C content, total phenolics content, total flavonoids content, and lycopene content, and agronomic traits such as external and internal fruit colour, fruit weight, firmness, fruit shape, stem scar size, locule number, and fruit wall thickness. Identification of QTLs allowed an estimate of gene number controlling each trait also allowed the relative contribution of each locus to trait phenotype and potential pleiotropic effects. The alleles for nutritionally and agronomically important traits identified in the present work can be used for development of high quality tomato hybrids with traditional introgression techniques and marker assisted selection.

Materials and methods

Plant materials

Both health-related and agronomically important traits were mapped in a BC_2F_2 mapping population that was developed by crossing *Lycopersicon esculentum* Mill. (syn: *Solanum lycopersicum* L.) (TA1166) as a recurrent parent with *L. hirsutum* Humb. and Bonpl. (syn: *Solanum habrochaites* S.Knapp & D.M.Spooner) (LA1223) as a donor. A

BC_2F_2 backcross population was used to increase the amount of *L. esculentum* Mill. genome in the population, so the phenotype of elite line could be more easily regained. LA1223 is an indeterminant wild tomato line with small, green fruits, while TA1166 a cultivated tomato line with large, red fruits. For this study, 10 tomato plants from each of the 152 individuals of the BC_2F_2 population were grown in Antalya by the seed company MULTI Tarım. Seeds were sown on 6 February 2007 and transplanted to the open field on 25 March. Plants were grown in double rows with 140 cm between wide rows and 50 cm between narrow rows. Double row planting is standard agricultural practice in coastal regions of Turkey as it provides plants with some shade to protect against sunburn. Within rows, plants were spaced at 40 cm intervals. Thus, plant density was 2.6 plants per m^2. Soil was well-drained and loamy in texture and 500 kg of 15:15:15 (N:P:K) fertiliser and 50 t of composted manure were applied per ha for basal fertilisation before transplanting. Plants were watered by drip irrigation with fertigation (1.4 dS m^{-1} EC value) at each irrigation using 1-2-1 fertiliser until first fruit set, 2-1-1 fertiliser until first fruit ripening, and 1-1-2 fertiliser after first fruit ripening. Fungicide was applied once during the growing season as a protective measure.

Tomato fruits were harvested from 10 plants for each line at the normal market stage in June-July 2007. After phenotypic characterisation for agronomic traits, tomato fruits were washed, about 1 kg of fruits of each sample were diced into pieces and mixed well. Then, tomato fruit mixtures were packed and stored at –20 °C until biochemical analyses were performed. As has been reported, there is no significant difference in antioxidant content of fresh and frozen tomato fruits (Toor et al. 2006).

Phenotypic characterisation

Eight agronomically important fruit traits were assessed for each member of the BC_2F_2 population as described in Frary et al. (2003). The studied agronomic traits were fruit weight, external and internal fruit colour, fruit firmness, fruit shape, stem scar, locule number, and fruit wall thickness. Fruit weight (FW) was determined by taking the average weight of 10 mature tomato fruits. External (EXC) and internal fruit colour (INC) were visually assessed for each line using a scale from 1 to 5 (1 = yellow or orange, 5 = most intense red). Fruit firmness (FIRM) was measured by hand squeezing of ripe tomato fruits using a scale of 1 to 5 (1 = soft, 5 = very firm). Fruit shape (FS) was determined by comparing the ratio of fruit length to fruit width using a scale from 1 to 5 (1 = round, 5 = elongated). Stem scar size (SSC) was measured based on stem scar diameter (1 = small, 5 = very large). Locule number (LN) of tomato fruit was determined by counting the locules of tomato fruit after transversely cutting the fruit. Fruit wall (WALL) or pericarp thickness was also determined using transverse sections of fruits using a scale from 1 to 5 (1 = thin, 5 = very thick) (Tanksley and Nelson 1996; Frary et al. 2003).

Total water soluble antioxidant capacity assay

For the total water soluble antioxidant (WAOX) capacity assay, 200 g of fruit was homogenised with 100 mL of cold distilled water for 2 min at +4 °C. Then, 10 g of puree was taken from the homogenate and diluted with 15 mL of cold distilled water. The homogenate was centrifuged at 3000 × *g* for 10 min at +4 °C. The WAOX capacity of tomato fruits was measured spectrophotometrically (Shimadzu, 1700 UV Visible Spectrophotometer, Japan) using the ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] decolourisation assay of Re et al. (1999). The ABTS radical cation stock solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulphate and was stored in the dark for 12-16 h. Before use, the ABTS•+ stock solution was diluted with phosphate buffered saline (PBS) at pH 7.4 to adjust its absorbance to 0.700 (±0.02) at 734 nm. Then, 2.5, 5, and 7.5 µL aliquots of tomato supernatant were mixed separately with 2 mL of ABTS radical cation solution, and decolourisation of blue-green ABTS•+ stock solution was kinetically monitored at 734 nm for 6 min at 30 °C. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard for construction of a standard curve. Each assay was repeated to give 3 replicates for each aliquot volume. The results were calculated as area under the curve (AUC) and expressed as µmol trolox kg^{-1} fresh weight (FW) of tomato fruits. To calculate AUC, the percent inhibition/concentration values for the extracts and trolox were plotted separately against the test periods (1, 3, 6 min) and the ratio of the areas

of curves for extracts and trolox was used to calculate the AUC value (Re et al. 1999).

Ascorbic acid content determination

Ascorbic acid (VITC) content of tomato was measured by AOAC 967.21 titrimetric method using 2,6-dichloroindophenol as reactive substance (Nielsen 2003). The extractions were prepared by homogenisation of 100 g of tomato with 115 mL of acetic acid-metaphosphoric acid extraction solution (8% acetic acid v/v and 3% metaphosphoric acid w/v) for 2 min at +4 °C. Afterwards, 25 g of tomato extract was taken from the homogenate and diluted to 100 mL with cold extraction solution. Then each homogenate was passed through filter paper and 15 mL of diluted sample was titrated against a 2,6-dichloroindophenol dye solution. For each tomato extract, the VITC content of 3 replicate samples was measured. The titrator was calibrated using commercial L-ascorbic acid and the results were expressed as mg ascorbic acid kg^{-1} FW of tomato fruit (Nielsen 2003).

Total phenolics content determination

The total phenolics (PHE) content was measured by using Folin-Ciocalteau as a reactive reagent and adapted from the method of Singleton and Rossi (1965). In this procedure, gallic acid was used for generation of a standard curve. Homogenates were prepared by blending 200 mL of cold distiled water with 100 g of tomato sample for 2 min at +4 °C. Then 2.5 g of homogenate was diluted with 20 mL of cold distilled water and centrifuged at 3000 × *g* for 10 min at +4 °C. After that, 2 mL of the supernatant was mixed with 10 mL of 2 N (10%) Folin-Ciocalteu and incubated for 3 min; then 8 mL of 0.7 M Na_2CO_3 was added. After 2 h of incubation at room temperature, the absorbance of the reaction mixture was measured at 765 nm in a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). There were 3 replicates for each sample. The PHE content was expressed as gallic acid equivalents (mg kg^{-1} FW) based on a gallic acid standard curve (Singleton and Rossi 1965).

Total flavonoids content determination

The total flavonoids (FLAV) content of tomato fruits was spectrophotometrically measured using the method described by Zhishen et al. (1999). Based on this procedure, epicatechin was used for generation of a standard curve. Tomato homogenates were prepared by blending 100 g of tomato sample with 200 mL of cold distilled water for 2 min at low speed in a Waring blender at +4 °C. Then 2.5 g of homogenate was diluted with 20 mL of cold distilled water and centrifuged at 3000 × *g* for 10 min at +4 °C. Then 1250 µL of clear supernatant was used for the measurement of the FLAV content. For this, 75 µL of 5% $NaNO_2$ was mixed with 1250 µL of tomato supernatant and then the mixture was incubated for 5 min. After that, 75 µL of 10% $AlCl_3$ was added to the mixture. After 1 min, 0.5 mL of 1 M NaOH and 0.6 mL of distilled water were added to the reaction mixture and the absorbance was measured at 510 nm in a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). There were 3 replicates for each sample. The FLAV content was calculated based on an epicatechin standard curve (mg kg^{-1} FW) (Zhishen et al. 1999).

Lycopene content determination

Lycopene (LYC) content was evaluated by using the method developed by Sadler et al. (1990). In this assay, homogenate was prepared by blending 100 g of tomato fruit with 200 mL of cold distilled water for 2 min at +4 °C. Three replicates of 3 g of tomato homogenates were diluted with 50 mL of hexane-acetone-ethanol (2:1:1, v:v:v) extraction buffer in a brown volumetric flask. Then these mixture were shaken on a rotary mixer for 30 min at 150 rpm at 25 °C in the dark. After agitation, samples were transferred into separation funnels and 10 mL of distilled water was added to the extract, and the samples were left for 4 h in the dark to separate polar and non-polar phases. LYC dissolved in the top, hexane layer. The top layer was taken and its absorbance was measured at 472 nm using a quartz cuvette in a spectrophotometer. The LYC content was expressed as mg kg^{-1} FW based on a lycopene standard curve (Sadler et al. 1990).

Genotypic characterisation

For molecular characterisation and QTL mapping of both antioxidant and agronomically important traits, Cleaved Amplified Polymorphic Sequence (CAPS) marker analyses were performed. CAPS are highly polymorphic, codominant, phenotypically neutral, and abundant molecular markers. In this technique, sequence-specific primers are used

to amplify a specific DNA region that contains restriction sites. After amplification of this DNA region, the incidence of variation/polymorphism is enhanced by using particular restriction enzymes that cleave the PCR products. Polymorphic markers were applied to the complete mapping population using the appropriate primer and restriction enzyme combinations in order to construct a molecular marker linkage groups map (Konieczny and Ausubel 1993).

Statistical analysis

Analysis of variance (ANOVA) and Fishers PLSD were used for statistical analysis of the data. Significance was determined at $P < 0.05$. Evaluation of correlation between the traits was done using the QGENE software (Nelson 1997). Chi-square analysis was performed in Excel 7. Single point regression analysis was performed to determine the association between molecular markers and each trait using QGENE (Nelson 1997). The effect of *L. hirsutum* alleles was calculated by subtracting the trait mean for individuals with at least one wild allele from the trait mean for individuals that were homozygous for *L. esculentum* alleles and dividing by the *L. esculentum* mean.

Results

Antioxidant traits

Total water soluble antioxidant (WAOX) capacity of fruit from *L. hirsutum* was 1.5-fold higher than the WAOX capacity of *L. esculentum*, a significant difference ($P < 0.05$, Table 1). The WAOX capacity of the BC_2F_2 population was normally distributed with continuous variation between 1618 and 5092 μmol trolox kg^{-1} FW, showing a 3.2-fold range in the population. The population mean was closer to the wild parental mean than the mean of *L. esculentum* (Table 1). The vitamin C (VITC) content of *L. hirsutum* and *L. esculentum* were nearly equal. In spite of the similarity of the 2 parental lines in VITC content, the BC_2F_2 population showed distinct segregation for the trait with values ranging from 80 to 320 mg kg^{-1} FW (Table 1). Interestingly, 84% of the population had higher VITC content than *L. hirsutum* and 73% of progeny had higher values than *L. esculentum*. The mean value of VITC content of the BC_2F_2 population was approximately 18% higher than the parents, 200 ± 4 mg kg^{-1} FW. The total phenolics (PHE) content of *L. hirsutum* was 1.5-fold higher than the PHE content of *L. esculentum* (Table 1) with a statistically significant difference. The PHE content of the BC_2F_2 population showed 3.2-fold range. The mean value of the PHE compounds for the population was intermediate between the parental lines. *L. hirsutum* had 1.5-fold higher total flavonoids (FLAV) content than cultivated tomato (Table 1). There was 5.3-fold difference between the lowest and the highest FLAV content of the population. The mean value of FLAV content for the BC_2F_2 population was 90.8 ± 3.6 mg kg^{-1} FW, slightly higher than FLAV content of *L. hirsutum*. The lycopene (LYC) content of *L. esculentum* was 22 times higher than that of *L. hirsutum* (Table 1). The BC_2F_2 population exhibited the highest variation for LYC among the antioxidant traits. The LYC content for the BC_2F_2 population ranged from 4 to 172 mg kg^{-1} FW (43-fold difference), while the mean value for the population was 69.5 ± 2.5 mg kg^{-1} FW.

Correlations between antioxidant traits

Moderate but statistically significant ($P < 0.05$) positive correlations were observed between some of the antioxidant traits (Table 2). The strongest positive correlations for antioxidant traits were found between WAOX capacity and PHE content ($r = 0.48$, $P < 0.0001$) and between WAOX capacity and VITC content ($r = 0.44$, $P < 0.0001$). These results were not surprising because both PHE and VITC content have great contributions to the WAOX capacity. An additional positive correlation was observed between VITC content and PHE content ($r = 0.36$, $P < 0.0001$).

Agronomic traits

The 2 parental lines showed extremely different values for fruit weight (FW), with a 40-fold difference. All progeny showed intermediate values for the trait ranging from 6.5 to 261.9 g (Table 1). The mean value of FW for the population was calculated as 95.7 ± 3.2 g. For both external (EXC) and internal (INC) fruit colour, *L. esculentum* had a moderate red fruit colour of 3 while *L. hirsutum* had green fruit (Table 1). The population showed a wide range of variation for both characters. *L. hirsutum* had softer fruit than *L. esculentum*. For the BC_2F_2 population, most individuals had firmness intermediate between the 2 parents (Table 1). In both parental lines, fruit shape

Table 1. Mean values and standard errors of parental lines and BC_2F_2 population for antioxidant and agronomical important traits. Values followed by different letters are significantly different ($P < 0.05$).

	L. esculentum	*L. hirsutum*	BC_2F_2 Population	
Trait	Mean ± SE	Mean ± SE	Mean ± SE	Range
WAOX (µmol trolox kg^{-1} FW)	2575 ± 123 a	3925 ± 11 b	3384 ± 49 c	1618-5092
VITC (mg kg^{-1} FW)	170 ± 13 a	160 ± 10 a	200 ± 4 b	80-320
PHE (mg kg^{-1} FW)	207.3 ± 0.7 a	303.1 ± 1.4 b	242.1 ± 4.3 c	140-454
FLAV (mg kg^{-1} FW)	54.6 ± 0.4 a	83.3 ± 2.9 b	90.8 ± 3.6 b	47-250
LYC (mg kg^{-1} FW)	89.4 ± 1.3 a	4.1 ± 0.1 b	69.5 ± 2.5 c	4-172
FW (g)	261.9	6.5	95.7 ± 3.2	6.5- 261.9
INC	3	1	2 ± 0.1	1- 4.5
EXC	3	1	2.2 ± 0.1	1- 5
FIRM	3.5	2	3.2 ± 0.1	1- 5
FS	1	1	1.31 ± 0.04	1- 4.5
SSC	5	1	3.7 ± 0.1	1- 5
LN	6	3	4.3 ± 0.1	2- 6
WALL	4.5	1	2.8 ± 0.1	1- 5

(FS) was spherical. However, there was good variation ranging from spherical to elongated for fruit shape in the BC_2F_2 population (Table 3). Nearly all progeny (88%) had round fruit shape similar to the 2 parents. In contrast, 12% of the mapping population had elongated fruit. The 2 parental lines were extremely different for stem scar size (SSC). There was a 5-fold difference between them, the SSC of *L. esculentum* was very large, whereas *L. hirsutum*'s SSC was very small (Table 1). Fruits of the BC_2F_2 population had intermediate stem scars. Fruit of *L. esculentum* had an average of 6 locules, whereas *L. hirsutum* had an average of 3 locules. The average fruit locule number (LN) for the population was intermediate with variation from 2 to 6 locules (Table 1). Cultivated tomato had much thicker pericarp or wall thickness (WALL) than the wild species. Among the BC_2F_2 population, average WALL was intermediate.

Correlation between agronomically important traits

The highest statistically significant positive correlation was observed between external and internal fruit colour as expected ($r = 0.88$, $P < 0.0001$) (Table 3). There was a positive correlation between SSC and FW ($r = 0.60$, $P < 0.0001$). SSC also showed high correlation with LN ($r = 0.58$, $P < 0.0001$). There was also a moderate significant correlation between FW and LN ($r = 0.50$, $P < 0.0001$). Elongated FS was correlated with fewer LN and smaller SSC (Table 3).

Genotypic characterisation and QTL mapping

To identify QTLs for both health-related and agronomically important traits, 70 CAPs and 2 SSR markers were tested on the 152 BC_2F_2 lines for genotypic characterisation. Out of the 70 CAPs and 2 SSRs markers that were mapped in the BC_2F_2 population, 14 of the markers (19%) fit the 29/32 AA : 3/32 Aa segregation ratio expected for a codominant marker after chi-square analysis ($P < 0.05$). A total of 58 of the markers (81%) were skewed toward the *L. hirsutum* genotypes, but there were no markers that were skewed toward the *L. esculentum* homozygous genotype.

A genetic linkage map was constructed for the 72 markers using the locations of the markers in a *L. pennellii* interspecific population as reference (Sol Genomics Network 2008). The number of markers per linkage group ranged from 3 (chromosomes 6 and 8) to 12 (chromosome 2) (data not shown). Overall, the map provided approximately 65% genome coverage. The poorest coverage was on chromosome 6, with only 9% of the genome represented by the 3 markers mapped on this chromosome. Five chromosomes (1,

Table 2. Correlations between antioxidant traits in the population. P value of each correlation is given in parentheses.

Trait	VITC	PHE	FLAV	LYC
WAOX	0.44 (0.0001)	0.48 (0.0001)	NS	–0.16 (0.05)
LYC	–0.16 (0.05)	NS	NS	
FLAV	NS	NS		
PHE	0.36 (0.0001)			

* Correlations with P value > 0.05 are considered to be non-significant (NS)

Table 3. Correlations between agronomical important traits in the population. P value of each correlation is depicted in parentheses.

Trait	FW	EXC	INC	FIRM	FS	SSC	LN
WALL	0.16 (0.05)	0.38 (0.0001)	0.38 (0.0001)	0.20 (0.02)	0.23(0.006)	0.18 (0.03)	NS
LN	0.50 (0.0001)	NS	NS	NS	–0.51 (0.0001)	0.58 (0.0001)	
SSC	0.60 (0.0001)	NS	NS	NS	–0.34 (0.0001)		
FS	–0.33 (0.0001)	0.21 (0.01)	0.19 (0.02)	NS			
FIRM	NS	NS	NS				
INC	NS	0.88 (0.0001)					
EXC	NS						

* Correlations with P value > 0.05 are considered to be non-significant (NS)

2, 5, 11, and 12) had at least 75% coverage with best coverage on linkage groups 2 (96%) and 5 (94%).

Single point regression analysis was performed to determine the association between molecular markers and each trait in the BC_2F_2 mapping population using QGENE (Nelson 1997). If more than one contiguous marker showed significant association with the same trait, it was assumed that only one locus was involved. In this study, a total of 75 significant ($P < 0.05$) QTLs were identified for all 13 characters. Table 4 lists the QTLs that were identified for each trait. Of the 75 QTLs, 28 (37%) were related with antioxidant traits, while 47 (63%) were associated with agronomically important traits.

QTLs for antioxidant traits

Six QTLs were identified for WAOX capacity (Table 4). The most significant locus was *waox12.1* on chromosome 12 with P = 0.0002. For this locus, the *L. hirsutum* allele was associated with a 12% increase in WAOX capacity. For 5 out of the 6 QTLs, as expected based on the values for the parental lines, *L. hirsutum waox* alleles enhanced the WAOX capacity. On the other hand, only 1 *L. esculentum waox* allele (*waox1.1*) was associated with higher WAOX capacity. VITC content was associated with 5 QTLs. The most significant *vitc* QTL was *vitc6.1*, marked by CT206, with P = 0.0005. The wild allele for this locus was associated with a 16% increase in VITC content. For *vitc1.1*, *vitc6.1*, and *vitc12.1*, *L. hirsutum* alleles were associated with higher VITC content for the loci on chromosomes 1, 6, and 12, while *L. esculentum* alleles were responsible for higher VITC content for the chromosome 2 QTLs. Five QTLs were detected for the PHE content. *phe6.1* was the most significant (P = 0.01) and was linked to marker CT206. Locus *phe7.1*, however, had the greatest effect on phenotypic variance as the *L. hirsutum* allele for this locus accounted for a 17% increase in phenolics content. For FLAV content, 4 QTL regions were

identified on the molecular marker map. The most significant QTL was *flav11.1* linked with TG36. The source of high FLAV content for the *flav11.1* locus was the *L. hirsutum* allele, which accounted for a 24% increase in FLAV content. In contrast, *L. hirsutum* alleles were associated with reduced FLAV content for the other 3 QTLs. Eight QTLs were identified for LYC content. *lyc8.1* and *lyc12.1* were the 2 most significant QTLs (Table 4). For *lyc3.1*, *lyc7.1*, *lyc8.1*, and *lyc12.1*, *L. esculentum* alleles were associated with an increase in LYC content, while for the rest of the QTLs *L. hirsutum* alleles were responsible for high LYC content. Of most interest were *lyc9.1* and *lyc10.1* as wild alleles at these loci were responsible for 37% and 46% increases in LYC content, respectively.

QTLs for agronomic traits

Three QTLs were identified for the FW and each QTL was located on different chromosomes (Table 4). *fw7.1* was the most significant QTL region for fruit weight and it was marked by both At2g42750 and At3g14910 with P = 0.00001. For this locus, the wild allele was associated with a 31% decrease in FW. Nine QTLs were identified for EXC on 6 different chromosomes. Chromosomes 4, 9, and 12 contained 2 *exc* QTLs, while chromosomes 1, 7, and 8 had one QTL each. The most significant QTL for EXC was *exc4.2* with P < 0.002. For *exc1.1*, *exc7.1*, *exc8.1*, *exc12.1*, and *exc12.2 L. hirsutum* allele were associated with decreased fruit colour; however, *exc4.1*, *exc4.2*, *exc9.1*, and *exc9.2* alleles from *L. hirsutum* were responsible for increased red colour. The wild alleles for the 2 loci on chromosome 9 increased EXC by 41% and 32%, respectively. For INC, 7 QTLs regions were identified. These were located on the same chromosomes as the *exc* QTLs. *inc8.1* linked with TG307 was the most significant QTL for INC with P = 0.0007. For *inc4.1* and *inc9.1 L. hirsutum* alleles were related to higher colour formation with these alleles increasing red colour by 22% and 30%, respectively. However, *L. esculentum* alleles increased INC for the other 5 loci. Seven QTLs were identified for FIRM. The most significant locus was *firm3.1*. *L. hirsutum* alleles were always associated with increased FIRM with effects as high as 21% for *firm2.1* and *firm2.2*. Four QTLs for FS were detected in this study. SSR40 was associated with *fs2.1*, the most significant QTL with P = 0.002. The source of elongated FS was *L. hirsutum* alleles. Seven QTLs on 7 different chromosomes were associated with SSC. The most significant QTL for SSC was *ssc2.1* with P = 0.00001. *L. hirsutum* alleles were associated with large SSC in only one case, *ssc11.1*. For all other SSC QTLs, the *L. hirsutum* alleles were responsible for formation of smaller SSC. Of most interest was *ssc2.1* for which the wild allele decreased SSC by 30%. The LN was associated with 6 QTLs on chromosomes. *L. esculentum* alleles were always associated with higher LN. Four QTLs were associated with WALL. For all of the QTLs, *L. esculentum* alleles enhanced the thickness of the pericarp. The most significant QTL for WALL was *wall12.1* with P < 0.003.

Discussion

Recently, consumers have shown great interest in the nutritional quality of fruit and vegetables. Antioxidants are chemical compounds that protect cells from the deleterious effect of oxidative stress that results from different cellular processes. Therefore, antioxidants prevent or decrease the risk of diseases associated with oxidative stress, such as cardiovascular diseases, cancers, and neurological disorders (Yao et al. 2004; Podsedek 2007). In addition to human health, antioxidant compounds also have several positive effects on plant health. For example, there is some evidence that there is a positive correlation between tolerance to salinity and antioxidant production in plants (Mittova et al. 2002; Mittova et al. 2004; Frary et al. 2010). Moreover, during pathogen-plant interactions, one of the early responses to pathogens is accumulation of reactive oxygen species (ROS) in the infection area (May et al. 1996). Production of antioxidant compounds could help the plant to protect itself from internal ROS accumulation. Therefore, tomato with increased antioxidant capacity will not only positively affect human health but will also positively affect the plant health and plant tolerance to biotic and abiotic stress conditions.

In the present study, *L. hirsutum* was used as a donor parent in order to increase both phenotypic and genotypic variation in the mapping population. *L. hirsutum* had significantly higher values than *L. esculentum* for all antioxidant traits, except VITC. This may be due to the fact that antioxidant compounds

Table 4. QTL identified for antioxidant and for agronomic traits, their location in the tomato genome, and any matches with previous studies. Table also shows the source of these QTL alleles and the effect of *Lycopersicon hirsutum* alleles over the traits.

Trait	QTL symbol	Marker	Chr	P	Effect of L. hirsutum allele (%)	Previously identified loci[a]
WAOX	*waox1.1*	At3g06050	chr1	0.0427	–7	
	waox5.1	T564	chr5	0.0158	9	
	waox6.1	CT206	chr6	0.0196	9	1
	waox8.1	TG307	chr8	0.0036	9	
	waox12.1	At2g06530	chr12	0.0002	12	
	waox12.2	At4g16580	chr12	0.0046	9	
VITC	*vitc1.1*	At4g00560	chr1	0.0505	8	
	vitc2.1	SSR40	chr2	0.0067	–14	
	vitc2.2	At4g37280	chr2	0.0047	–12	2
	vitc6.1	CT206	chr6	0.0005	16	
	vitc12.1	At2g06530	chr12	0.0199	9	1.2
PHE	*phe1.1*	At2g15890	chr1	0.0362	8	
	phe6.1	CT206	chr6	0.0144	11	
	phe7.1	At1g55870	chr7	0.0174	17	1
	phe9.1	At5g06130	chr9	0.0479	10	1
	phe12.	At2g06530	chr12	0.0177	9	
FLAV	*flav2.1*	T266	chr2	0.0247	–26	
	flav3.1	At1g61620	chr3	0.0487	–23	
	flav5.1	At5g20180	chr5	0.0441	–9	
	flav11.1	TG36	chr11	0.0166	24	
LYC	*lyc2.1*	At4g33985	chr2	0.042	21	
	lyc3.1	At5g51110	chr3	0.0101	–26	1
	lyc7.1	At2g32970	chr7	0.0077	–19	
	lyc8.1	TG307	chr8	0.0001	–30	
	lyc9.1	At2g29210	chr9	0.0236	37	
	lyc10.1	TG566	chr10	0.001	46	3
	lyc11.1	At4g22260	chr11	0.0029	18	
	lyc12.1	At2g06530	chr12	0.0001	–28	1
FW	fw2.1	At4g33985	chr2	0.0002	–30	6
	fw3.1	At3g47640	chr3	0.0044	–23	
	fw7.1	At2g42750	chr7	0.0001	–31	
EXC	*exc1.1*	At5g13030	chr1	0.0051	–23	
	exc4.1	At3g16150	chr4	0.0362	20	
	exc4.2	At1g47830	chr4	0.0017	31	5
	exc7.1	At2g32970	chr7	0.0514	–13	
	exc8.1	TG307	chr8	0.0055	–22	
	exc9.1	At3g09925	chr9	0.0041	41	
	exc9.2	At2g29210	chr9	0.0444	32	
	exc12.1	TG180	chr12	0.0186	–18	

Table 4. Continued.

Trait	QTL symbol	Marker	Chr	P	Effect of L. hirsutum allele (%)	Previously identified loci[a]
INC	*exc12.2*	At2g06530	chr12	0.0057	–20	
	inc1.1	At5g13030	chr1	0.0093	–22	
	inc4.1	At1g47830	chr4	0.0311	22	4.5
	inc7.1	T671	chr7	0.0147	–19	4
	inc8.1	TG307	chr8	0.0007	–27	4
	inc9.1	At3g09925	chr9	0.0076	30	
	inc12.1	TG180	chr12	0.0052	–22	
	inc12.2	At2g06530	chr12	0.002	–24	
FIRM	firm2.1	SSR40	chr2	0.0148	21	
	firm2.2	T266	chr2	0.0155	21	
	firm2.3	At4g37280	chr2	0.026	17	
	firm3.1	At5g49970	chr3	0.0117	18	
	firm4.1	At1g71810	chr4	0.0162	19	
	firm5.1	CT138	chr5	0.0161	20	4
	firm8.1	At5g41350	chr8	0.0422	15	
FS	fs1.1	At4g00560	chr1	0.0439	14	
	fs2.1	SSR40	chr2	0.0017	31	7
	fs3.1	At1g61620	chr3	0.0425	24	
	fs7.1	At2g42750	chr7	0.0276	17	4
SSC	ssc1.1	T1422	chr1	0.0201	–15	
	ssc2.1	At4g33985	chr2	0.0001	–30	
	ssc3.1	At3g47640	chr3	0.0003	–25	
	ssc7.1	At2g42750	chr7	0.0006	–19	
	ssc8.1	TG307	chr8	0.0331	–12	
	ssc11.1	TG36	chr11	0.0424	13	
	ssc12.1	TG180	chr12	0.0169	–13	
LN	ln2.1	At4g33985	chr2	0.0017	–15	8
	ln3.1	At3g47640	chr3	0.0013	–14	
	ln4.1	At1g71810	chr4	0.0285	–10	
	ln7.1	At2g42750	chr7	0.006	–10	
	ln10.1	At3g13235	chr10	0.0318	–7	
	ln12.1	TG180	chr12	0.0123	–9	
WALL	wall6.1	CT206	chr6	0.0438	–14	
	wall8.1	TG307	chr8	0.0133	–14	
	wall11.1	CT269	chr11	0.0147	–13	
	wall12.1	At2g06530	chr12	0.0025	–16	

[a] References are coded: 1 = Rousseaux et al. (2005). 2 = Stevens et al. (2007). 3 = Tanksley et al. (1992). 4 = Bernacchi et al. (1998). 5 = Monforte et al. (2001). 6 = Frary et al. (2000). 7 = Liu et al. (2002). 8 = Lippman and Tanksley (2001).

have crucial roles in plant defence systems and, during natural selection, alleles that are responsible for higher production of antioxidant compounds may have accumulated in wild species. In contrast, *L. esculentum* has been domesticated and artificially selected for agronomic traits, and may have lost some of the favourable antioxidant alleles. The presence of favourable antioxidant alleles in *L. hirsutum* was revealed by QTL analysis. Among the 28 antioxidant QTLs, 18 loci (64%) had *L. hirsutum* alleles that were associated with increased antioxidant trait values. The positive effects of *L. hirsutum* alleles over the antioxidant traits ranged from 8% (*phe1.1* and *vitc1.1*) to 46% (*lyc10.1*). Interestingly, the *L. hirsutum* alleles for *lyc10.1* and *lyc9.1* showed the highest phenotypic effect on LYC content, 46% and 37%, respectively. *L. hirsutum* has green fruit even in its ripe stage; thereby this result is most likely due to the transgressive segregation of lycopene alleles, which enhanced LYC content in the BC_2F_2 population. These findings are not new; Bernacchi et al. (1998) and Monforte and Tanksley (2000) also found that *L. hirsutum* alleles could be used to improve red colour in tomato fruit. Such findings reinforce the idea that wild germplasm can contain favourable alleles that are masked by an undesirable phenotype.

There was a slight tendency of *L. hirsutum* alleles to have negative effects on agronomic traits. For the 47 agronomic parameter QTLs (FS excluded because neither round nor elongated FS can be considered as unfavourable), 19 loci (44%) had *L. hirsutum* alleles that were responsible for improvement of the traits. On the other hand, a total of 24 identified *L. hirsutum* alleles (56%) negatively affected the agronomically important traits. This was expected because *L. hirsutum* as a wild parent contained many undesired traits in terms of horticultural aspects such as low FW and green fruit colour. The highest negative effect of *L. hirsutum* alleles was for the *fw2.1* allele with a 30% decrease in FW. In some cases, however, *L. hirsutum* alleles were associated with increased value of some agronomically important traits even when the parental line was inferior for these traits such as INC and EXC. For example, *inc9.1* and *exc9.1* alleles from *L. hirsutum* positively affected these traits by 41 and 30%, respectively. Again, this is because of transgressive segregation of alleles in the population. In other words, different combinations of alleles from the parents can lead to progeny that can exceed both parental lines for a trait of interest. As a result, the phenotype of the wild species does not always reflect its genetic potential. Thus, the use of molecular marker-based techniques can reveal the real potential of this exotic germplasm and also reveal novel genes or alleles that can be used for improvement of existing cultivar types.

Colocalisation of QTLs

A total of 75 QTLs were identified for both antioxidant and agronomically important traits on the tomato genome map. Some of the QTLs were colocalised in the same genomic region. One of the most notable colocalisations was observed among INC, EXC, and LYC content. All of the QTLs that were identified for INC always colocalised with EXC and also *exc9.2* colocalised with *lyc9.1*. In addition, colour and lycopene loci on chromosomes 7, 8 and 12 were located in the same genomic regions. Because lycopene pigment concentration determines the red colour of tomato fruit, most probably these 3 traits are controlled by the same genes. This also clarified the high and positive correlation between these characters. Similar association between INC and EXC was also identified by Fulton et al. (2000) and by Doğanlar et al. (2002). For antioxidant traits, *waox 6.1*, *vitc6.1*, and *phe6.1* were located on the same chromosomal location and *waox12.1*, *vitc12.1*, and *phe12.1* were colocalised on chromosome 12. A positive correlation was also seen among these antioxidant traits. For example, there were positive correlations between WAOX capacity and PHE content and between WAOX capacity and VITC content. VITC and PHE content are water soluble antioxidants; therefore, genes that enhance these traits would also be expected to increase the WAOX capacity. Previous studies also indicated similar positive correlations between PHE content and WAOX capacity in tomato and pepper (Hanson et al. 2004; Rousseaux et al. 2005; Toor et al. 2006; Frary et al. 2008; Frary et al. 2010). An additional positive correlation was observed between VITC content and PHE content, this correlation was also described in pepper by Frary et al. (2008). Colocalisation of some of the VITC and PHE loci could explain why these

2 antioxidant characters are positively correlated. As expected, there was no significant correlation between LYC content and other antioxidant traits. This was not surprising because LYC is a lipid soluble antioxidant, thereby it is not expected to contribute to WAOX capacity.

For agronomic characterisation, highly significant correlations among LN, FW, and SSC were observed (Table 3). This is expected as fruit with more locules tend to be larger and have larger SSC; a similar result was also observed by Doğanlar et al. (2002). Colocalisation of these traits on the molecular marker linkage map (*ln2.1*, *fw2.1*, and *ssc2.1*, *ln7.1* and *fw7.1*, *ln12.1*, and *ssc12.1*) adds support to this hypothesis. Thus, these multiple QTLs may represent fewer loci with pleiotropic effects.

Reliability of identified loci

The accuracy of QTL mapping is highly dependent on the size and type of mapping population, the resolution of linkage map and environmental conditions. In the present study a total of 75 QTLs were identified for both antioxidant and agronomically important traits on the tomato genome map. Seventeen loci (23% of QTLs) identified in the present study were also identified in previous studies. The *waox6.1* was identified by Rousseaux et al. (2005) in the same location on chromosome 6 in *L. pennellii* introgression lines. The *vitc2.2* QTL region was identified by Stevens et al. (2007). In addition, the *vitc12.1* QTL on chromosome 12 was identified in approximately the same map position in these 2 previous studies (Rousseaux et al. 2005; Stevens et al. 2007). The *phe7.1* and *phe9.1* mapped to similar locations as *phe* QTLs previously identified by Rousseaux et al. (2005). The *phe7.1* was of special interest because the *L. hirsutum* allele at this locus was associated with the greatest increase in PHE content. For LYC content, *lyc9.1* and *lyc10.1* were of the most interest as wild alleles at these loci were responsible for 37% and 46% increases in LYC content, respectively. The *lyc3.1* and *lyc12.1* matched loci that were identified by Rousseaux et al. (2005) in the same map region. The *Delta* mutation, which results in reddish orange fruit, maps to a similar location on chromosome 12, suggesting that *Delta* might be a candidate locus for this QTL (Rousseaux et al. 2005). In addition, the never ripe mutant of tomato, *nr*, has been mapped to the same region of chromosome 10 as *lyc10.1* (Tanksley et al. 1992). For fruit colour, *exc4.2* was previously detected by Monforte and Tanksley (2000). Monforte and Tanksley (2000) also reported the *inc4.1* QTL region for INC in their study. In addition, *inc7.1* and *inc8.1* QTLs were in similar regions as colour QTL identified by Bernacchi et al. (1998). EXC and INC QTL on the top of chromosome 12 also colocalised with the *Delta* fruit colour mutant of tomato. The *fw2.1*, located on chromosome 2, matched the location of *fw2.2*, a major FW QTL that was cloned by Frary et al. (2000). The *firm5.1* QTL region for FIRM was also reported by Bernacchi et al. (1998). For FS loci, Liu et al. (2002) and Bernacchi et al. (1998) identified *fs2.1* and *fs7.1*, respectively. Lastly, the *ln2.1* QTL for LN was also identified by Lippman and Tanksley (2001).

The presence of associations between molecular markers and genes of interest indicates the potential usefulness of marker assisted selection (MAS) for improvement of these traits. If a marker is tightly linked with a desired trait, the possibility that the marker and locus will be transmitted together is very high due to low recombination frequency. Therefore, screening of the population with a marker linked to the desired trait makes it feasible to select individuals that have the desired trait or traits without phenotypic characterisation. In addition, MAS can also be used for negative selection, which means that undesired traits can be eliminated from the population. MAS does not require mature plants, thereby selection can be done at the seedling stage with a higher efficiency of selection. By using MAS, requirements for time, space, and labour are greatly reduced. In the present study, marker TG566 linked with *lyc10.1* (46% allelic effect, P = 0.001) and At2g06530 linked with *waox12.1* (12% allelic effect, P = 0.0002), *vitc12.1* (9% effect, P = 0.02), and *phe12.1* (9% effect, P = 0.02) may be candidates for MAS to improve antioxidant content of tomato using *L. hirsutum* alleles. For agronomic traits, the most significant markers were At3g09925 linked with both *exc9.1* (41% allelic effect, P = 0.004) and *inc9.1* (30% effect, P = 0.008), and At1g47830, which was associated with both *exc4.2* and *inc4.1*. MAS also can be used for negative selection; for example, At4g33985 was linked with a *fw2.1* QTL

that negatively affected FW (approximately 30% reduction in weight P = 0.0002). Therefore, progeny that possess the *L. hirsutum* allele for this marker could be eliminated through MAS. Finally, the genetic linkage map that was constructed in the present study could also be useful in other tomato or even other solanaceous species populations because linkage between some of the markers and traits may have been preserved during genome evolution.

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