

## A QTL CONTROLLING STEM MORPHOLOGY AND VASCULAR DEVELOPMENT IN *LYCOPERSICON ESCULENTUM* × *LYCOPERSICON HIRSUTUM* (SOLANACEAE) CROSSES IS LOCATED ON CHROMOSOME 2<sup>1</sup>

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The vascular tissue of higher plants is organized into a continuous and unified system that undergoes a transition between two highly differentiated structures, the root and the shoot. This transition was studied in tomato by investigating the genetic basis of morphological variation between *Lycopersicon esculentum* and *L. hirsutum* LA407. Our analysis concentrated on morphology in stem cross sections, and we detected heritable genetic differences in an inbred backcross population having *L. esculentum* as the recurrent parent and LA407 as the donor parent. Inbred backcross line (IBL) 2353 contained a donor segment from chromosome 2 and retained features of the LA407 stem vascular morphology. Marker-trait analysis of vascular structure in a cross between IBL 2353 and *L. esculentum* showed significant ( $0.0001 \leq P \leq 0.0375$ ) associations between markers on chromosome 2 and the size of primary vascular bundles, the shape of the vascular system, and the thickness of the secondary vascular tissue. Families with LA407 DNA for the markers on chromosome 2 had larger primary vascular bundles, more developed secondary vascular tissue, and a triangular vascular shape. These results suggest that the distal portion of chromosome 2 in LA407 contains a locus or loci affecting vascular morphology and development.

**Key words:** *Lycopersicon*; molecular markers; quantitative trait loci mapping; Solanaceae; vascular anatomy; vascular transition.

Although the vascular tissue of higher plants is organized into a continuous system, the anatomy of the vascular tissue present in roots and stems differs markedly. The tomato root is a radial protosteles, comprising a solid column of vascular tissue with the phloem located peripheral to the xylem (Rasa and Esau, 1961). In contrast, the vascular bundles in the stem occur as distinct longitudinal strands located toward the periphery. The vascular strands are clearly delimited from the pith and cortex, and in each cross section there is a ring of vascular tissue separated by medullary rays at regular intervals (Hayward, 1938). The vascular bundles in the stem of tomato have a bicollateral endarch arrangement, in which the oldest xylem elements (protoxylem) are closest to the center of the axis and vascular bundles have phloem located on both sides of the xylem. The primary vascular bundles of the stem are pronounced near the base of the leaf primordium, where one extension forms a vein of the leaf while the other follows a perpendicular course along the stem axis (Hayward, 1938).

There is a region of vascular transition through the hypocotyl where the exarch, radial protosteles bundle of the root is reoriented into the bicollateral endarch bundles of the stem.

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This vascular transition occurs in three main phases. First, the protosteles of the root divides, and the two resulting metaxylem portions differentiate separately (Thiel, 1933). The metaxylem approaches the steles periphery while the protoxylem is more centrally located. At the time of the first diarch division, phloem is located on the inner and outer faces of each xylem group (Thiel, 1933). Next, the two xylem units divide again, forming the four vascular bundles that are present in the hypocotyl. Finally, after the vascular transition from root to stem is complete, the vascular tissue of the stem is roughly triangular in shape and contains three primary vascular bundles offset by three secondary vascular bundles.

Traditionally, secondary growth is said to begin with the initiation of the vascular cambium. In contrast to other woody plants, secondary growth is present in the stem of tomato even before the completion of internode elongation but does not occur close to the shoot apical meristem (Thompson and Heimsch, 1964). Tomato therefore has a continuous cylinder of procambium present early in development. The vascular cambium arises from the procambium located in the center of vascular bundles and from the interfascicular cells located between vascular bundles (Esau, 1977). A stem exhibiting secondary growth will have its primary xylem toward the center of the stem, the primary phloem pushed outward by new vascular tissue, and a differentiated secondary xylem and phloem. Secondary vascular bundles originate in the stem and have no direct connection with the leaf. In tomato, both the primary and secondary vascular bundles are bicollateral bundles.

Although the features of stem anatomy, vascular transition, and secondary growth have been described for tomato, there remain unanswered questions and problems of interpretation. For example, the process through which the four bundles of the hypocotyl and the three primary bundles of the stem are

reconciled is unclear. The hypocotyl creates a continuous link between two highly differentiated tissue groups, the root and the shoot. Although Hayward (1938) described the completion of the hypocotyl-stem transition as an anastomosis of vascular bundles at or below the cotyledonary node, this explanation is unsatisfactory. The region of transition does not represent a fusion of tissues derived from two independent meristems. Rather, it represents a continuum of differentiation that occurs as the root and shoot meristems become spatially separated. The initiation of the phyllotaxy, leaf primordia, and the processes of secondary growth may all play a role in completing the transition in both space and time.

Studies of tomato varieties that compare anatomical development and morphology were suggested as a means to resolve disagreements in the literature concerning the development of the tomato stem (Thompson and Heimsch, 1964). Differences in the description of stem anatomy and xylem tissues appear for the varieties 'Yellow Plum' and 'Marglobe', but variation in sampling patterns, sampling duration, and interpretation of the anatomical features between the two studies makes it difficult to generalize about the anatomical changes that occur during the growth and development of the tomato stem (Vening, 1949; Thompson and Heimsch, 1964). Despite the potential of comparative studies to elucidate patterns of development in the stem and vascular system there have been no comparisons between cultivated tomato varieties or the many related wild *Lycopersicon* species.

In order to study the development of the tomato stem we investigated morphological variation within and between *L. esculentum* Mill. processing tomato varieties and the wild species accession *L. hirsutum* Humb. and Bonpl., LA407. Our studies concentrated on stem morphology and vascular development during the transition from hypocotyl to stem. We observed differences in the spatial occurrence of the transition that are heritable and may be the result of spatial cues, different rates of development, or both. We provide evidence that a genetic approach may help identify genes that control features of vascular development and stem anatomy.

## MATERIALS AND METHODS

**Tomato genotypes and populations**—In the course of these studies we included several tomato genotypes and two segregating populations. Seed from LA407, progeny, or lines in the segregating populations and *L. esculentum* processing tomato varieties Ohio 88119, Ohio 86120, Hunt 100, and Peto 95-43 were grown in a completely randomized design for all experiments with replication in both space and time. The genetic stocks LA2705 (VFNT Cherry, sp) and LA1221 (VFNT Cherry, sp+) were included as controls for the self-pruning (sp) gene that affects plant habit (Pnueli et al., 1998). The LA407 IBC population consists of 64 BC<sub>2</sub>S<sub>5</sub> lines and has been described in detail previously (Francis et al., 2001; Kabelka, Franchino, and Francis, 2002). Briefly, the LA407 IBC population was created by crossing *L. hirsutum* LA407 to Hunt 100 followed by a backcross to Hunt 100. Individual BC<sub>1</sub> lines were crossed to Peto 95-43; BC<sub>2</sub> lines were then advanced by self-fertilization and single-seed descent for five generations. The second segregating population was developed by crossing inbred backcross line (IBL) 2353 from the LA407 IBC population to the variety Ohio 86120. Both IBL 2353 and Ohio 86120 have a determinant growth habit (sp). A single hybrid individual was self-pollinated to produce a segregating F<sub>2</sub> population and individual F<sub>2</sub> plants were self-pollinated to obtain F<sub>2,3</sub> derived families.

**Growth conditions**—All tomato plants were seeded into 288-well flats containing Metro Mix 360 soil media. Plants were grown in a greenhouse with a day temperature of 23.8° ± 3°C and a night temperature of 18.3° ± 3°C

with a 14/10 light/dark period. Supplemental high-intensity discharge lighting was provided when natural light fell below 200 W/m<sup>2</sup>. Plants were fertilized with a standard 20-20-20 fertilizer applied twice a week at a concentration of 200 ppm. Twenty-eight days after seeding, tomato plants were transplanted into 10.2-cm plastic pots containing Metro Mix 360 soil media. Twenty-two days after transplanting, ultrathin hand sections were taken between the cotyledons and the first vegetative node and between subsequent vegetative nodes. Under our growth conditions, plants had 6–8 vegetative nodes but had not yet set flowers.

**Imaging stem vascular morphology**—Stem sections were taken between the soil and cotyledons and between subsequent nodes for comparisons of *L. esculentum* genotypes and *L. hirsutum* LA407. For subsequent populations, stem sections were taken between the cotyledons and the first vegetative node and between the first and second vegetative nodes. For experiments designed to compare *L. esculentum* with *L. hirsutum* LA407 and progeny in the IBC population, sections were fixed in Farmer's fluid (10 mL acetic acid, 60 mL 70% ethanol, and 30 mL chloroform) until completely clear, then stained with 0.03% Trypan Blue. A Diagnostic Instruments (Sterling Heights, Michigan, USA) dissecting microscope was used for observation, and images were collected using the 2VS-474 Imaging System (Carl Zeiss, Thornwood, New York, USA). All measurements and observations were taken with an Olympus SZH10 light microscope connected to a CCD camera for analysis of the F<sub>2,3</sub> population and parents. For this population, sections were visualized without fixation or staining. Measurements were taken directly from the microscope using a linear eyepiece recticle. Digital images were saved for each stem section and indexed by line or family number, position of the stem section, and magnification.

For each of the tomato genotypes, sections were taken between the cotyledons and the first vegetative node and between subsequent vegetative nodes. The overall shape of the vascular system (triangular or circular), secondary vascular thickness, length of primary vascular bundles, and width of primary vascular bundles were measured for each stem section. Primary vascular bundle width was measured parallel to the secondary vascular tissue, while primary vascular bundle length was measured from the edge of the secondary vascular tissue projecting in toward the pith.

**Molecular marker analysis**—DNA was extracted using a hexadecyltrimethylammonium bromide (CTAB) protocol for all experiments (Burnham et al., 2002). Differences between parents were detected using both polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) as genetic markers. The entire LA407 inbred backcross (IBC) population was genotyped with 67 molecular markers. The genetic marker data were used for vascular trait evaluation and genetic mapping using statistical methods described in detail by Kabelka, Franchino, and Francis (2002). The LA407 IBC population was genotyped with a minimum of two markers per chromosome arm.

The molecular markers used to genotype IBL 2353, Ohio 86120, and the 2353 F<sub>2</sub> population were derived from the bottom of chromosome 2 (Table 1). A genetic linkage map for the LA407 introgression in IBL 2353 was created with five markers using 148 individuals from the IBL 2353 × Ohio 86120 F<sub>2</sub> population with the MAPMAKER software program (Lander et al., 1987). MAPMAKER uses the log likelihood ratios to calculate recombination and gene order between linkage groups. The Kosambi mapping function was used to convert recombination frequency to map distances in centimorgans (Kosambi, 1944).

**Statistical analysis**—Analysis of variance (ANOVA) and mean comparisons of germplasm and lines were performed with the SAS General Linear Models (GLM) procedure. Mean separations were based on the least significant difference (LSD) using the experimentwise error and were performed only after a significant *F* test was obtained. Mixed-model analysis of variance was used for analyses that included both fixed and random effects and to obtain mean square estimates for calculating heritability. For all analyses using the SAS procedure MIXED, restricted maximum likelihood estimation (REML) was specified. When trait scoring was qualitative, nonparametric tests

TABLE 1. Molecular markers and PCR conditions.

Marker	Primer	Annealing temperature (°C)	Cycles	Size (base pairs)	Restriction enzyme
CT059	F 5'TTGTAGTGGCAAGTGTACGGG3' R 5'TCATATCGGGAGAAGGTGCGTG3'	60°	36	506	None
TG620	Restriction fragment length polymorphism			1500	Dra I
TG492	F 5'TGGAGAAGGTTCAAAGGGAACG3' R 5'GGCCAAGGATATTTCTCAAGG3'	57°	36	900	Mnl I
TG091	F 5'TGCAGAGCTGTAATATTTAGAC3' R 5'CGGTCTCAGTTGCAACTCAA3'	60°	36	600	Dra I
CT094	F 5'GGGAAAACCTGGCAGCTAA3' R 5'TGCCCAATTATGTGGCAGTA3'	57°	36	1000	Dra I

for marker-trait association were performed using a Wilcoxon test for two-sample data and a Kruskal-Wallis test for multi-sample data, analogous to the approach described by Sandbrink et al. (1995). For these tests, the SAS procedure "PROC NPAR1WAY" was used with the Wilcoxon test specified.

**Experimental plan and statistical model for the inbred backcross population**—Replicate lines from the IBC population were sectioned in two completely randomized experiments conducted over time. The number of loci affecting quantitative traits was estimated in the IBC population using the approach of Eskridge and Coyne (1996). When trait scoring was qualitative, gene number estimates were based on a chi-square test for goodness-of-fit to expected segregation ratios for 1–4 loci. Broad-sense heritability was estimated for each trait separately according to Cotterill (1987), and standard errors for heritability were calculated according to Hallauer and Miranda (1988).

For tests of marker-trait association, the statistical model for quantitative traits scored in the IBC population was

$$X_{ijk} = \mu + R_i + M_j + G_k(M_j) + \epsilon_{ijk}$$

where  $X_{ijk}$  is the trait value of the  $k^{\text{th}}$  genotype of the  $j^{\text{th}}$  marker class in the  $i^{\text{th}}$  replication,  $\mu$  is the population mean,  $R_i$  is the effect of the  $i^{\text{th}}$  replicated experiment,  $M_j$  is the effect of the  $j^{\text{th}}$  marker class,  $G_k(M_j)$  is the effect of the  $k^{\text{th}}$  genotype within the  $j^{\text{th}}$  marker class and  $\epsilon_{ijk}$  is the experimental error. Degrees of freedom were calculated via the Satterthwaite option (Neter, Wasserman, and Kutner, 1990). The appropriate  $F$  test for marker was equal to  $M/G_k(M_j)$ . Marker was considered as a fixed effect while replication and genotype within marker were considered random effects.

**Experimental plan and statistical models for  $F_{2,3}$  population**—Parents for the population, IBL 2353 and Ohio 86120, were compared in order to determine which characters to pursue in the segregating population. The comparison was replicated twice in time with 40 individuals planted for IBL 2353 and Ohio 86120 in both replications. Mean separations for genotypic differences were performed after significance was detected in the statistical model

$$X_{ij} = \mu + G_i + R_j + (G_i \times R_j) + \epsilon_{ij}$$

where  $X_{ij}$  is the vascular trait value for  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  replication,  $\mu$  is the population mean,  $G_i$  is the effect of the  $i^{\text{th}}$  genotype,  $R_j$  is the effect of  $j^{\text{th}}$  replicated experiment,  $(G_i \times R_j)$  is the genotype by replication interaction, and  $\epsilon_{ij}$  is the residual error term. Genotype was considered as a fixed effect, while genotype by replication interactions and replication were considered as random effects.

Measurements that were significantly different between IBL 2353 and Ohio 86120 were investigated in the  $F_{2,3}$  population. In order to determine the optimal sampling strategy for this experiment, a power analysis was performed on seven  $F_{2,3}$  families in each marker class with 16 individuals sampled per family. The variance associated with molecular markers for traits measured multiple times per section (e.g., the length and width of each bundle) is

$$\sigma^2_M = (\sigma^2_{F(M)}/F) + (\sigma^2_{IF(M)}/IF) + (\sigma^2_{error}/IFR)$$

where  $M$  = marker,  $F$  = family,  $I$  = individual within family, and  $R$  = number of measurements taken per stem section. The variance associated with molecular markers for vascular traits measured once per stem section (overall vascular shape and secondary vascular thickness) is

$$\sigma^2_M = (\sigma^2_{F(M)}/F) + (\sigma^2_{error}/IF)$$

Estimates were obtained for  $\sigma^2_{F(M)}$ ,  $\sigma^2_{IF(M)}$  and  $\sigma^2_{error}$  by using the SAS procedure "PROC NESTED." Sampling strategies using different numbers of individuals within family (1–16) and different numbers of families (5–40) per marker class were analyzed graphically in order to devise a strategy that would minimize the variation associated with marker class ( $\sigma^2_M$ ) while sampling a reasonable number of progeny.

For the main experiment we sampled a minimum of five  $F_3$  individuals per family and a minimum of 20 families per marker class. For tests of marker-trait association within the population, the statistical model was

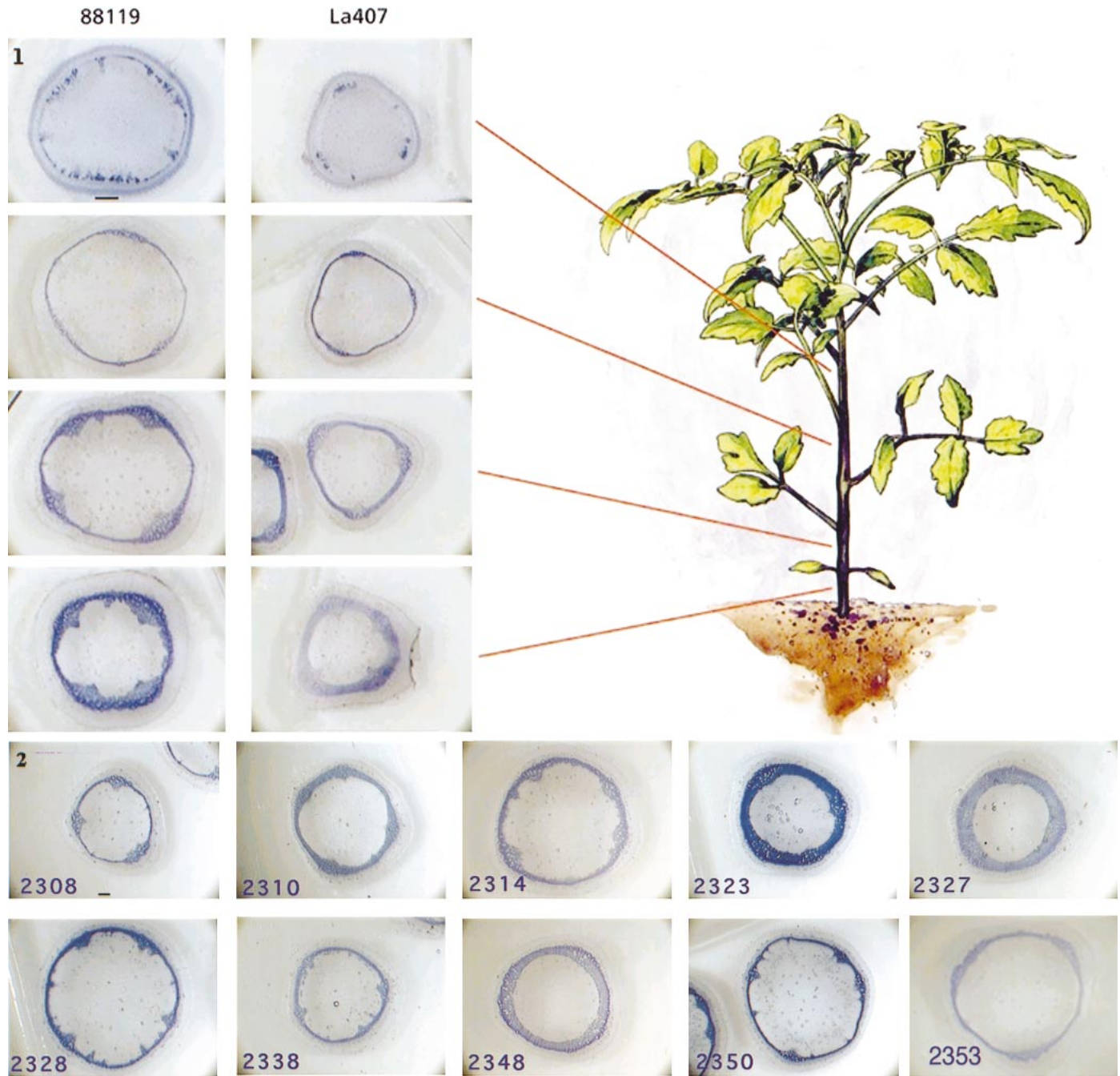
$$X_{ij} = \mu + M_i + F_j + (M_i \times F_j) + \epsilon_{ij}$$

where  $X_{ij}$  is the vascular trait value for the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  family,  $\mu$  is the population mean,  $M_i$  is the effect of the  $i^{\text{th}}$  genotype,  $F_j$  is the effect of the  $j^{\text{th}}$  family,  $M_i \times F_j$  is the marker genotype by family interaction, and  $\epsilon_{ij}$  is the residual error term. Molecular markers were considered fixed effects while genotype by family interactions were considered random effects.

## RESULTS

**Comparison of *L. hirsutum* and *L. esculentum***—Quantitative and qualitative differences were observed between the stem morphology and vascular system of *L. hirsutum* LA407 and the *L. esculentum* processing tomato genotypes (Fig. 1). Differences between *L. esculentum* processing tomato varieties were not observed. The morphology of the self-pruning (sp and sp+) controls was not significantly different, and the vascular anatomy of these VFNT Cherry genetic stocks was more similar to *L. hirsutum* than the processing tomato genotypes (data not shown). Due to the early seedling stages sampled in our studies, it would be inappropriate to compare the results presented here with earlier descriptions for 'Yellow Plum' and 'Marglobe' (Venning, 1949; Thompson and Heimsch, 1964) as these reports are based on anatomy and morphology in mature plants.

Sections through stem tissue between nodes and progressing up the stem reveal that the triangular morphology is established earlier in LA407 than in the *L. esculentum* processing genotypes we examined. The triangular vascular morphology consists of three primary vascular bundles offset by three secondary vascular bundles enclosed by triangular secondary vascular tissue. In contrast, the vascular morphology of the hypocotyl region is square to circular in shape and consists of four primary vascular bundles. In *L. esculentum* genotypes,



Figs. 1–2. 1. Vascular transition from root to stem in *Lycopersicon esculentum* line ‘88119’ and *L. hirsutum* accession LA407. The red lines indicate internode where stem sections were taken. Note triangular vascular structure achieved by the first vegetative node in *L. hirsutum*. Bar scale = 1 mm. 2. Morphological variation present within the LA407 IBC population. Stem sections were taken between the cotyledons and the first vegetative node. Scale bar = 1 mm.

the four vascular bundles persist through the first vegetative node, while in LA407 the triangular vascular pattern is present in the internode between the cotyledon and first vegetative node (Fig. 1). Other vascular differences between the two species include a thicker, more developed secondary xylem in LA407 and more diffuse primary vascular bundles in *L. esculentum* genotypes (Fig. 1).

**Analysis of inbred backcross population**—The theoretical expectation was that each of the 64 BC<sub>2</sub>S<sub>5</sub> lines will contain

12.5% of the donor LA407 parent genome and 87.5% of the domesticated *L. esculentum* genome, though the recovery of *L. hirsutum* RFLP- and PCR-based markers suggested a greater bias toward the *L. esculentum* parent (Kabelka, Franchino, and Francis, 2002). The IBC population showed considerable variation for stem shape and vascular morphology (Fig. 2). The observed phenotypic distribution was consistent with genetic models of 2–3 loci for the quantitative traits secondary xylem thickness and vascular bundle length ( $P < 0.05$ ). These traits had heritabilities in the IBC population of 0.43 (SE =

TABLE 2. Comparison of vascular morphology between IBL 2353 and variety Ohio 86120.

Trait	P	2353 Mean	86120 Mean
Bundle width 0/1	0.2565	1.206	1.155
Bundle width 1/2	0.0103	1.326	1.201
Bundle length 0/1	<0.0001	0.934	0.712
Bundle length 1/2	0.0719	0.978	0.938
2° Vascular thickness 0/1	0.0162	0.192	0.155
2° Vascular thickness 1/2	<0.0001	0.232	0.132
Vascular shape 0/1	0.2160	1.680	2.000
Vascular shape 1/2	<0.0001	1.160	1.878

Note: Annotation 0/1 refers to stem sections taken between the cotyledons and the first vegetative node, whereas 1/2 refers to stem sections taken between the first and second vegetative nodes. All measurements for bundle length, bundle width, and secondary vascular thickness are reported in millimeters. A triangular vascular shape was scored as a “1,” while circular vascular shape was scored as a “2.” Statistical analysis of qualitative scores was based on nonparametric tests while the genotype means reflect the mean of 40 plants in two experimental replicates.

0.25) and 0.32 (SE = 0.17), respectively. The inheritance of the triangular morphology associated with *L. hirsutum* LA407 stems was consistent with both two (chi-square 1.056, 1 df) and three-locus models (chi-square 1.88, 1 df) and had a heritability of 0.53 (SE = 0.36). Stem diameter and the relative area of the stem occupied by pith also appeared to be under genetic control, but were not investigated in subsequent generations. These traits are therefore not discussed further.

Statistical analysis of marker-trait linkage suggested that a locus on the bottom of chromosome 2 was associated with the thickness of the secondary xylem, bundle length, and triangular morphology from *L. hirsutum* LA407 (TG620 and

TG091,  $P < 0.001$ ). Marginally nonsignificant associations ( $0.1 > P < 0.05$ ) were detected for markers on chromosomes 4 (TG049) and 7 (TG572).

**Comparison of IBL 2353 and Ohio 86120**—IBL 2353 was chosen for further analysis due to its determinate growth habit, the presence of the *L. hirsutum* donor segment from chromosome 2, and the lack of other *L. hirsutum* donor segments in the genome. Ohio 86120 was chosen as representative of the processing tomato genotypes examined. Statistically significant differences ( $P \leq 0.0162$ ,  $P \leq 0.0001$ ) were found between IBL 2353 and Ohio 86120 for secondary vascular thickness in both stem sections (Table 2). Statistically significant differences were also shown to exist for vascular bundle width and overall vascular shape between the first and second vegetative nodes ( $P \leq 0.0103$ ,  $P \leq 0.0001$ ) and for vascular bundle length between the cotyledons and the first vegetative node ( $P \leq 0.0001$ , Table 2). IBL 2353 displayed thicker, more developed secondary vascular tissue, and a triangular vascular shape was obtained earlier in space and time, whereas Ohio 86120 displayed thinner secondary vascular tissue and a circular vascular shape. Line 2353 also displayed wider and longer primary vascular bundles than Ohio 86120 (Table 2, Fig. 3).

**2353  $F_{2,3}$  population analysis**—A genetic linkage map was created for the TG492, TG091, CT059, CT094, and TG620 molecular markers using 148  $F_2$  individuals in an  $F_2$  intercross population derived from crossing IBL 2353 to variety Ohio 86120. In comparison to the other molecular markers analyzed, TG620 is located nearest to the bottom of chromosome 2, while TG492 is located farthest away from TG620. TG620 is separated from CT094 by 3.9 centimorgans (cM); CT094 is

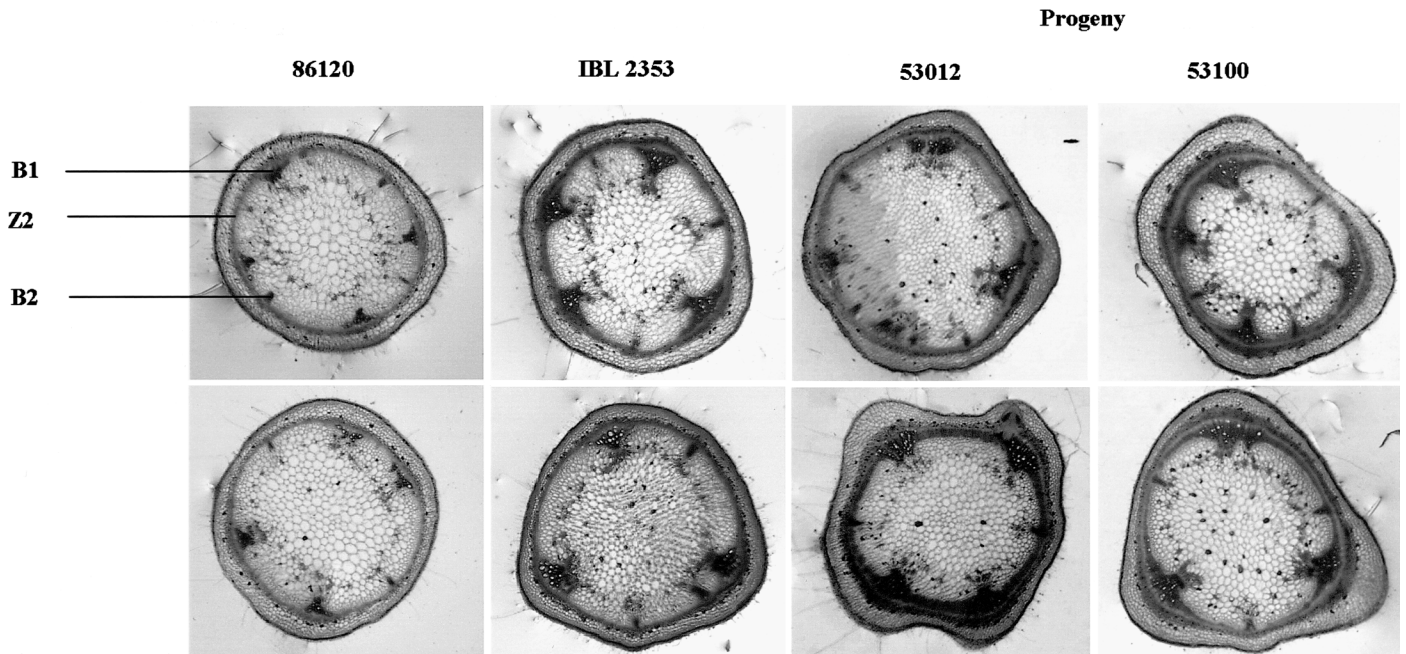


Fig. 3. Vascular structure for variety Ohio 86120, IBL 2353, and segregating progeny. Progeny 53012 has *Lycopersicon esculentum* DNA and 53100 has *L. hirsutum* DNA for all molecular markers. The top panel shows stem sections taken between the cotyledons and the first vegetative node. The bottom panel shows stem sections taken between the first and second vegetative nodes. B1 = primary vascular bundle; B2 = secondary vascular bundle; Z2 = secondary vascular tissues; scale bar = 1 mm. Primary vascular bundle length was measured from the edge of the secondary vascular tissues projecting in toward the pith. Primary vascular bundle width was measured parallel to the secondary vascular tissues.

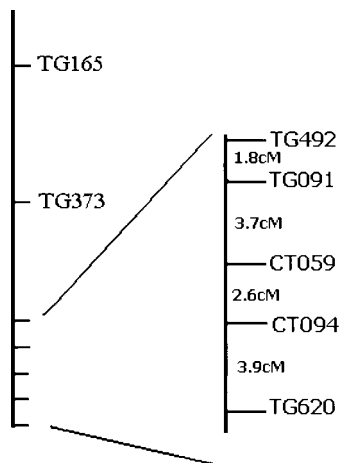


Fig. 4. Composite genetic map of chromosome 2 from IBL 2353  $\times$  variety Ohio 86120  $F_2$  population. Markers TG616, TG165, TG373, TG091, and TG620 were placed onto the LA407 IBC population to identify LA407 donor segments on chromosome 2. IBL 2353 contained LA407 DNA for TG091 and TG620. Molecular markers TG492, TG091, CT059, CT094, and TG620 were used for mapping and vascular marker-trait analysis in the IBL 2353  $\times$  variety Ohio 86120  $F_2$  population.

separated from CT059 by 2.6 cM; CT059 is separated from TG091 by 3.7 cM; and TG091 is separated from TG492 by 1.8 cM (Fig. 4).

The moderate heritability estimates obtained in the IBC population suggested that measurements of vascular phenotypes would require replication in subsequent generations in order to confirm the existence of a QTL on chromosome 2. We concentrated on those traits that were significantly different between IBL 2353 and Ohio 86120 and used  $F_{2,3}$  families to provide replicated measures. To determine the optimal sampling strategy for analyzing vascular traits in the  $F_{2,3}$  population, a pilot study was performed and variance components were estimated for the marker model. The goal of this analysis was to determine an efficient balance between sampling individuals within families and between families. We determined that five individuals per family provided sufficient within-family replication and that the number of families examined should be 20 or greater per marker class. This sampling strategy minimized the variance associated with marker classes.

The marker-trait analysis for stem morphology and vascular structure in the IBL 2353  $\times$  Ohio 86120 population showed a highly significant ( $P \leq 0.0001$ ) association between markers CT059 and CT094 and primary vascular bundle length between the cotyledons and the first vegetative node (Table 3). Molecular marker TG091 showed a significant association ( $P \leq 0.0375$ ) between the first and second vegetative nodes for secondary xylem thickness. Molecular markers CT094 and

TG620 showed significant associations ( $P \leq 0.0167$ ,  $P \leq 0.0152$ ) between the first and second vegetative nodes for the overall shape of the vascular system. Mean separation statistics were performed and  $F_3$  families homozygous for LA407 DNA at CT059, CT094, TG620, and TG091 had longer primary vascular bundles, thicker secondary vascular tissue, and the triangular stem shape that predominates when the hypocotyl to stem transition is complete (Fig. 3).  $F_3$  individuals homozygous for *L. esculentum* DNA at these markers had shorter primary vascular bundles, thinner, less developed secondary vascular tissue, and a circular vascular shape (Fig. 3).

## DISCUSSION

Heritable differences in the spatial occurrence of the hypocotyl to stem transition were observed between LA407 and *L. esculentum* processing genotypes and in subsequent segregating populations. The observed differences include the establishment of a roughly triangular vascular shape with three primary vascular bundles offset by three secondary vascular bundles closer to the cotyledons in LA407. The triangular vascular shape matches the description of stem morphology associated with mature plants as described by Thompson and Heimsch (1964). In addition, the primary vascular bundles and secondary vascular tissue are thicker in LA407, suggesting that secondary growth begins earlier. The differences observed between *L. hirsutum* LA407 and processing tomato genotypes may be due to spatial cues, different rates of development, or both.

There are many striking phenotypic differences between wild and cultivated tomatoes as a result of generations of selection. The most dramatic differences are manifested as increases in fruit size and shape, growth habit, and leaf and flower morphology. The majority of these traits are quantitative in nature, showing continuous phenotypic distributions. *Lycopersicon hirsutum* is a perennial and indeterminate tomato species that produces an abundance of vegetative growth in comparison to the domesticated tomato genotypes grown for the processing tomato industry. It is plausible that the root-to-stem vascular transition is completed earlier in LA407 due to its large growth habit and need for additional xylem and phloem cells. The vascular differences observed between *L. esculentum* processing tomato genotypes and LA407 may result from a more rapid root-to-stem vascular transition in LA407 or a retention of juvenile characters due to selection in *L. esculentum* genotypes.

Although the features of stem anatomy and vascular transition have been investigated and described for many plant species, little is known regarding the molecular mechanisms controlling vascular tissue patterning and differentiation. A large body of evidence indicates that auxin is a major signal involved in several aspects vascular ontogeny (Aloni, 1987;

TABLE 3. Marker-trait analysis in tomato IBL 2353  $\times$  variety Ohio 86120  $F_{2,3}$  population.

Trait	TG492 $P$	TG091 $P$	CT059 $P$	CT94 $P$	TG620 $P$
Bundle width 1/2	0.8628	0.5365	0.7362	0.3654	0.2164
Bundle length 0/1	0.2001	0.0933	<0.0001	<0.0001	0.6726
2° Vascular thickness 0/1	0.1700	0.0980	0.0552	0.6703	0.5302
2° Vascular thickness 1/2	0.1089	0.0375	0.0886	0.08245	0.1442
Vascular shape 1/2	0.2382	0.8590	0.2059	0.0167	0.0152

Note: Annotation 0/1 refers to stem sections taken between the cotyledons and the first vegetative node, whereas 1/2 refers to stem sections taken between the first and second vegetative nodes.

Sachs, 2000). Mutants that interfere with multiple aspects of vascular development have been identified in *Arabidopsis thaliana*, allowing for positional cloning of their wild-type homologues. The INTERFASCICULAR FIBERLESS (IFL1) gene encodes a homeodomain-leucine zipper (HD-LZ) protein responsible for interfascicular fiber differentiation in inflorescence meristems (Zhong and Ye, 1999). IFL1, ATHB-8, -9, -14, and -15 are involved in vascular tissue patterning and differentiation and belong to the HD-ZIP III family of transcription factors (Sessa et al., 1998; Baima et al., 2000). Further studies will need to be conducted to determine if homologues of Arabidopsis HD-ZIP III genes are involved in the patterning of vascular tissue in other plant species.

We provide evidence that a genetic approach will help identify loci that control features of vascular development and stem anatomy in tomato. This approach has been successful in mapping quantitative trait loci (QTLs) controlling vascular bundle number in *Oryza sativa* L. (Sasahara, Fukuta, and Fukuyama, 1999). The phenotypic distribution within the LA407 IBC population was consistent with two to three gene models for the traits associated with stem morphology and vascular development. This population further served to identify a segment of LA407 DNA on chromosome 2 containing QTL(s) that contribute to stem morphology and vascular structure. The limited number of lines in the IBC population restricted our ability to detect linkage with more loci from *L. hirsutum*, and it is likely that several undetected QTLs contribute to the differences observed between LA407 and *L. esculentum* genotypes. In support of this hypothesis, LA407 plants develop the triangular morphology earlier in space and time than IBL 2353 (Figs. 1 and 3). However, IBL 2353 retained features of the vascular morphology associated with LA407, had longer and wider primary vascular bundles in cross section, thicker secondary vascular tissues, and a triangular vascular shape obtained by the first vegetative node (Table 2, Fig. 3). In contrast, *L. esculentum* variety Ohio 86120 had shorter and smaller primary vascular bundles, thinner secondary vascular tissues, and a circular vascular shape with four bundles persisting through the first vegetative node (Table 2, Fig. 3). Analysis of F<sub>2,3</sub> families derived from a cross between IBL 2353 and Ohio 86120 confirmed the role of the distal portion of chromosome 2 in controlling aspects of stem morphology and vascular development.

It is worth noting that the same region of chromosome 2 also contains a locus from *L. hirsutum* LA407 conferring resistance to bacterial canker of tomato (Kabelka, Franchino, and Francis, 2002). Resistance to bacterial canker is correlated with the LA407 vascular morphology and QTLs for both traits map to the same location on chromosome 2 (data not shown). Bacterial canker is a systemic disease of tomato caused by the gram-positive bacterium, *Clavibacter michiganensis* subsp. *michiganensis*. In infected plants, bacteria proliferate and move in the xylem. A longer incubation period and decreased rate of symptom progression have been documented for bacterial canker with increasing plant age (Forster and Echandi, 1973; Chang, Ries, and Pataky, 1992). It is interesting to speculate that the locus on chromosome 2 may result in decreased symptom progression due to an increased rate of vascular development. However, statistical correlations do not confirm function and further work will be necessary in order to accept this hypothesis.

Several major fruit mass and fruit size QTLs are also located on chromosome 2 of tomato. Molecular-marker analysis of a

cross between yellow pear and the round-fruited wild species, *Lycopersicon pimpinellifolium* LA1589, revealed that pear-shaped fruit is largely determined by a major locus, *ovate*, on chromosome 2 (Butler, 1952; Barton et al., 1955; Ku et al., 1999). Fruit mass and shape QTL (fw2.1, fs2.1, fw2.2, fs2.2, fw2.3, and fs2.b) have been mapped to the lower portion of chromosome 2 using segregating populations derived from crosses to the wild tomato species *L. hirsutum* and *L. pennellii* (Eshed and Zamir, 1995; Bernacchi et al., 1998; Frary et al., 2000). In addition, an exerted stigma character was mapped to the lower portion of chromosome 2 using an interspecific backcross population derived from *L. esculentum* × *L. hirsutum* f. *typicum* (Bernacchi and Tanksley, 1997). The large number of loci that control morphological characters in this region emphasize the importance of the distal portion of chromosome 2 during the transition from the wild to domesticated tomato.

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