Journal of Experimental Botany, Vol. 58, No. 6, pp. 1339–1349, 2007
doi:10.1093/jxb/erl301 Advance Access publication 5 February, 2007
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Morphological variation in tomato: a comprehensive study of quantitative trait loci controlling fruit shape and development

Marin Talbot Brewer[1], Jennifer B. Moyseenko[1], Antonio J. Monforte[2] and Esther van der Knaap[1,*]

[1] *Department of Horticulture and Crop Science, The Ohio State University/OARDC, 1680 Madison Avenue, Wooster, OH 44691, USA*

[2] *IRTA Carretera de Cabrils Km 2, E-08348 Cabrils, Barcelona, Spain*

Received 13 August 2006; Revised 10 November 2006; Accepted 12 December 2006

Abstract

Variation in fruit morphology is a prevalent characteristic among cultivated tomato. The genetic and developmental mechanisms underlying similarities and differences in shape between the fruit of two elongated tomato varieties were investigated. Fruit from two F_2 populations constructed from either *Solanum lycopersicum* cv. Howard German or cv. Banana Legs crossed with *S. pimpinellifolium* accession LA1589, and one BC_1 population constructed with *S. lycopersicum* Howard German as the recurrent parent, were analysed for shape by using a new software program Tomato Analyzer. Quantitative trait loci (QTLs) controlling 15 individual shape attributes were mapped by both single and multitrait composite interval mapping in each population. In addition, principal components analysis and canonical discriminant analysis were conducted on these shape attributes to determine the greatest sources of variation among and between the populations. Individual principal components and canonical variates were subjected to QTL analysis to map regions of the genome influencing fruit shape in the cultivars. Common and unique regions, as well as previously known and novel QTLs, underlying fruit morphology in tomato were identified. Four major loci were found to control multiple fruit shape traits, principal components, and canonical variates in the populations. In addition, QTLs associated with the principal components better revealed regions of the genome that varied among populations than did the QTL associated with canonical variates. The QTL identified can be compared across additional populations of tomato and other fruit-bearing crop species.

Key words: Development, fruit shape, principal components analysis, quantitative trait loci, tomato.

Introduction

Fruit of tomato (*Solanum lycopersicum*) are diverse in size and shape, ranging from small and round to large and variably shaped. A prevalent morphological feature distinguishing many cultivated varieties from undomesticated accessions is an elongated fruit shape. The major loci that have been identified as contributing to an elongated shape in tomato are *sun* (Van der Knaap and Tanksley, 2001; Van der Knaap *et al*., 2002, 2004), *ovate* (Ku *et al*., 1999; Liu *et al*., 2002; Van der Knaap *et al*., 2002), and *fs8.1* (Grandillo *et al*., 1996; Ku *et al*., 2000).

Two tomato varieties that exhibit extreme fruit shape characteristics are *S. lycopersicum* cultivars Howard German and Banana Legs. As a result of the *sun* locus, both varieties bear fruit that are elongated in shape (Brewer *et al*., 2006). However, fruit of Howard German are tapered, whereas those of Banana Legs are slightly curved with a rounded and often indented tip (Fig. 1). It was of interest to determine whether the shapes of the fruit are controlled by similar or different loci and to understand the genetic and molecular mechanisms that contribute to shape variation in these cultivars. Dissimilarity in

* To whom correspondence should be addressed. E-mail: vanderknaap.1@osu.edu

Abbreviations: ANOVA, analysis of variance; BLF_2, Banana Legs F_2 population; CDA, canonical discriminant analysis; CV, canonical variate; $HGBC_1$, Howard German BC_1 population; HGF_2, Howard German F_2 population; PC, principal component; PCA, principal components analysis; PCR, polymerase chain reaction; QTL, quantitative trait locus; RFLP, restriction fragment length polymorphism.

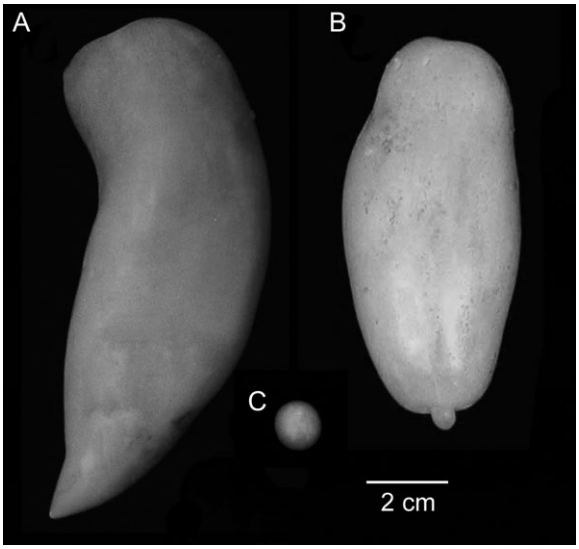

Fig. 1. Images of parental fruit (A) *S. lycopersicum* cv. Howard German, (B) *S. lycopersicum* cv. Banana Legs, and (C) *S. pimpinellifolium* accession LA1589. The size bar represents 2 cm.

fruit shape is the result of differential growth processes which probably occur during formation of the ovary, or after anthesis during the formation of the fruit. Thus, uncovering the genes responsible for this phenotypic variation will provide insight into developmental pathways that control fruit formation.

Critical factors limiting accurate phenotypic analysis and scoring of fruit morphology attributes are objectivity and precision. In a previous study, terms and mathematical descriptors were developed for fruit shape attributes and implemented into the software program Tomato Analyzer (Brewer *et al*., 2006). The application provides quick and objective measurements of morphological features and permits reproducible phenotypic evaluations of populations. Thus, fruit shape features analysed with Tomato Analyzer allow for extensive comparisons among populations, researchers, and even different crops (Brewer *et al*., 2006).

In this study, Tomato Analyzer was used to measure fruit shape attributes in three tomato populations. These attributes correspond to various morphological features of the fruit and were mapped as quantitative trait loci (QTLs) in each of the populations. Additionally, an attempt was made to identify the attributes that contributed most to the variation. Therefore, principal components analysis (PCA) and canonical discriminant analysis (CDA) were conducted on the 15 fruit shape features that were measured. PCA and CDA are statistical methods that use data compression to simplify a complex set of terms by expressing multiple variables, such as numerous fruit shape traits, as components or variates, respectively. For PCA, the first principal component (PC) represents the greatest amount of variation resulting from a combination of all of the variables. The second PC represents the next greatest source of variation, and so on. PCA is an effective tool to identify the attributes that contribute most to the phenotypic variation across populations, whereas CDA identifies the greatest sources of variation that differentiates populations. Additionally, analysis of variance (ANOVA) can be conducted on individual PCs to determine if they can be used to distinguish populations. The PCs and canonical variates (CVs) can be subjected to quantitative trait analyses to allow for better detection of shape QTLs (Langlade *et al*., 2005). For example, QTLs for one trait may not appear large in effect, but in combination with other traits the effect of the locus may become more pronounced.

It was expected that several of the fruit morphology QTLs would coincide with one another. In addition, it was expected that due to genetic background effects, some shape features would be apparent in the parents and advanced backcross populations but not in F_2 populations. Thus, instead of focusing on individual attributes, the main objectives of this study were to identify regions of the genome controlling fruit shape, including common and unique as well as previously known and novel loci. Furthermore, QTLs detected by single and multiple trait composite interval mapping were compared and contrasted with QTLs underlying the major sources of morphological variation represented by the PCs and CVs.

Materials and methods

Plant material

Two F_2 populations were constructed from crosses between one of two elongated *S. lycopersicum* cultivars (Banana Legs or Howard German) and a wild species, *S. pimpinellifolium* accession LA1589 (Fig. 1). The Banana Legs F_2 population (BLF_2) consisted of 99 plants and the Howard German F_2 population (HGF_2) consisted of 130 plants. Both populations were grown simultaneously in the same greenhouse in the summer of 2003. A BC_1 population ($HGBC_1$) was constructed using *S. lycopersicum* Howard German as the recurrent parent. This population contained 100 individuals and was grown in the field in Wooster, OH, USA in the summer of 2003. For all three populations, eight representative fruit were harvested from each plant. Fruit were weighed, cut longitudinally, and scanned at 300 dpi (dots per inch). Images were saved as JPEG files for phenotypic analyses. Due to the larger fruit of the BC_1 population, images had to be adjusted to a lower resolution of 100 dpi to accommodate automated phenotypic analysis with Tomato Analyzer (Brewer *et al*., 2006).

Phenotypic analysis

The Tomato Analyzer software program version 2.1.0.0, which can be downloaded from the laboratory website (http://www.oardc.ohio-state.edu/vanderknaap/), was used for all phenotypic measurements. After making necessary adjustments to individual fruit in an image, analyses were conducted using the batch mode feature of the software application (Brewer *et al*., 2006). Attributes that were segregating within the populations by visual observation were selected for analysis and exported by Tomato Analyzer for further

QTL analysis. The attributes selected for measurement are listed in Fig. 2A and are briefly described here. Fruit shape index (acronym=fs) was defined as the ratio of highest fruit height to widest width (H/W) (Fig. 2B). Fruit shape triangle (tri) was measured as the ratio of the proximal end width to distal end width, w_1/w_2 (Fig. 2C). The distal and proximal end widths were measured at two settings, which were 5% from both the distal and proximal ends of the fruit and 20% from both the distal and proximal ends of the fruit. Heart shape (hrt) is a function of three characteristics: the location of the maximum width, the shoulder height (Fig. 2F), and the degree of tapering (Fig. 2G). To determine the shape at the distal end of the fruit, angle (dan), blockiness (dblk), and indentation area (diar) were measured. The angle of the distal fruit end was measured by determining the slope via regression along the boundary on both sides of the fruit at the distal end (Fig. 2C, D). The angle was measured at the point where the lines intersected and was expressed in degrees, where 180° was flat, >180° was indented, and <180° was pointed. The distal end angle was measured at three settings, which were 2, 5, and 20% from the tip of the fruit. Blockiness was calculated as the ratio of the width close to the distal end of the fruit to the mid-width, w_2/W_m (Fig. 2D). Distal end blockiness was measured at both the 5% and 20% settings. Distal end indentation area was determined as the ratio of the indentation area to the total fruit area (Fig. 2D, inset).

Many proximal end features were also measured in the three populations. Proximal end angle (pan), blockiness (pblk), indentation area (piar), and shoulder height (psh) were measured to describe fruit shape at the stem end of the fruit. The angle of the proximal fruit end was measured where lines from the shoulder points to the site of pedicel attachment intersect (Fig. 2E), where 180° is flat and >180° is concave. Blockiness was calculated as the ratio of the width closest to the proximal end of the fruit to the mid-width, w_1/W_m (Fig. 2E). The width closest to the proximal end was selected at 5% and 20% from the top of the fruit. The proximal end indentation area was measured as the ratio of the indentation area to the total fruit area (Fig. 2F). Shoulder height was calculated as the height of the shoulders of the fruit (h_1, h_2) relative to the maximum fruit height (Fig. 2F). Additional details of the algorithms can be found in Brewer *et al*. (2006).

Genotypic and statistical analysis

Total genomic DNA was isolated from young leaves as described by Bernatzky and Tanksley (1986) and Fulton *et al*. (1995). The genetic maps were constructed with a combination of mostly restriction fragment length polymorphism (RFLP)- and a few polymerase chain reaction (PCR)-based markers using MAPMAKER v2.0 and the Kosambi mapping function (Kosambi, 1944; Lander *et al*., 1987). Additional information on RFLP- and PCR-based markers, including map location and primer information, can be found on the Solanaceae Genomics Network website (http://www.sgn.cornell.edu). The molecular linkage map contained 111 markers across the 12 tomato chromosomes for both the HGF_2 and BLF_2 populations, and 108 markers for the $HGBC_1$ population. The resulting maps spanned ~1200, 1130, and 1330 cM, resulting in average marker distances of 11, 10, and 12 cM for the HGF_2, BLF_2, and $HGBC_1$ populations, respectively. In both F_2 populations, distortion of allelic segregation was detected at the top of chromosome 7.

A

Fruit Shape Trait		Acronym	Measured as:	Image
Fruit shape index		fs	maximum height/maximum width	B
Fruit shape triangle		tri	proximal width/distal width (5%, 20%)	C
Fruit shape heart		hrt	function of shoulder height, taperness, and the location of the maximum width, (Brewer *et al*. 2006)	F,G
Distal fruit end				
	angle	dan	angle of intersect (2%, 5%, 20%)	C,D
	blockiness	dblk	distal width (5%, 20%)/mid-width	D
	indentation area	diar	indentation area relative to total fruit area	D
Proximal fruit end				
	angle	pan	angle from shoulders to pedicel	E
	blockiness	pblk	proximal width (5%, 20%)/mid-width	E
	indentation area	piar	indentation area relative to total fruit area	F
	shoulder height	psh	shoulder height relative to total fruit height	F

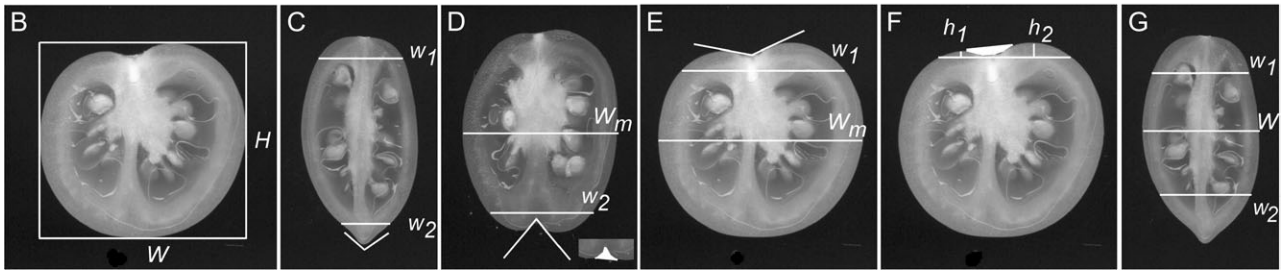

Fig. 2. Fruit shape attributes measured by Tomato Analyzer in the three populations. (A) Fruit shape attributes, acronyms used in QTL analysis, a brief description of the algorithm used to measure the attribute, and the image that corresponds to a visual representation of the method by which the attribute is measured. (B) Fruit shape index: ratio of maximum height to width, H/W. (C) Fruit shape triangle: the ratio of proximal width to distal width, w_1/w_2. Distal fruit end shape angle was measured at 5% above the tip from the fruit. (D) Distal fruit end blockiness: ratio of fruit width at the distal end to mid-width, w_2/W_m. Distal fruit end shape angle at position 5% above the tip from the fruit. Inset: distal end indentation area relative to total fruit area. (E) Proximal fruit end shape angle. Proximal fruit end blockiness: ratio of fruit width at the proximal end to mid width, w_1/W_m. (F) Proximal fruit end indentation: shoulder height, $(h_1+h_2)/2\times H$. Proximal fruit end indentation: area, indentation area relative to total fruit area. (G) Tapering function of the attribute fruit shape heart: $1-w_2/W+w_1/W$. w_2=average width below the widest width W; w_1=average width above the widest width W.

Segregation distortion was also detected at the top of chromosome 11 in the HGF_2 population. Segregation distortion was in favour of the wild parent allele in both regions.

The broad sense heritability (H^2) of each attribute was calculated by using the variances displayed by the F_2 and the LA1589 parental lines. Variances displayed by the *S. lycopersicum* parental lines were not used in the analysis to avoid scaling effects since the trait means in the cultivated lines were much higher than the trait means in the HGF_2, BLF_2, and parental LA1589, with the exception of hrt (Table 1). The variance of the F_2 (V_{F2}) represented both genetic (V_G) and environmental (V_E) variances, whereas LA1589 is a fixed genotype, therefore its variance is only reflected by V_E. V_G was calculated as $V_G = V_{F_2} - V_E$, and H^2 as:

$$H^2 = \frac{V_G}{V_{F_2}}$$

Correlation coefficients, PCA, and CDA were conducted with SAS V9 (SAS Institute Inc., Cary, NC, USA). PCA was conducted across the HGF_2 and BLF_2 populations, and PCA in the BC_1 population was based on the eigenvectors derived from the F_2 populations. For PC1, PC2, and PC3, the eigenvectors from each attribute were multiplied by the standardized trait value in the BC_1 and summed to determine the PC values for each individual component.

QTL analysis was performed by composite interval mapping (Zeng, 1993, 1994) and for subsets of attributes by multitrait composite interval mapping (Jiang and Zeng, 1995). For each trait, PCA, and CDA values, composite interval mapping was performed using model six with five marker cofactors selected by forward regression and a 10 cM window size, as implemented in Windows QTL Cartographer v2.5 (Wang *et al*., 2006). Permutation tests were conducted 1000 times at a significance level of 0.05 to determine QTL threshold levels (Churchill and Doerge, 1994). QTLs above the significance threshold determined by the permutation tests were considered significant. In addition, QTLs below the significance level, but with a LOD >3.0 that overlapped with significant fruit shape QTLs in the same population (different attribute) or a different population (same attribute), were listed as well. Additive and dominance effects and percentage phenotypic variance explained by the QTLs (R^2) were estimated with Windows QTL Cartographer at the highest probability peaks. Multitrait composite interval mapping was used jointly to map QTLs affecting several fruit shape attributes. Traits were grouped based on high correlations (Supplementary Table S1 available at *JXB* online).

Results and discussion

The fruit of *S. lycopersicum* cv. Howard German and *S. lycopersicum* cv. Banana Legs have similar morphological features as well as subtle differences in shape (Fig. 1). Tomato Analyzer was used to measure 15 shape attributes (listed in Fig. 2) on four fruit from each parent (Table 1). The largest differences in shape between Howard German and Banana Legs fruit compared with LA1589 fruit were found for fruit shape index, triangle shape at 5%, and distal end angle at 2, 5, and 20%. As expected, the cultivated fruit had a greater fruit shape index, a more pronounced triangle shape at 5%, which measured the very tip of the fruit, and were more angled at the distal end than the round wild relative. The most noticeable differences in shape between Howard German and Banana Legs were found for triangle shape at 20%, and the proximal shape features shoulder height, angle, and indentation area (Table 1). Thus, Howard German was determined to have a greater triangle shape at 20%, and more pronounced shoulders, indentation, and angles at the proximal end than Banana Legs.

Table 1. *Mean fruit shape attribute values and broad sense heritablity in Howard German and Banana Legs F_2 populations*

Values are given as the mean (±SD).

Trait category	Attribute[a]	Howard German[b]	Banana Legs[b]	LA1589[b]	HGF_2[c]	BLF_2[c]	H^{2d}	
							HGF_2	BLF_2
Fruit shape	fs	2.26 (±0.21)	2.23 (±0.16)	1.02 (±0.02)	1.14 (±0.24)	1.08 (±0.17)	0.99	0.99
	tri5	2.34 (±0.75)	2.87 (±0.38)	1.11 (±0.12)	1.34 (±0.3)	1.35 (±0.29)	0.84	0.83
	tri20	1.75 (±0.29)	1.18 (±0.11)	1.03 (±0.03)	1.08 (±0.06)	1.09 (±0.04)	0.74	0.50
	hrt	0.37 (±0.10)	0.28 (±0.15)	0.03 (±0.05)	0.524 (±0.17)	0.229 (±0.13)	0.92	0.84
Distal fruit end shape	dblk5	0.31(±0.06)	0.21 (±0.05)	0.41 (±0.02)	0.45 (±0.05)	0.43 (±0.05)	0.87	0.85
	dblk20	0.61 (±0.11)	0.73 (±0.03)	0.79 (±0.01)	0.8 (±0.03)	0.79 (±0.02)	0.86	0.75
	dan2 (°)	118 (±18)	87 (±25)	169 (±15)	174 (±23)	170 (±17)	0.58	0.20
	dan5 (°)	64 (±15)	59 (±41)	159 (±9)	162 (±24)	159 (±13)	0.86	0.50
	dan20 (°)	48 (±10)	51 (±15)	107 (±4)	97 (±16)	100 (±13)	0.94	0.90
	diar	0 (±0)	0 (±0)	0 (±0)	0.023 (±0.0788)	0.0076 (±0.031)	1.00	1.00
Proximal fruit end shape	pblk5	0.70 (±0.19)	0.60 (±0.07)	0.45 (±0.03)	0.57 (±0.05)	0.56 (±0.03)	0.57	0.25
	pblk20	1.05 (±0.11)	0.86 (±0.06)	0.82 (±0.02)	0.86 (±0.03)	0.86 (±0.02)	0.50	0.00
	psh	0.0091 (±0.0053)	0.006 (±0.0074)	0.002 (±0.0003)	0.0069 (±0.79)	0.0053 (±0.59)	1.00	1.00
	pan (°)	207 (±16)	187 (±8)	180 (±0)	185 (±5)	184 (±4)	1.00	1.00
	piar	0.0047 (±0.0045)	0.0006 (±0.0006)	0 (±0)	0.0011 (±0.17)	0.0008 (±0.11)	1.00	1.00

[a] Trait acronyms associated with a number (tri, dblk, pblk) indicate the setting at which the width measurement was taken. For example, 5=5% (pblk), 95% (dblk), and both 5% and 95% (tri) from the proximal end. For distal end angle (dan), the number indicates the position along the boundary at which the slope was calculated. For example, 2=2% above the tip.

[b] Four field-grown fruit of the parental lines were analysed by Tomato Analyzer.

[c] Eight fruit of each plant in the F_2 population were analysed by Tomato Analyzer. The mean across the entire population is presented.

[d] Heritability was calculated by using the variances of the attribute displayed by the parental LA1589 and the respective F_2 population.

To determine the genetic basis for these similarities and differences, three populations constructed by crosses of the parents (Fig. 1) were examined for variation in shape and map position of QTLs controlling shape. The shape attributes measured by Tomato Analyzer (Fig. 2) were selected based on visual observations of phenotypes segregating in the populations. Furthermore, the settings for distal end angle, distal and proximal end blockiness, and triangle were chosen based on the settings that maximized phenotypic variation as indicated by the significance of the underlying QTLs. The mean, standard deviation, and heritability of each attribute are shown in Table 1. High heritability was observed for several attributes such as fruit shape index, triangular shape at 5%, heart shape, distal end blockiness, and distal end angles at 5% and 20%. Some attributes showed the maximum heritability of 1. However, this value may be an overestimation due to the lack of variance for these attributes in the parental line LA1589. Correlation coefficients between the attributes indicated that many features were highly significantly correlated (Supplementary Table S1 available at *JXB* online). Interestingly, attributes which were correlated could be divided into two groups. One group of correlated attributes consisted mainly of distal fruit end features, and a second group consisted mainly of proximal fruit end features (Supplementary Table S1 available at *JXB* online).

Fruit shape QTLs

The QTL analyses of the 15 shape attributes indicated numerous loci controlling fruit shape. A total of 36, 32, and 27 QTLs were found to control individual shape traits in the HGF_2, BLF_2, and $HGBC_1$, respectively. Approximately 18% of the QTLs overlapped across all three populations, whereas ~56% of the QTLs were only found in one population (Supplementary Tables S2–S4 available at *JXB* online; Fig. 3). Additionally, overlapping loci controlled many of the shape traits (Fig. 3), which could be due to pleiotropy or a result of measuring the same shape feature which was implied by the high

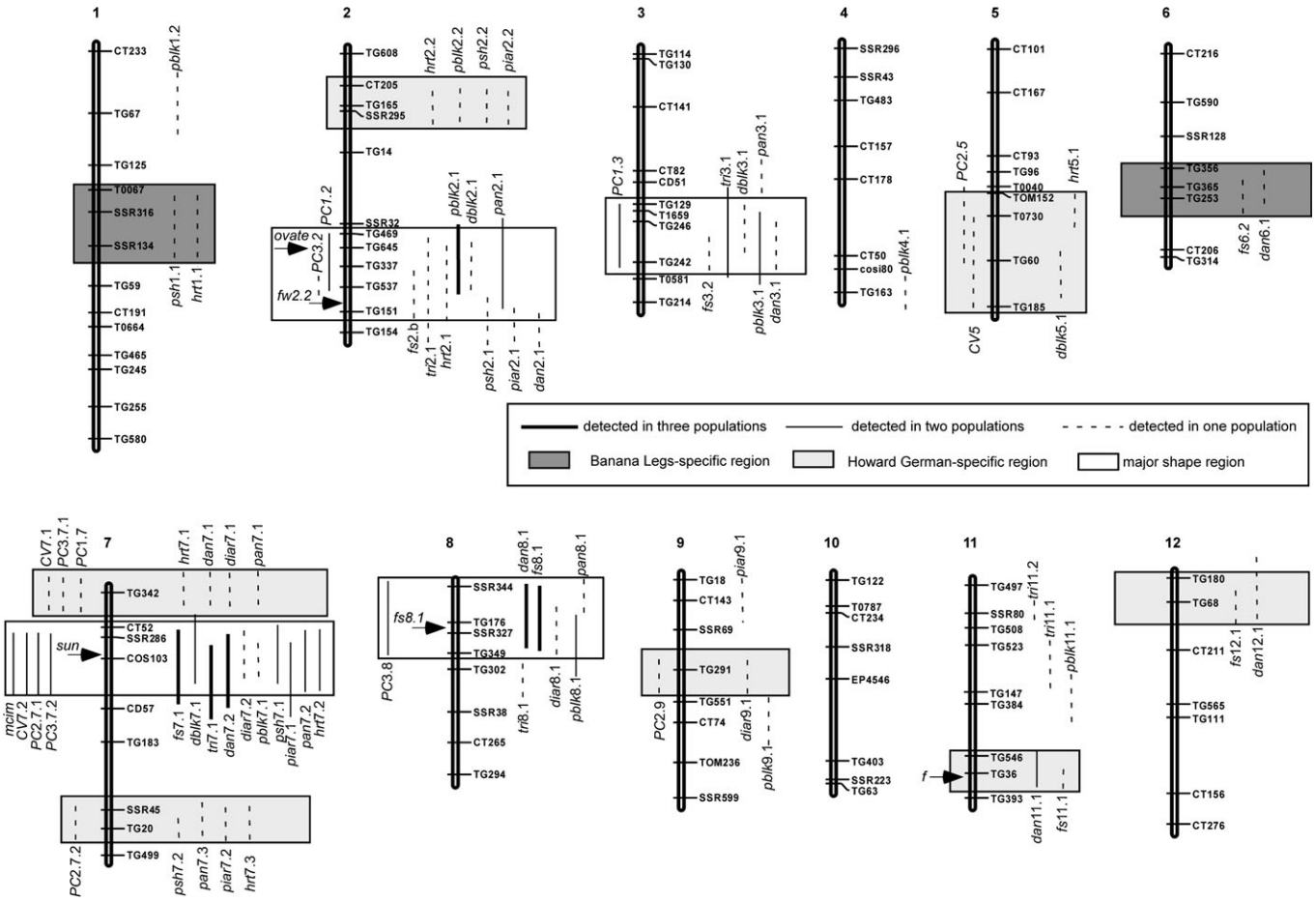

Fig. 3. QTL map position of fruit shape attributes. Composite molecular linkage map of RFLP- and PCR-based molecular markers used in the Howard German and Banana Legs F_2 populations and Howard German BC_1 population. Individual trait QTLs are indicated to the right of each chromosome. PC, CV, and multiple composite interval mapping (mcim) QTLs are indicated to the left of each chromosome. QTLs identified in all three populations are indicated by the thick vertical line; QTLs identified in two populations are indicated by the thin vertical line; QTLs identified only in one population are indicated by the dashed line.

correlation observed among many attributes (Supplementary Table S1 available at *JXB* online). Following adjustment for pleiotropic QTLs, a total of 21 loci were identified as controlling fruit shape in these three populations (Fig. 3). QTLs common to all three populations were detected on chromosomes 2, 7, and 8. Two groups of correlated traits (Supplementary Table S1 available at *JXB* online) were used for joint analysis by multitrait composite interval mapping. In both cases, the only significant QTL overlapped with the major fruit shape locus *sun* on chromosome 7 (Fig. 3; Supplementary Table S6 available at *JXB* online). Similar results were obtained when all attributes were jointly analysed (data not shown).

Common regions controlling shape

The most noticeable region of the genome controlling fruit shape was located near the top of chromosome 7 overlapping with the marker COS103 (Fig. 3). The QTLs detected in this region coincided with the well-known *sun* locus, which contributes to an elongated fruit shape (Van der Knaap *et al*., 2004). In all three populations, QTLs were detected for fruit shape index (*fs7.1*), distal end angle at 20% (*dan7.2*), and triangle shape (*tri7.1*) (Supplementary Tables S2–S4 available at *JXB* online). The *S. lycopersicum* allele contributed to a more elongated, angled, and triangle-shaped fruit. QTLs for distal end blockiness (*dblk7.1*) and distal end indentation area (*diar7.2*) were also detected in this region. The distal end blockiness QTL was present in both F_2 populations, and the distal end indentation area QTL was detected in only the BLF_2 population. In addition to distal end features, the *sun* locus affected multiple proximal end attributes including blockiness (*pblk7.1*), shoulder height (*psh7.1*), indentation area (*piar7.1*), and angle (*pan7.2*) (Fig. 3). Proximal end shoulder height, indentation area, and angle QTLs were detected in both F_2 populations, whereas a QTL for blockiness was detected in the $HGBC_1$ population. An increase in the size of the proximal end features shoulder height, indentation area, and indentation angle was conferred by the *S. pimpinellifolium* allele. A heart shape QTL (*hrt7.2*) was also detected in this region in both F_2 populations. A greater heart shape value at this locus was conferred by the *S. pimpinellifolium* allele, which may be due to the large effect of this allele on shoulder height, an important component of the heart shape algorithm (Brewer *et al*., 2006). Interestingly, correlation coefficients were found to be high between fruit shape index and many other attributes (Supplementary Table S1 available at *JXB* online). This observation was consistent with the presence of the overlapping QTLs at *sun*, including the joint multitrait QTL, and supported the notion that this locus, which is best known for its role in controlling fruit shape index, controlled several other shape features as well.

Another locus with QTLs that were common among all three populations was *fs8.1*, a well-studied fruit shape locus controlling fruit elongation (Grandillo *et al*., 1996; Ku *et al*., 2000). Loci were detected in all three populations at the top of chromosome 8 near the markers SSR327 and TG176 (Fig. 3). In this study, *fs8.1* and *dan8.1*, which controlled distal end angle at 20%, were detected in all three populations. However, the effect of *fs8.1* was much larger in the $HGBC_1$ than the F_2 populations where the locus controlled 16% of the phenotypic variation (Supplementary Table S4 available at *JXB* online) compared with 7% and 6% of the phenotypic variation in the HGF_2 (Supplementary Table S2 available at *JXB* online) and BLF_2 (Supplementary Table S3 available at *JXB* online), respectively. QTLs for proximal end blockiness (*pblk8.1*) were detected in both of the Howard German populations, whereas QTLs for distal end indentation area (*diar8.1*) and proximal end angle (*pan8.1*) were detected in the HGF_2 and $HGBC_1$, respectively.

The third region controlling fruit shape in all three populations was detected near the bottom of chromosome 2 near markers TG537, TG337, and TG645 (Fig. 3). A QTL for proximal end blockiness (*pblk2.1*) was detected in all three populations. A proximal end angle QTL (*pan2.1*) was detected in the HGF_2 and $HGBC_1$ populations. Additionally, triangle shape (*tri2.1*), fruit shape index (*fs2b*), and distal end blockiness (*dblk2.1*) QTLs were detected in the BLF_2, and a heart shape (*hrt2.1*) QTL was detected in the HGF_2. Fruit were blockier, more heart-shaped, more elongated, and more triangle-shaped as a result of the *S. lycopersicum* allele at these loci. Additionally, the large-fruited parent conferred larger indentation angles at the proximal end. Other loci on chromosome 2 responsible for variation in fruit shape coincided with the markers TG151 and TG154. However, due to its close proximity, this locus may coincide with the locus centred on TG337 (Fig. 3). The region near TG151 and TG154 affected proximal end shoulder height (*psh2.1*) and indentation area (*piar2.1*) in the HGF_2, and distal end angle at 20% (*dan2.1*) in the BLF_2. Larger proximal end indentations were conferred by the *S. lycopersicum* allele, while greater distal end angles, or flatter fruit, were conferred by the *S. pimpinellifolium* allele.

Known fruit morphology genes that map to the lower half of chromosome 2 include *ovate* (Liu *et al*., 2002) and *fw2.2* (Frary *et al*., 2000). The *ovate* allele that confers an elongated, pear-shaped fruit is not present in Howard German or Banana Legs (data not shown). Thus, it is unlikely that *ovate* controls fruit shape in this region of the genome. However, the large-fruited allele of *fw2.2* is present in the two cultivated parents. Larger fruit often display more extreme shapes (Van der Knaap and Tanksley, 2003), so it is conceivable that shape QTLs identified on the lower part of chromosome 2 were controlled by a pleiotropic fruit size locus.

A fourth important region of the genome responsible for fruit shape variation in both F_2 populations was found at the bottom of chromosome 3 near TG246 and TG242 (Fig. 3). However, no QTLs were detected in this region in the $HGBC_1$. Triangle shape (*tri3.1*) and proximal end blockiness (*pblk3.1*) were detected in both HGF_2 and BLF_2. Fruit shape index (*fs3.2*) and distal end angle at 20% (*dan3.1*) were detected in only the HGF_2, whereas distal end blockiness (*dblk3.1*) was detected in only the BLF_2. Other fruit size and shape QTLs, including *fw3.2* (Grandillo *et al*., 1999; Van der Knaap and Tanksley, 2003) and a fruit shape locus *ljfs3.1* (Van der Knaap *et al*., 2002), have been detected here and could represent coinciding QTLs.

Unique regions controlling fruit shape

In addition to regions containing many common QTLs, there were also several regions containing QTLs specific to either the Howard German (HGF_2/$HGBC_1$) or Banana Legs (BLF_2) populations. When only considering the locations containing two or more QTLs, the region at the top of chromosome 2, at the top and bottom of chromosome 7, at the bottom of chromosome 11, and at the top of chromosome 12 contained loci that were detected only in the F_2 and BC_1 populations derived from Howard German (Fig. 3). The region harbouring Howard German-specific QTLs was found on chromosome 2 where multiple fruit shape loci overlapped with the markers TG165 and SSR295 (Fig. 3). The region contained QTLs for heart shape (*hrt2.2*), proximal end blockiness (*pblk2.2*), shoulder height (*psh2.2*), and indentation area (*piar2.2*). The *pblk2.2* locus was detected in the HGF_2 (Supplementary Table S2 available at *JXB* online), whereas the remaining loci were found in the $HGBC_1$ (Supplementary Table S4 available at *JXB* online). A more indented fruit at the proximal end resulted from the *S. lycopersicum* allele at this locus. At the top of chromosome 7 coinciding with the marker TG342, QTLs were found for distal end angle (*dan7.1*) and distal end indentation area (*diar7.1*) in the HGF_2 (Supplementary Table S2 available at *JXB* online), and heart shape (*hrt7.1*) and proximal end angle (*pan7.1*) in the $HGBC_1$ (Supplementary Table S4 available at *JXB* online). QTLs for these attributes were also found at the *sun* locus, which maps ~20 cM away from TG342. However, these QTLs were most probably not due to *sun*, since the effects at these loci came from different parental alleles. For distal end angle, for example, the additive effects indicated that the *S. lycopersicum* allele conferred a smaller angled, more pointed fruit at COS103 (*dan7.2*) and a larger or less pointed to indented fruit at TG342 (*dan7.1*). Additionally, a larger angle at the proximal end was conferred by the *S. lycopersicum* allele at TG342, whereas the larger angle and more indented fruit controlled by COS103 was conferred by the *S. pimpinellifolium* allele at that marker (Supplementary Tables S2 and S4 available at *JXB* online). At the bottom of chromosome 7, QTLs coinciding with TG20 were found in the HGF_2 population. These QTLs controlled the proximal end features shoulder height (*psh7.2*), angle (*pan7.3*), indentation area (*piar7.2*), and heart shape (*hrt7.2*). Greater values for these proximal end features were conferred by the *S. lycopersicum* allele. Interestingly, the locus detected on the bottom of chromosome 7 controlled proximal end shape attributes similar to those controlled by the locus on the top of chromosome 2. Furthermore, since these loci were detected in the HGF_2 and/or $HGBC_1$ and not in the BLF_2 population, this result suggested that these two regions contained QTLs that control the subtle differences in fruit shape between Howard German and Banana Legs.

With the exception of the region on chromosome 11 near TG36, these Howard German-specific regions were not previously known to contain highly significant fruit shape QTLs. The region detected on chromosome 11 near TG36 coincides with the *f* locus known to control locule number (Barrero and Tanksley, 2004), *ljfs11.1* known to control the eccentricity of the fruit (Van der Knaap *et al*., 2002), and *fw11.3*, which controls fruit size (Lippman and Tanksley, 2002).

Regions that were specific for the BLF_2 population were found toward the centre of chromosome 1 and toward the lower end of chromosome 6 (Fig. 3). These regions were also not known to have major effects on fruit shape. Additional unique QTLs shown in Fig. 3 were found in other regions, but will not be discussed in detail since these QTLs were only detected once and not confirmed across multiple traits or populations.

Principal components analysis and canonical discriminant analysis

The means of the individual traits were subjected to ANOVA, which showed that only distal end blockiness at 5% and 20% was significantly different between the F_2 populations (Supplementary Table S5 available at *JXB* online). To determine if a combination of attributes could better explain phenotypic diversity, PCA and CDA were conducted across the HGF_2 and BLF_2 on the 15 measured shape attributes. PCA is useful for determining which combinations of attributes are contributing to phenotypic diversity, and CDA, which is similar to PCA, is a powerful tool to reveal which combinations of shape features can distinguish different populations.

The first three PCs contributed to a total of 82% of the variation in the HGF_2 and BLF_2 (Fig. 4A–C). Traits with eigenvectors >0.25 were considered the most significant contributors to each PC and are listed in Fig. 4. PC1, which represented 35% of the variation, was most affected by triangle shape at 20% and 5%, distal end blockiness at 20% and 5%, proximal end blockiness at 20% and 5%,

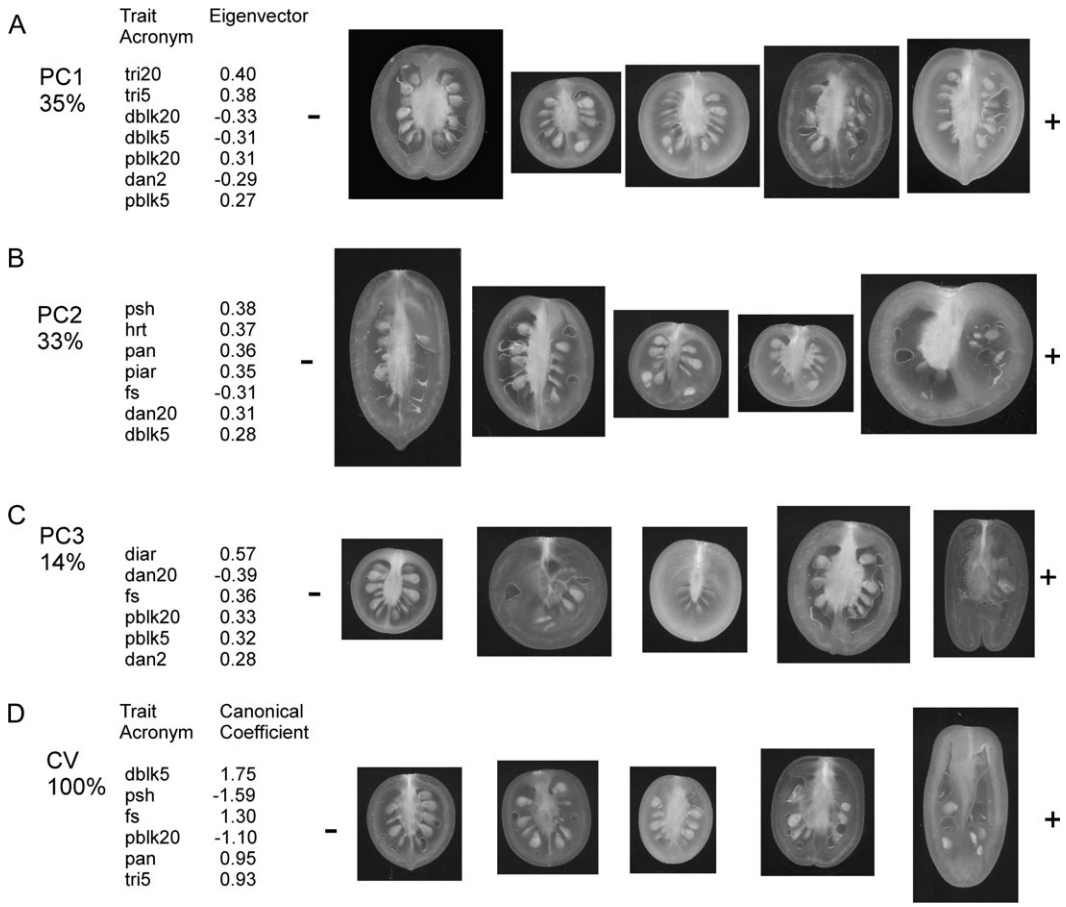

Fig. 4. Principal components analysis and canonical discriminant analysis conducted on the Howard German and Banana Legs F_2 populations. (A–C) The amount of the variability accounted for by each principal component (PC) is listed along with the acronym and eigenvector of the traits that contributed most to each PC (|eigenvector| >0.25). Images display fruit as the PC value increased from the lowest value (–) to the highest value (+) across the two populations. (D) The first canonical variate (CV) represented 100% of the variability between the F_2 populations. The acronym and canonical coefficient of the traits that contributed most to the CV (|canonical coefficient| >0.75). Images display fruit as the CV value increased from the lowest value (–) to the highest value (+) across the two populations.

and distal end angle at 2% (Fig. 4A). Shape attributes with negative eigenvectors forced the PCs in a negative direction, which is exemplified by the shape of fruit on the left, while attributes with positive eigenvectors pushed the PC values in a positive direction as represented by fruit shape displayed on the right of Fig. 4A. For PC1, for example, triangle shape 5% and 20% and proximal end blockiness 5% and 20% had positive eigenvector values. Thus, fruit with a more triangular and blockier shape at the proximal end resulted in an increase of the PC value, which is depicted by the rightmost fruit. Distal end blockiness 5% and 20%, and distal end angle 2% had negative eigenvector values indicating that fruit with smaller PC1 values were blockier, flatter, and more indented at the distal end, which is represented by the fruit to the left (Fig. 4A). PC2 represented 33% of the variation and was most affected by shoulder height, heart shape, proximal end angle, proximal end indentation area, fruit shape index, distal end angle at 20%, and distal end blockiness at 5% (Fig. 4B). Comparison of the eigenvectors and the change in fruit morphology from the most negative to the most positive PC2 value indicated that this component measured shape from elongated with a rounded proximal end to squat with an indentation at the proximal end (Fig. 4B). PC3, which represented 14% of the variation, was most affected by distal end indentation area, distal end angle at 20% and 2%, fruit shape index, and proximal end blockiness at 20% and 5% (Fig. 4C). This component measured fruit from spherical in shape to very blocky and elongated with an indentation at the distal end. To determine which of the PCs was differentiating the two populations, ANOVA was conducted, which indicated that only PC3 distinguished the HGF_2 and BLF_2 populations (P=0.015; Supplementary Table S5 available at *JXB* online). When considering the differences at the population level, the HGF_2 fruit averaged a more positive PC3 value, indicating they were more similar to the fruit on the right (Fig. 4C), whereas the fruit from the BLF_2 population averaged a more negative value, similar in shape to the fruit on the left.

Another method to identify combinations of attributes that distinguish the two F_2 populations is CDA. The first CV was significant (P=0.003) and represented 100% of the variance between the F_2 populations. The attributes with the greatest contribution to the first CV were distal end blockiness at 5%, proximal end shoulder height, fruit shape index, proximal end blockiness at 20%, proximal end angle, and triangle shape at 5% (Fig. 4D). Thus, collectively, these attributes best distinguished the two F_2 populations. The range of CV1 from negative to positive showed that fruit shape ranged from slightly blocky at the proximal end and tapered with a tip at the distal end to elongated and blocky at both ends (Fig. 4D). Most individuals from the BLF_2 had lower CV1 values similar to the fruit depicted on the left (Fig. 4D), while individuals from the HGF_2 had higher CV1 values and were represented more by fruit on the right. Interestingly, this shape difference was also noted for PC3 (Fig. 4C), which is the PC that distinguished the two populations. Thus, while only two shape attributes were significantly different between the F_2 populations, ANOVA of the PC and CDA demonstrated that combinations of attributes could clearly distinguish the two populations at a significant level.

To determine whether PCs and CVs were controlled by the same or additional loci compared with individual trait QTLs and whether QTLs of larger effect could be identified, the first three PCs and the first CV were subjected to QTL analysis. PC1 was primarily described by blockiness at the distal and proximal ends and triangle shape, and was controlled by QTLs on chromosome 2 (*pc1.2*) near TG645 and on chromosome 3 (*pc1.3*) near TG242 and TG246 in both F_2 populations (Fig. 4A, Supplementary Table S6 available at *JXB* online). These QTLs overlapped with loci where multiple individual fruit shape QTLs were detected, including ones that contributed most significantly to PC1 (Fig. 3). Importantly, although the individual trait QTLs on chromosomes 2 and 3 were not as significant as those controlled by the *sun* locus, these two regions of the genome were responsible for the largest amount of variation among the F_2 populations when all attributes were combined. All PC1 QTLs had positive additive effects, indicating that an increase in PC1 at these loci was conferred by the *S. lycopersicum* allele. Therefore, alleles from the large-fruited parent contributed to the fruit shape displayed by the rightmost fruit in Fig. 4A, whereas the alleles of the wild relative contributed to the fruit shape displayed to the left.

A highly significant QTL was detected for PC2 in both F_2 populations on chromosome 7 near marker COS103, overlapping with *sun* (Supplementary Table S6 available at *JXB* online). Importantly, the PC2 QTLs also coincided with many QTLs from individual fruit shape traits that contributed to PC2. An increase in PC2 was conferred by the *S. pimpinellifolium* allele and resulted in fruit that were squat and indented at the proximal end. Furthermore, increases in fruit shape index resulted in a decrease of the value of PC2 (Fig. 4A), which was consistent with the *S. lycopersicum* allele of *sun* conferring a more elongated fruit. Another minor effect QTL for PC2 was detected on chromosome 7 near TG20 in the HGF_2. Importantly, this QTL coincided with individual fruit shape loci that were detected in the same HGF_2 population that controlled proximal end fruit shape features.

PC3 was described by a combination of attributes that could distinguish the F_2 populations. The attributes that contributed most to PC3 were distal end indentation area, distal end angle at 20%, and fruit shape index (Fig. 4C). Surprisingly, the values for these attributes did not differ much in the Howard German and Banana Legs parental fruit (Table 1), suggesting that despite different genetic background effects, the final shape is similar. Although PC3 was controlled by both similar and different QTLs, most of these QTLs were below the significance level as indicated by the permutation tests (Supplementary Table S6 available at *JXB* online). The two loci of largest effect were found on the top of chromosomes 7 and 8 but only in the HGF_2. Furthermore, *pc3.7.1* and *pc3.8* overlapped with distal end indentation area QTLs *diar7.1* and *diar8.1*, respectively, which might confirm the importance of these loci in controlling this attribute (Fig. 3).

QTLs were also identified by subjecting the first CV as a trait in QTL analysis. These QTLs were expected to highlight the differences in fruit shape between the F_2 populations, which could be mediated by different loci or by loci with a different effect depending on the genetic background. A QTL overlapping with *sun* (*cv7.2*) was detected in both populations; however, remaining QTLs were only detected in the HGF_2 population (Supplementary Table S6 available at *JXB* online). Interestingly, the QTL at the top of 7, *cv7.1*, coincided with *diar7.1* and *dan7.1* in the HGF_2 population and with the QTL detected for PC3, which is an additional suggestion that this QTL was responsible for shape differences between the populations and is separate from the *sun* locus. The CV QTLs coincided with individual trait QTLs in the F_2 populations (Fig. 3).

The eigenvectors for each trait derived from PCA that was conducted in the F_2 populations were used to calculate corresponding PC values for the $HGBC_1$ population in order to compare the PCs across all three populations. This was done in order to determine if the same or different QTLs could be detected in a differently structured population. The $HGBC_1$ was not included in the original PCA because the difference in the population structure would have accounted for most of the variability. For example, the shapes of the individuals from the backcross population were more extreme and they were larger in size since the majority of the alleles came from the large-fruited parent. For either PC1 or PC2, none of the same

QTLs were detected in either the F_2 or the BC_1 population. However, the changes in fruit shape controlled by each PC were the same in the BC_1 as those observed in the F_2 populations (data not shown). In the BC_1, a QTL at the top of chromosome 7 (*pc1.7*) overlapping with TG342 was detected for PC1 (Supplementary Table S7 available at *JXB* online). For PC2, QTLs were found on chromosome 5 (*pc2.5*) near T0730 and on chromosome 9 (*pc2.9*) near TG291. These QTLs overlapped with QTLs for heart shape (*hrt5.1*) and distal end indentation area (*diar9.1*), respectively (Fig. 3), that were also detected in the $HGBC_1$. Fruit that were flatter and more indented at the proximal end were also more indented at the distal end, which explained the overlap of *diar9.1* and *pc2.9*. Similarly, indented shape at the proximal end resulted in enhanced heart shape features, which explained the overlap of *pc2.5* and *hrt5.1*. These overlapping loci confirmed the importance of these regions in controlling fruit shape. The only PC QTL overlapping in both of the F_2 and the BC_1 was *pc3.8*, which coincided with *fs8.1* (Fig. 3). Interestingly, whereas the *fs8.1* locus had a relatively small effect on fruit shape index in the HGF_2 (LOD=5.4, R^2=0.07), the same locus had a greater effect on the PC3 in the HGF_2 (LOD=9.3, R^2=0.26), albeit that the QTL was just below the significance level determined by the permutation test, and an even larger effect on PC3 in the $HGBC_1$ (LOD =11.5, R^2=0.36). Thus, PCA enhanced the effect and increased the significance of this locus because *fs8.1* controlled multiple traits. Additionally, the effect of *fs8.1* on fruit shape index was more pronounced in a BC_1 than in an F_2 population, indicating that this locus required additional large-fruited alleles to exert its effect on fruit elongation.

One of the unexpected results was that *sun* was not detected for PC2 in the $HGBC_1$, especially since this locus had such a large effect on this component in the F_2 populations. Besides affecting fruit shape index, *sun* also displayed large effects on proximal end features in the F_2. However, the effect on the proximal end features was conferred by the small-fruited parent. The alleles of the small-fruited parent were under-represented in the backcross population, which may explain the lack of significance found at *sun*. Thus, it can be concluded that similar QTLs will probably not control the same PCs in differently structured populations.

Conclusions

By comparing fruit morphology QTLs in three separate populations, many regions of the tomato genome controlling fruit shape were identified. Some of these regions overlapped among all three populations, while others were unique to populations derived from either Howard German or Banana Legs, indicating that these loci may be contributing to shape variation between these varieties. Many attributes showed overlapping map positions, which indicated pleiotropic effects of the locus. For example, the fruit shape locus *sun* controlled fruit shape index as well as triangle shape and distal end angle in all three populations. This result is a strong indication that the locus controls these attributes in various genetic backgrounds which, in fact, was confirmed using near isogenic lines that differ at the *sun* locus (results not shown). Additionally, while many shape attributes that mapped to the bottom of chromosome 2 were detected in only one population, the proximal end blockiness attribute was detected in all three populations. Therefore, proximal end blockiness will probably be the most reliable attribute to map across populations and in progeny studies.

Interestingly, some regions specific to either the Howard German or Banana Legs populations (Fig. 3) coincided with QTLs for individual attributes that were also different between the large-fruited parents (Table 1). For example, the values for triangle shape at 20%, and the proximal end features shoulder height, angle, and indentation area were different between the Howard German and Banana Legs parent fruit. QTLs for these individual attributes overlapped with five of the cultivar-specific regions located on chromosome 1, the top of chromosome 2, chromosome 5, the top of chromosome 7, and the bottom of chromosome 7. This result suggests that these regions are responsible for the shape differences between Howard German and Banana Legs. Moreover, these loci seem to control whether a fruit has more pronounced shoulders and indentations at the proximal end.

PCA was useful in determining the greatest sources of variation and combination of attributes that contributed to this variation. Furthermore, whereas QTL analysis of the 15 individual attributes resulted in an average of 33 loci per population and the multiple trait composite interval mapping detected only one locus (*sun*), mapping of the PC resulted in a QTL map with the modest number of 11 loci across all three populations. Importantly, the PC QTLs overlapped with fruit shape attribute QTLs that were contributing to the component, indicating that PC QTLs accurately identified regions of the genome controlling the same traits that comprised the component. Thus, PCA is very useful to simplify QTL analyses that are intended to evaluate the impact of many attributes that segregate in populations and outperformed the multiple trait QTL analysis at least in these populations.

The results presented here, on 15 fruit morphology attributes that collectively described shape variation, provides an unprecedented detail on the diversity in tomato fruit morphology and its genetic control. Moreover, by employing Tomato Analyzer, these attributes can be measured in additional tomato populations and other fruit crops which would permit for the first time extensive morphological comparisons across species.

Supplementary data

Tables presented in the supplementary section available at *JXB* online show correlation matrix of traits, and detailed QTL results (LOD score for the interval, most significant marker, percentage of the phenotypic variance explained) for the individual attributes, the PCA and the CDA.

Acknowledgements

We thank Jason Dickey and Cheryll Radovich for their excellent technical assistance in harvesting and scanning fruit, and genotyping the tomato populations. We also acknowledge Lee Duncan and John Elliott for their help maintaining the plants in the greenhouse and the field. This research was supported by the National Science Foundation (no. DBI-0227541). Salaries and research support were also provided by State and Federal Funds assigned to the Ohio Agricultural Research and Development Center, the Ohio State University. AJM was partly supported by funds from Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, Spain) and Departament d'Universitats, Recerca i Societat de la Informació (Generalitat de Catalunya, Spain).

References

Barrero LS, Tanksley SD. 2004. Evaluating the genetic basis of multiple-locule fruit in a broad cross-section of tomato cultivars. *Theoretical and Applied Genetics* **109,** 669–679.

Bernatzky R, Tanksley SD. 1986. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics* **112,** 887–898.

Brewer MT, Lang L, Fujimura K, Dujmovic N, Gray S, van der Knaap E. 2006. Development of a controlled vocabulary and software application to analyze fruit shape variation in tomato and other plant species. *Plant Physiology* **141,** 15–25.

Churchill GA, Doerge RW. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* **138,** 963–971.

Frary A, Nesbitt TC, Frary A, *et al.* 2000. *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. *Science* **289,** 85–88.

Fulton TM, Chunwongse J, Tanksley SD. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter* **13,** 207–209.

Grandillo S, Ku HM, Tanksley SD. 1996. Characterization of *fs8.1*, a major QTL influencing fruit shape in tomato. *Molecular Breeding* **2,** 251–260.

Grandillo S, Ku HM, Tanksley SD. 1999. Identifying the loci responsible for natural variation in fruit size and shape in tomato. *Theoretical and Applied Genetics* **99,** 978–987.

Jiang C, Zeng ZB. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* **140,** 1111–1127.

Kosambi DD. 1944. The estimation of map distances from recombination values. *Annals of Eugenics* **12,** 172–175.

Ku HM, Doganlar S, Chen KY, Tanksley SD. 1999. The genetic basis of pear-shaped tomato fruit. *Theoretical and Applied Genetics* **99,** 844–850.

Ku HM, Grandillo S, Tanksley SD. 2000. *fs8.1*, a major QTL, sets the pattern of tomato carpel shape well before anthesis. *Theoretical and Applied Genetics* **101,** 873–878.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1,** 174–181.

Langlade NB, Feng X, Dransfield T, Copsey L, Hanna A, Thebaud C, Bangham A, Hudson A, Coen E. 2005. Evolution through genetically controlled allometry space. *Proceedings of the National Academy of Sciences, USA* **102,** 10221–10226.

Lippman Z, Tanksley SD. 2001. Dissecting the genetic pathway to extreme fruit size in tomato using a cross between the small-fruited wild species *Lycopersicon pimpinellifolium* and *L. esculentum* var. giant heirloom. *Genetics* **158,** 413–422.

Liu J, Van Eck J, Cong B, Tanksley SD. 2002. A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. *Proceedings of the National Academy of Sciences, USA* **99,** 13302–13306.

Van der Knaap E, Lippman ZB, Tanksley SD. 2002. Extremely elongated tomato fruit controlled by four quantitative trait loci with epistatic interactions. *Theoretical and Applied Genetics* **104,** 241–247.

Van der Knaap E, Sanyal A, Jackson S, Tanksley SD. 2004. High-resolution fine mapping and fluorescence *in situ* hybridization analysis of *sun*, a locus controlling tomato fruit shape, reveals a region of the tomato genome prone to DNA rearrangements. *Genetics* **168,** 2127–2140.

Van der Knaap E, Tanksley SD. 2001. Identification and characterization of a novel locus controlling early fruit development in tomato. *Theoretical and Applied Genetics* **103,** 353–358.

Van der Knaap E, Tanksley SD. 2003. The making of a bell pepper-shaped tomato fruit: identification of loci controlling fruit morphology in Yellow Stuffer tomato. *Theoretical and Applied Genetics* **107,** 139–147.

Wang S, Basten CJ, Zeng Z-B. 2006. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.

Zeng ZB. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Sciences, USA* **90,** 10972–10976.

Zeng ZB. 1994. Precision mapping of quantitative trait loci. *Genetics* **136,** 1457–1468.