© Indian Academy of Sciences

RESEARCH ARTICLE

Preservation of *Solanum pimpinellifolium* genomic fragments in recombinant genotypes improved the fruit quality of tomato

SABINA L. MAHUAD[1], GUILLERMO R. PRATTA[1]*, GUSTAVO R. RODRIGUEZ[1], ROXANA ZORZOLI[2] and LILIANA A. PICARDI[2]

[1]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and*
[2]*Consejo de Investigaciones de la Universidad Nacional de Rosario (CIUNR) Cátedra de Genética, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, CC 14 S2125ZAA, Zavalla (Santa Fe), Argentina*

Abstract

Five recombinant inbred lines obtained from the F_2 generation of an interspecific cross between cultivar, Caimanta (Cai, *Solanum lycopersicum*) and wild accession, LA722 (P, *S. pimpinellifolium*) were crossed to obtain the second cycle hybrids (SCH). Eleven fruit quality traits were assessed in evaluating phenotypic variability among genotypes P, Cai, F_1 (Cai × P), five RILs, and 10 SCH. One of the five recombinant inbred lines and three SCH had higher values than P, as the best genotype for shelf life. Sequence-related amplified polymorphism was used as the molecular method for detecting polymorphism among these 18 genotypes. The percentage of polymorphism in RILs and SCH was 61% and 66% respectively. Moreover, some bands detected in P were present in SCH. Several multivariate analyses were performed to find agreement between the phenotypic variability observed for fruit quality traits and the polymorphism obtained from sequence-related amplified polymorphism markers. A general Procrustes analysis estimated that there was a consensus proportion of 75% between phenotypic and molecular data. There was considerable preservation of some bands from the wild genotype, which could increase the variability in fruit quality traits in populations where the genetic diversity is limited.

[Mahuad S. L., Pratta G. R., Rodriguez G. R., Zorzoli R. and Picardi L. A. 2013 Preservation of *Solanum pimpinellifolium* genomic fragments in recombinant genotypes improved the fruit quality of tomato. *J. Genet*. **92**, 195–203]

Introduction

In recent years, an important reduction of genetic variability was observed in several fruit quality traits of the cultivated tomato (*Solanum lycopersicum*). Several reports (reviewed by Foolad 2007) point out that wild germplasm is an important source to increase the genetic background of this species. The wild species *S. pimpinellifolium* could be an interesting contributor to genetic variability in improving fruit quality traits. Rodriguez *et al.* (2006) obtained 17 recombinant inbred lines (RILs) from the F_2 generation of an interspecific cross between the wild accession LA722 from *S. pimpinellifolium* and the Argentinean cultivar Caimanta (Cai) (*S. lycopersicum*). During the selfing process, a divergent-antagonistic selection programme was carried out for fruit weight as well as fruit shelf life (SL). Subsequently, these RILs were explored as a genetic source to improve fruit quality traits and to look for new genetic variability for these traits at the phenotypic and molecular levels (Pratta *et al.* 2011).

It is known that molecular markers could be useful to verify genetic variability in unknown germplasm. Monforte and Tanksley (2000) indicated the importance of these tools to identify genes of interest in species whose phenotypic characteristics are undesirable from a productive point of view. In addition, Bredemeijer *et al.* (1998) pointed out that the scanty genetic diversity observed in the cultivated tomato is reflected in the low level of polymorphism found in proteins, isozymes and in most of the DNA markers. Grandillo and Tanksley (1996a,b) working with restriction fragment length polymorphism (RFLP) markers, microsatellites (simple sequence repeat, SSR) and random amplification of polymorphic DNA (RAPD), in a cross between *S. lycopersicum* and *S. pimpinellifolium*, detected significant associations between molecular markers and many important agronomic quantitative traits. Other reports demonstrated the

*For correspondence. E-mail: gpratta@unr.edu.ar.
Sabina L. Mahuad and Guillermo R. Pratta contributed equally to this work.

Keywords. plant genetic resources; second cycle hybrids; SRAP; generalized Procrustes analysis; molecular markers.

importance of SSR to determine levels of polymorphism and to identify different cultivars (Smulders *et al.* 1997; He *et al.* 2003). Li and Quiros (2001) developed a simple marker technique known as sequence-related amplified polymorphism (SRAP) that preferably amplifies open reading frames (ORFs) mostly identifying expressible sequences. This system was mainly developed for the family Brassicaceae, but it has also been used in other species (potatoes, apple, citrus fruits, cherry, plum-tree, garlic, lettuce, celery, pumpkin, tomato and artichoke) where different percentages of polymorphism were detected among various genotypes at either interspecific or intraspecific levels (Li and Quiros 2001; Ferriol *et al.* 2004; Ruiz *et al.* 2005; Cravero *et al.* 2007; Gulsen *et al.* 2007).

Generally, to improve an agronomic trait in prebreeding phases or in any programme, breeders are interested in classifying genotypes into groups as homogeneous as possible but with the highest heterogeneity among them. Currently multivariate methods have a discriminatory power to characterize genotypes by phenotypic and molecular markers, although it remains unclear whether molecular markers would be useful to detect differences between closely related genotypes (Ruiz *et al.* 2005).

In the present study, we evaluated several phenotypic traits that define fruit quality in five of the 17 RILs obtained by Rodriguez *et al.* (2006) and in 10 hybrids obtained by crossing them in a diallel design. SRAP markers were also obtained for all these genotypes. As a pilot experiment, different types of multivariate analysis were performed to verify whether the classification by phenotypic and DNA markers have a significant consensus to group these genotypes according to their genetic variability.

Material and methods

Plant material

The assay was carried out at the field station 'J. F. Villarino' (Universidad Nacional de Rosario, Zavalla, Argentina 33°S 61°W). Five elite RILs: ToUNR1, ToUNR9, ToUNR8, ToUNR15 and ToUNR18, of the 17 obtained by Rodriguez *et al.* (2006) were crossed following a complete diallel design without reciprocal crosses (Griffing 1956) to obtain the second cycle hybrids (SCH) (Kearsey and Pooni 1996) (SCH: F_1 (ToUNR18 × ToUNR1), F_1 (ToUNR18 × ToUNR15), F_1 (ToUNR18 × ToUNR9), F_1 (ToUNR18 × ToUNR8), F_1 (ToUNR1 × ToUNR15), F_1 (ToUNR1 × ToUNR9), F_1 (ToUNR1 × ToUNR8), F_1 (ToUNR15 × ToUNR9), F_1 (ToUNR15 × ToUNR8), F_1 (ToUNR9 × ToUNR8)). This plant material was obtained from the experiments performed by Marchionni Basté *et al.* (2010) to obtain several genetic parameters in this new genetic background. The parental genotypes Cai of *S. lycopersicum* and the accession LA722 (P) of *S. pimpinellifolium* together with their F_1 (Cai × P) were also included in the trial. Fifteen seeds of each of these 18 genotypes were sowed in seedling trays in July, and after thirty days, 10 of the best seedlings ($n = 180$) were transplanted to a greenhouse in a complete randomized design. Agronomic practices were performed according to the standard recommendations for this area (Rodriguez *et al.* 2006).

Phenotypic traits

Tomato fruits were evaluated at two stages: (i) breaker stage (10% red surface with the first signs of carotenoids accumulation ($n = 2570$ fruits) and (ii) ripening stage (90% red surface) as defined by Nuez (1991) ($n = 1300$ fruits). In the stage (i) the following traits were evaluated: weight (W, g), height (H, cm), diameter (D, cm), shape index (S, ratio H/D), and shelf life (SL) measured as days from harvest until the beginning of fruit softening (Schuelter *et al.* 2002). Fruits were stored on a shelf at 25 ± 3°C to evaluate SL trait. In the stage (ii) fruits were evaluated for firmness (FR) measured on the equatorial plane in two opposite areas of the fruit with a durometer Durofel DFT 100 Digital Firmness Tester (Agro-Technologie, Forges Les Eaux, France) with a tip of 0.10 cm^2, soluble solids content (SS, in °Brix, determined by a hand refractometer in the homogenized juice from the pericarp tissue), titratable acidity (TA, grams of citric acid / 100 grams of homogenized juice), pH and colour, which was determined through the chroma index by calculating the a/b ratio (C, where a and b are the absorbencies at wave length of 540 and 675 nm, respectively) and the reflectance percentage (L) which indicates darkness or lightness of colour and ranges from black (0) to white (100). The parameters L, a, and b were determined using Chroma Meter CR-400 (Konica-Minolta, Tokyo, Japan). The colour value parameters were calculated by averaging the measurements obtained from three readings in the equatorial zone of each fruit.

Molecular markers

DNA was extracted once the third true leaf was fully expanded. It was isolated from a minimum of three samples of the 18 genotypes. Total DNA was extracted from 40 mg young frozen leaf tissue using a DNA extraction Kit Wizzard® Genomic DNA Purification kit for plant tissue (Promega, Madison, WI, USA). DNA quality and concentration were measured by agarose gel 1% with 0.5× TBE (Tris/boric acid/EDTA) stained with ethidium bromide and compared with a standard DNA lambda phage (Promega, USA). The protocol reported by Li and Quiros (2001) and Li *et al.* (2003) was used to obtain SRAP markers. Nine different primers were employed in these five forward and reverse combinations: C1, ME1 - EM2; C2, ME2 - EM1; C3, ME2 - GA34; C4, ME8 - SA14; C5, OD34 - DC1. The primer sequences used were: forward primer, ME1: 5′-TGAGTCC AAACCGGATA-3′, ME2: 5′-TGAGTCCAAACCGGAGC-3′, ME8: 5′-TGAGTCCTTTCCGGTGC-3′, OD34: 5′-CAAT CAGGGCGTAGCAGT-3′, and the reverse primer, EM1: 5′-GACTGCGTACGAATTCAAT-3′, EM2: 5′-GACTGCGT ACGAATTCTGC-3′, GA34: 5′-CCAAATGGAACAAAAT

Table 1. Fruit quality traits in 18 genotypes of tomato.

	W	SL	H	D	S	L	C	FR	pH	SS	TA
Genotype	X ± S.E	X ± S.E	X ± S.E	X ± S.E	X ± S.E	X ± S.E	X ± S.E	X ± S.E	X ± S.E	X ± S.E	X ± S.E
1	137.62 ± 14.60 j	12.38 ± 1.13 a	5.86 ± 0.23 l	6.56 ± 0.24 j	0.90 ± 0.03 bc	44.35 ± 0.70 i	0.83 ± 0.04 a	49.73 ± 1.87 de	5.29 ± 0.11 f	5.67 ± 0.39 a	0.23 ± 0.02 a
2	1.81 ± 0.14 a	15.70 ± 0.73 bcd	1.30 ± 0.03 a	1.48 ± 0.04 a	0.88 ± 0.01 b	40.57 ± 0.30 h	1.03 ± 0.03 b	53.77 ± 1.03 f	4.59 ± 0.05 bcd	8.50 ± 0.04 f	0.60 ± 0.02 gh
3	10.67 ± 1.21 g	16.59 ± 0.71 abcd	2.34 ± 0.09 gh	2.63 ± 0.09 gh	0.90 ± 0.01 bc	37.04 ± 0.20 cd	1.13 ± 0.02 cd	46.97 ± 1.03 abcd	4.58 ± 0.03 bcd	6.96 ± 0.11 bc	0.37 ± 0.01 bcd
4	21.66 ± 1.35 i	24.79 ± 1.18 hi	2.85 ± 0.09 k	3.55 ± 0.06 i	0.81 ± 0.02 a	39.81 ± 0.43 g	1.04 ± 0.04 b	54.17 ± 1.82 f	4.96 ± 0.02 e	5.54 ± 0.08 a	0.32 ± 0.01 b
5	2.34 ± 0.21 b	17.98 ± 0.62 cde	1.42 ± 0.04 b	1.58 ± 0.06 a	0.90 ± 0.01 bc	40.42 ± 0.24 gh	1.14 ± 0.02 cd	54.55 ± 0.83 f	4.51 ± 0.05 abc	9.30 ± 0.27 g	0.65 ± 0.01 h
6	3.81 ± 0.32 cd	18.72 ± 0.43 def	1.63 ± 0.04 c	1.83 ± 0.06 bc	0.89 ± 0.01 bc	37.09 ± 0.07 cd	1.37 ± 0.01 h	53.39 ± 0.61 f	4.35 ± 0.02 a	7.04 ± 0.06 bc	0.79 ± 0.02 i
7	11.59 ± 1.04 gh	14.05 ± 0.75 ab	2.22 ± 0.07 fg	2.84 ± 0.09 h	0.79 ± 0.02 a	38.81 ± 0.35 f	1.12 ± 0.03 c	54.32 ± 0.96 f	4.61 ± 0.05 cd	7.91 ± 0.11 e	0.44 ± 0.01 ef
8	13.72 ± 0.72 h	21.11 ± 1.20 efgh	2.77 ± 0.07 jk	2.83 ± 0.06 h	0.97 ± 0.02 d	36.54 ± 0.28 bc	1.15 ± 0.02 cd	52.47 ± 0.82 ef	4.72 ± 0.03 d	7.69 ± 0.08 de	0.37 ± 0.02 bcde
9	3.36 ± 0.26 c	18.77 ± 0.93 def	1.67 ± 0.07 c	1.80 ± 0.04 b	0.93 ± 0.02 bcd	38.09 ± 0.17 e	1.27 ± 0.01 g	48.89 ± 1.03 cde	4.42 ± 0.04 ab	8.09 ± 0.28 ef	0.55 ± 0.04 g
10	5.81 ± 0.20 e	26.50 ± 1.27 i	2.02 ± 0.03 d	2.17 ± 0.03 de	0.94 ± 0.02 b	35.39 ± 0.17 a	1.18 ± 0.01 cde	47.15 ± 0.86 bcd	4.50 ± 0.03 abc	8.26 ± 0.08 ef	0.59 ± 0.02 g
11	5.80 ± 0.23 e	24.60 ± 1.24 hi	1.90 ± 0.03 de	2.21 ± 0.05 d	0.88 ± 0.01 cd	37.82 ± 0.20 e	1.19 ± 0.01 def	55.71 ± 1.15 f	4.65 ± 0.05 cd	7.09 ± 0.07 cd	0.39 ± 0.02 bcde
12	7.31 ± 0.55 ef	21.99 ± 0.67 fgh	2.09 ± 0.07 ef	2.40 ± 0.07 ef	0.89 ± 0.01 bc	37.96 ± 0.21 e	1.25 ± 0.02 fg	45.53 ± 1.35 abc	4.49 ± 0.04 abc	5.49 ± 0.14 a	0.42 ± 0.03 def
13	3.50 ± 0.22 c	15.47 ± 0.75 abcd	1.64 ± 0.04 c	1.84 ± 0.04 bc	0.89 ± 0.01 bc	37.46 ± 0.10 de	1.39 ± 0.01 h	52.39 ± 0.89 ef	4.62 ± 0.05 cd	6.89 ± 0.13 bc	0.55 ± 0.02 g
14	7.37 ± 0.32 f	21.42 ± 0.84 efgh	2.11 ± 0.03 ef	2.36 ± 0.04 e	0.91 ± 0.01 bc	36.29 ± 0.17 b	1.24 ± 0.02 efg	43.31 ± 1.50 a	4.50 ± 0.04 abc	7.20 ± 0.07 cd	0.55 ± 0.02 g
15	10.62 ± 0.99 g	24.45 ± 1.20 ghi	2.59 ± 0.09 ij	2.59 ± 0.11 fg	1.02 ± 0.02 e	37.11 ± 0.17 cd	1.15 ± 0.02 cd	54.85 ± 1.32 f	4.54 ± 0.04 bc	6.48 ± 0.12 b	0.44 ± 0.03 ef
16	10.42 ± 0.66 g	20.58 ± 1.06 ef	2.47 ± 0.06 hi	2.57 ± 0.05 fg	0.97 ± 0.02 d	36.99 ± 0.12 cd	1.23 ± 0.02 efg	44.87 ± 1.29 ab	4.74 ± 0.05 d	7.31 ± 0.10 cd	0.33 ± 0.01 bc
17	4.62 ± 0.31 d	20.67 ± 0.98 efg	1.89 ± 0.05 d	1.96 ± 0.05 c	0.98 ± 0.01 d	36.86 ± 0.20 bcd	1.26 ± 0.02 g	49.00 ± 1.24 cde	4.62 ± 0.04 cd	7.23 ± 0.54 cd	0.40 ± 0.03 cdef
18	10.64 ± 0.95 g	14.68 ± 0.88 abc	2.41 ± 0.09 ghi	2.63 ± 0.08 gh	0.92 ± 0.03 bc	37.39 ± 0.24 de	1.19 ± 0.03 def	43.86 ± 1.59 ab	4.65 ± 0.06 cd	7.35 ± 0.13 cd	0.46 ± 0.01

Mean values (X) ± Standard error (S.E.) for fruit quality traits Weight (W), Shelf Life (SL), Height (H), Diameter (D), Shape index (S), Reflectance (L), a/b index (C), Firmness (FR), pH (pH), Soluble Solids content (SS) and Titratable Acidity (TA) for the 18 genotypes. 1, Cai; 2, P; 3, F_1 (Cai × P); 4, ToUNR1; 5, ToUNR8; 6, ToUNR9; 7, ToUNR15; 8, ToUNR18; 9, F_1 (ToUNR9 × ToUNR8); 10, F_1 (ToUNR1 × ToUNR8); 11, F_1 (ToUNR1 × ToUNR9); 12, F_1 (ToUNR1 × ToUNR15); 13, F_1 (ToUNR15 × ToUNR8); 14, F_1 (ToUNR15 × ToUNR9); 15, F_1 (ToUNR18 × ToUNR1); 16, F_1 (ToUNR18 × ToUNR8); 17, F_1 (ToUNR18 × ToUNR9); 18, F_1 (ToUNR18 × ToUNR15). Different letters are significant differences ($P < 0.05$) by Duncan test mean values are not transformed values.

GATG-3′, SA14: 5′-TTACCTTGGTCATACAACATT-3′, DC1: 5′-TAAACAATGGCTACTCAAG-3′. Separation of amplification fragments was done on 5% polyacrylamide gels (w/v) at 50 W for 4 h. DNA bands were visualized using Silver Staining kit (NO_2Ag), SILVER SEQUENCE™ Staining Reagents (Promega). The size of PCR products was determined by comparison with molecular weight marker 50 marker (50 to 500 bp) of biodynamics.

Data analysis

Mean values and standard errors for 11 phenotypic traits were calculated in 18 genotypes. The normal distribution of each trait was verified according to Shapiro–Wilk test (1965). Those traits without a normal distribution were transformed using the logarithmic function. Mean values were compared by one-way analysis of variance (ANOVA) and a Duncan test ($P < 0.05$) (Sokal and Rohlf 1967). With the aim of minimizing the total within-cluster variance and maximizing the between-cluster variance for these quantitative evaluations, the clustering method proposed by Ward (1963) was applied with the Euclidean distance, and the cophenetic correlation coefficient was obtained. The principal component analysis (PCA) was also applied on the standardized variables with the software Info-Gene (Balzarini and Di Renzo 2003).

SRAP markers

A range of variation of 500 to 150 bp bands was analysed in this assay. Each band was scored as present (1) or absent (0). In order to obtain the percentage of polymorphism (%P), the ratio number of polymorphic bands / number of total observed bands (TB) was calculated. The %P was first calculated by comparing the genotypes Cai and P with the F_1 (Cai × P) and then taking these patterns as reference, the five RILs were compared with 10 SCH. We also recorded the percentage of *de novo* bands (%*dN*B) as bands that were observed in F_1 (Cai × P) but not observed in any parental genotype (or vice versa, as absence). In a similar way, we also recorded *de novo* bands (*dN*B) as those that were present in RILs but not in the genotypes Cai, P and F_1 (Cai × P). Also, bands observed in the SCH that were absent in RILs and the genotypes Cai, P and F_1 (Cai × P) were considered *dN*B. Due to the dichotomic assessment of these variables, the Jaccard (1901) distances were calculated from the data matrix of presence/absence of bands for the five primer combinations in the 18 genotypes. Then, genotypes were grouped by the average linkage method which is particularly useful for cluster based on similarity coefficients. Principal coordinate analysis (PcoA) was applied in order to obtain Gower distance and to perform the following joint analysis (Gower 1966).

Joint analysis

A GPA was performed using Info-Gene (Balzarini and Di Renzo 2003) software. This method allows placing together the information about the morphological and molecular markers to obtain a consensus configuration from PCA and PcoA (Gower 1975). GPA has several advantages as it is a fairly straightforward approach to shape correspondence and also is a practical solution for very similar object alignment.

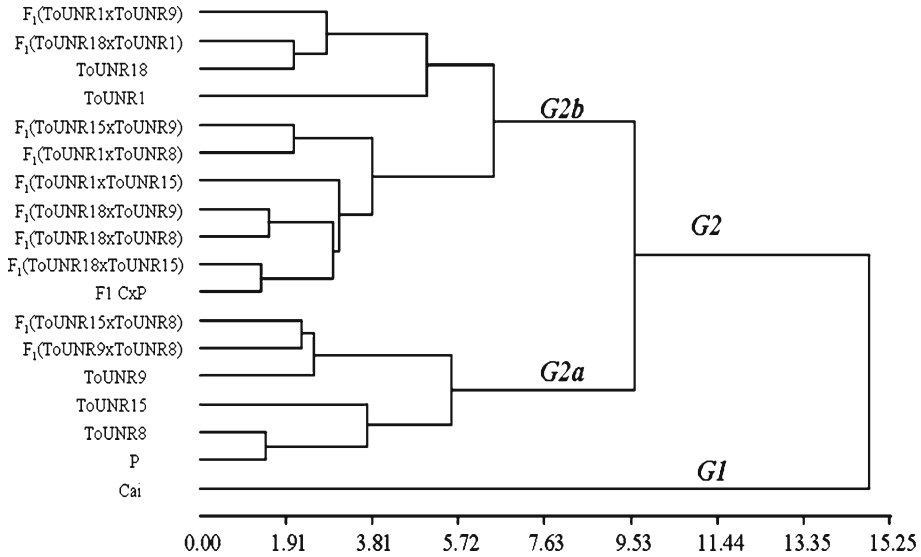

Figure 1. Cluster analysis of the parents *S. lycopersicum* cv. Caimanta (Cai) and *S. pimpinellifolium* LA722 (P), the F_1(CaixP), the five recombinant inbred lines (RILs) and the 10 second cycle hybrids (SCH) using 11 fruit quality traits: W, weight; SL, self life; H, height; D, diameter, L, reflectance; S, shape index; C, a/b index; FR, firmness; SS, soluble solids content; TA, titratable acidity; G1, G2, G2a and G2b, groups of genotypes according to cluster analysis.

Table 2. Principal components analysis of fruit quality traits in 18 genotypes of tomato.

	PC 1		PC 2	
Variable	Coefficient	Correlation	Coefficient	Correlation
W	0.39	0.94	$-4.8\ 10^{-3}$	−0.01
SL	−0.14	−0.34	0.39	0.54
H	0.39	0.95	0.16	0.21
D	0.40	0.97	0.09	0.13
S	−0.08	−0.19	0.46	0.64
L	0.31	0.75	−0.40	−0.55
C	−0.34	−0.82	0.17	0.23
FR	0.01	0.02	−0.44	−0.60
pH	0.39	0.94	0.03	0.04
SS	−0.23	−0.54	−0.34	−0.47
TA	−0.30	−0.72	−0.31	−0.43

Principal Component (PC1 and PC2) for the fruit quality traits; Weight (W), Shelf Life (SL), Height (H), Diameter (D), Shape index (S), Reflectance (L), a/b index (C), Firmness (FR), pH (pH), Soluble Solids content (SS) and Titratable Acidity (TA). Cophenetic correlation = 0.97; coefficient, contribution to the original variable to the PC; correlation, correlation coefficient between the original variable and the PC.

Results

Evaluation of the phenotypic traits

To characterize the 18 different genotypes phenotypically, the mean values and standard errors for the fruit quality traits of all genotypes were calculated (see table 1). All variables had a normal distribution except W ($w = 0.37$; $P < 0.001$), D ($w = 0.71$; $P < 0.001$) and H ($w = 0.70$; $P < 0.001$), consequently they were transformed to be included in ANOVA. Highly significant differences ($P < 0.0001$) between Cai and P genotypes, and between these genotypes and the F_1 (Cai × P), were found for all fruit quality traits. This hybrid genotype was intermediate respect to the parental genotypes excepting for the following traits: fruit SL, S, L, C, FR and pH. Moreover, there were also significant differences ($P < 0.0001$) among RILs for the majority of the traits. Figure 1 shows the cluster analysis of the 18 genotypes. The cophenetic correlation coefficient was 0.79. Further, the higher distance (14.8) was observed between Cai and the rest of the genotypes. At this distance two groups were defined: G1, which was integrated only by Cai and G2, was integrated by the remaining genotypes. The discriminatory traits at this clustering level were W ($F = 618.9$; $P < 0.0001$), H ($F = 64.4$; $P < 0.0001$), D ($F = 60.2$; $P < 0.0001$), L ($F = 20.2$; $P = 0.0004$), C ($F = 13.2$; $P = 0.0022$), and pH ($F = 24.0$; $P = 0.0002$). Three different clusters (G1, G2a and G2b) were defined at a distance of 9.62. In this case, the main discriminatory traits were SL ($F = 7.3$; $P < 0.006$) and TA ($F = 9.5$; $P < 0.0021$). On the other hand, the results obtained through the PCA showed that the first two components account for 70% of the total variation, PC_1 explaining 52.8% and PC_2 explaining 17.1%. The PC_1 was mainly explained by the following traits: W, H, D, pH and C, while PC_2 was by SL, S, and FR (table 2 ; figure 2). The cophenetic correlation was 0.97. As found in cluster analysis (see figure 1), Cai genotype was far away from the distribution of the rest of the genotypes, together with ToUNR1. Both these

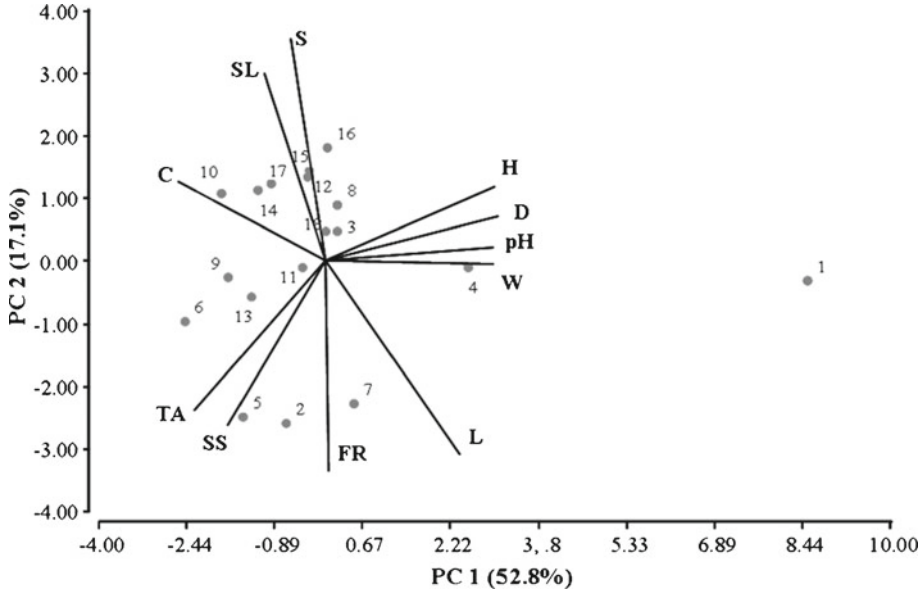

Figure 2. Biplot of principal component (PC) analysis of the parents *S. lycopersicum* cv. Caimanta (Cai) and *S. pimpinellifolium* LA722 (P), the F_1(Cai × P), the five RILs and the 10 SCH using 11 fruit quality traits (see figure 1 for traits). 1, Cai; 2, P; 3, F_1 (Cai × P); 4, ToUNR1; 5, ToUNR8; 6, ToUNR9; 7, ToUNR15; 8, ToUNR18; 9, F_1 (ToUNR9 × ToUNR8); 10, F_1 (ToUNR1 × ToUNR8); 11, F_1 (ToUNR1 × ToUNR9); 12, F_1 (ToUNR1 × ToUNR15); 13, F_1 (ToUNR15 × ToUNR8); 14, F_1 (ToUNR15 × ToUNR9); 15, F_1 (ToUNR18 × ToUNR1); 16, F_1 (ToUNR18 × ToUNR8); 17, F_1 (ToUNR18 × ToUNR9); 18, F_1 (ToUNR18 × ToUNR15).

Table 3. SRAP in 18 genotypes of tomato.

	Cai / P	F_1 (Cai × P)	RILs	SCH
TB	57	57	59	64
PB	19	25	36	42
*dN*B		6	5	5
%TP	33	44	61	66

Total number of Bands (TB), Polymorphic Bands (PB), *de novo* bands (*dN*B) and Total Percentage of Polymorphism (%TP) for the five combinations of primers in *S. lycopersicum* cv. Caimanta (Cai) and *S. pimpinellifolium* LA722 (P), the F_1(CaixP), the 5 Recombinant Inbred Lines (RILs) and the 10 Second Cycle Hybrids (SCH).

genotypes had the greatest inertia. Additionally, when the types of angles were analysed (see figure 2), a positive correlation of SL with S was found whereas there was no association with L and FR. Otherwise, W was associated with H, D, and pH but not with C. Neither SL nor W were associated with FR. According to these results and those showed in table 1, ToUNR1 was the genotype with the largest values for W, H, D, and pH when compared with the wild progenitor P. A similar situation occurs with ToUNR8 having the largest SS. A great number of SCH were associated with high values for SL, S, and C traits.

Evaluation of polymorphisms with SRAP markers

Profiles obtained for Cai and P genotypes where compared with the five primer combinations revealing that these genotypes showed 33% of polymorphisms over a total of 57 bands (table 3). Similar total number of bands were obtained for F_1 (Cai × P), but the number of polymorphic bands was higher than in the parental genotypes. An important fact was that the total %P was increased owing to the presence/absence of *dN*B. The TB for RILs and SCH genotypes were 59 and 64 bands respectively. The %P for SCH genotypes was double when compared with Cai and P genotypes (table 3). Again, the presence/absence of *dN*B produced this increase in molecular polymorphism. Both, RILs and SCH, had 8% of %*dN*B. Cluster analysis for SRAP markers had a cophenetic correlation of 0.90. Four different groups were identified at a distance of 0.58 (figure 3). Group B had higher number of genotypes, including P, four RILs, and those SCH for which ToUNR1 was a female parent, together with the hybrid F_1 (ToUNR18 × ToUNR8). The remaining SCH were in group C, while the F_1 (Cai × P) was in group A together with Cai genotype. Finally, ToUNR9 was alone in group D.

The PcoA for SRAP markers, using Gower distance showed that three principal coordinates were necessary to explain the variation detected by SRAP markers. These three coordinates account for 61% of the total variation, explaining the first one as 32% while the other two principal coordinates accounted for the 19% and 10% respectively.

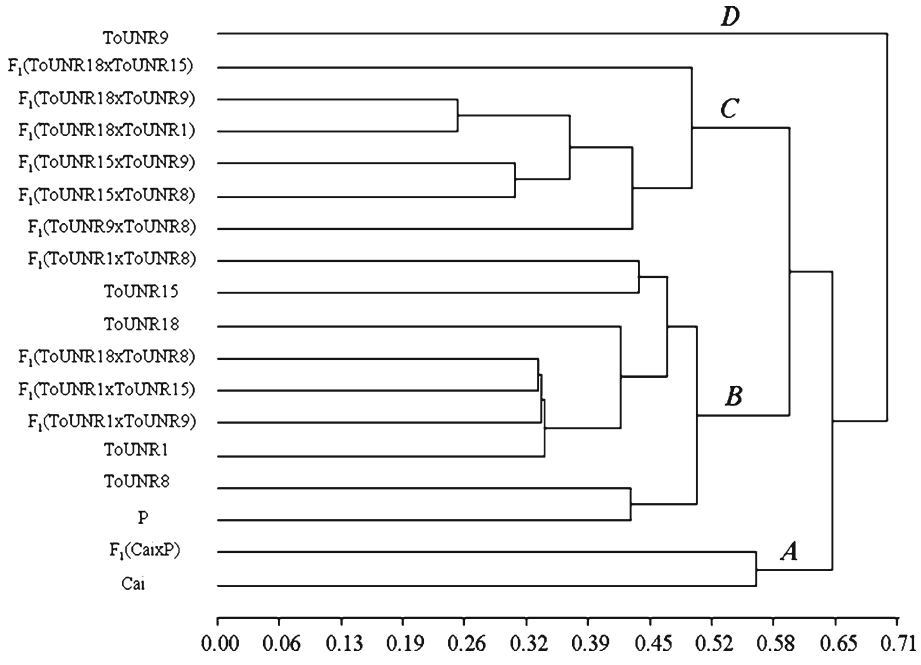

Figure 3. Cluster analysis of the parents *S. lycopersicum* cv. Caimanta (Cai) and *S. pimpinellifolium* LA722 (P), the hybrid F_1 (Cai × P), the five RILs and the 10 SCH using SRAP profiles generated from five primer combinations. A, B, C and D, groups of genotypes according to cluster analysis.

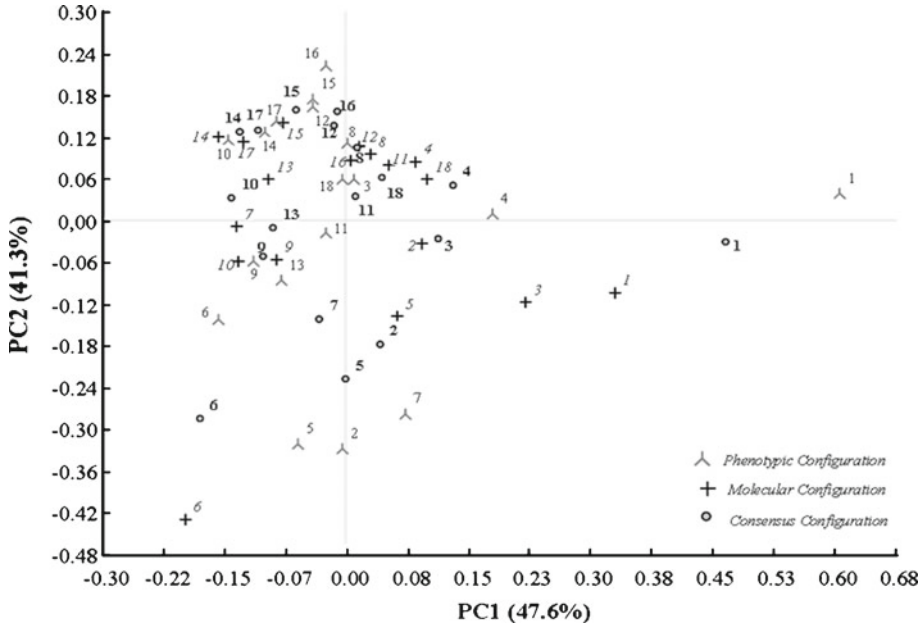

Figure 4. Biplot of *S. lycopersicum* cv. Caimanta (Cai) and *S. pimpinellifolium* LA722 (P), the F_1 (Cai × P), the five RILs and the 10 SCH in the plane formed by the first two axes of GPA. 1, Cai; 2, P; 3, F_1 (Cai × P); 4, ToUNR1; 5, ToUNR8; 6, ToUNR9; 7, ToUNR15; 8, ToUNR18; 9, F_1 (ToUNR9 × ToUNR8); 10, F_1 (ToUNR1 × ToUNR8); 11, F_1 (ToUNR1 × ToUNR9); 12, F_1 (ToUNR1 × ToUNR15); 13, F_1 (ToUNR15 × ToUNR8); 14, F_1 (ToUNR15 × ToUNR9); 15, F_1 (ToUNR18 × ToUNR1); 16, F_1 (ToUNR18 × ToUNR8); 17, F_1 (ToUNR18 × ToUNR9); 18, F_1 (ToUNR18 × ToUNR15).

Ten bands carried by the wild genotype LA722 mostly contributed to the molecular variability observed in this essay. These results and those obtained in the PCA for phenotypic traits were needed for the joint analysis. The aim of PGA was to find some agreement within the information obtained from the phenotypic and molecular data. In this analysis two components explained almost 89% of the total variance contributed by both phenotypic traits and molecular markers: CP_1 explains 47.6% while the CP_2 explained 41.3% (see figure 4). Table 4 shows the consensus values for each genotype among the phenotypic and molecular markers under study. The average consensus value between

Table 4. ANOVA of generalized procrustes analysis (GPA) to combine information from 11 phenotypic markers and five SRAP primer combinations in 18 genotypes of tomato.

Genotype	Consensus	Residual	Total	CP
Cai	0.452	0.053	0.505	0.895
P	0.070	0.049	0.119	0.591
F_1 (Cai x P)	0.029	0.038	0.067	0.428
ToUNR1	0.049	0.015	0.064	0.760
ToUNR8	0.106	0.025	0.131	0.811
ToUNR9	0.228	0.042	0.271	0.843
ToUNR15	0.056	0.071	0.127	0.441
ToUNR18	0.032	0.011	0.043	0.748
F_1 (ToUNR9 × ToUNR8)	0.052	0.027	0.079	0.659
F_1 (ToUNR1 × ToUNR8)	0.058	0.034	0.092	0.634
F_1 (ToUNR1 × ToUNR9)	0.008	0.013	0.021	0.375
F_1 (ToUNR1 × ToUNR15)	0.045	0.012	0.057	0.793
F_1 (ToUNR15 × ToUNR8)	0.031	0.026	0.058	0.544
F_1 (ToUNR15 × ToUNR9)	0.069	0.007	0.076	0.914
F_1 (ToUNR18 × ToUNR1)	0.064	0.008	0.071	0.890
F_1 (ToUNR18 × ToUNR8)	0.060	0.022	0.083	0.731
F_1 (ToUNR18 × ToUNR9)	0.061	0.007	0.068	0.895
F_1 (ToUNR18 × ToUNR15)	0.038	0.032	0.070	0.543
Total	1.508	0.492	2.000	0.754

S. lycopersicum cv. Caimanta (Cai) and *S. pimpinellifolium* LA722 (P), the F_1(CaixP), the five recombinant inbred lines (RILs) and the 10 second cycle hybrids (SCH). CP, consensus proportion.

phenotypic and molecular data (CP) was 75.4%. The F_1 (ToUNR15 × ToUNR9) was the genotype with highest level of consensus (91.4%), followed by Cai genotype, F_1 (ToUNR18 × ToUNR9) and F_1 (ToUNR18 × ToUNR1), while the lowest values were for the F_1 (ToUNR1 × ToUNR9) (37.5%) and ToUNR15 (44.1%). The remaining RILs had values higher than 70%.

Discussion

The divergent-antagonistic selection for fruit weight and SL performed during six generations gave 17 RILs significantly differing for phenotypic traits related to fruit quality. Parental RILs to be crossed in a diallel design were selected according to the classification performed by Rodriguez *et al.* (2006). In the present experiment we were interested in finding whether these new hybridizations (SCH) display new kind of variability for fruit quality traits. Moreover, our aim was to verify if this potential genetic variability is detected in similar degree through the phenotypic and molecular characterization.

Results in table 1 showed that SCH have a wide phenotypic variability in the expression of the 11 phenotypic traits evaluated in fruits. Further, we can see that the SCH showed traits with higher values than parental RILs. For example, the F_1 (ToUNR1 × ToUNR8) had the higher value for SL among all genotypes, even higher than the wild parental genotype. Also RILs, ToUNR1 and ToUNR18, had higher SL than the initial parents of this new genetic background (Cai and P genotypes). RILs showed a greater phenotypic variability than SCH for the W trait. The genotypes ToUNR1 and ToUNR18 are those with greater W and they are even higher than F_1 (Cai × P). As Tanksley (1993) has pointed out, such an effect could be due to the dominance effect of the wild genes for fruit weight. For FR trait, although a great majority of RILs have similar values than P genotype, the SCH displayed a wide range of values. In addition, in the set of SCH, there were FR mean values significantly higher than those of Cai and P, these new genotypes becoming a wide phenotypic variation for that trait. With respect to colour, the hybrid F_1 (ToUNR15 × ToUNR8) was different to the other SCH and similar to the best RIL, ToUNR9. Finally, ToUNR8 had the highest SS content, that was even superior to the wild progenitor P. Such differences are a strong evidence of a wide phenotypic variability among these new hybridizations.

Cluster analysis showed the similarity of the wild progenitor P, three RILs (ToUNR8, ToUNR9 and ToUNR15) and two of the SCH (F_1 (ToUNR15 × ToUNR8) and F_1 (ToUNR9 × ToUNR8)). This analysis showed that RILs grouped with P, i.e., three of these five new genotypes were more similar to the wild progenitor rather than to the cultivated one. Also, the hybrid F_1 (Cai × P) grouped with the other two RILs and the remaining eight SCH. It should be noted that these results obtained through the cluster analysis are consistent with those phenotypic values presented in table 1.

Molecular analysis appears to corroborate that SCH have preserved ADN fragments from this wild genotype of *S. pimpinellifolium*. The degree of polymorphism expressed by SRAP showed that 10 bands of the wild genotype P were detected in most of the SCH genotypes (C1, 419pb, 383pb, 316pb, 287pb, 216pb, 194pb; C2, 240pb C3, 175pb; C4, 236pb, 234pb). This could be evidence of the high degree of variability that wild genotype contributed to this new genetic background. The variability generated in the first cross between Cai and P was possibly preserved through the divergent-antagonistic selection process performed by Rodriguez *et al.* (2006) to obtain these RILs. Since SRAP markers would detect coding sequence polymorphisms, these results could provide relevant information about regions from *S. pimpinellifolium* that are possible source of variability for fruit quality traits. Cluster analysis using these molecular markers complemented the information provided by SRAP. In fact this analysis showed that 50% of the 18 genotypes are close to the wild genotype according to their level of polymorphism. Cluster analysis from phenotypic data included P and the interspecific F_1 with all RILs and SCH, but cluster analysis from molecular data grouped the interspecific F_1 with Cai though preserved the association of RILs and SCH with P, excepted by ToUNR9. Although using other distance measures and/or clustering methods might identify an entirely different clustering pattern, genetic prepotency (i.e. the ability of one parent to transmit more characteristics to its offspring than the other parent) of P genome is clearly noticeable from both phenotypic and molecular results.

Finally, we used a method that allows grouping genotypes as well as identifying them as associated in similar but not identical manners. GPA allowed obtaining the consensus matrices of a different nature to achieve a single configuration. According to Gower (1975), the quantification of a possible consensus provides information on appropriateness of the configurations produced by each set of variables (morphological and molecular) in a genetic background. Bramardi *et al.* (2005) showed the usefulness of the GPA, using as a model 34 accessions of cucumber characterized by 16 quantitative and qualitative variables with RAPD markers. This analysis has demonstrated the ability of the GPA to characterize simultaneously a set of accessions with heterogeneous characters. These authors pointed out that GPA is a suitable tool for the characterization of germplasm. In our data, we obtained an overall estimated value equal to 75% (see table 4) indicating that the correlation between the variation of phenotypic values and polymorphisms detected by SRAP markers are in good agreement for characterizing these genotypes. Data obtained among the SCH genotypes have shown a wider variability of the consensus estimation provided by GPA. Perhaps one of the possible causes could be the highest percentage of polymorphism detected in these genotypes due to *dNB*. The overall estimated value of 75% suggests that there is a strong consensus between the order obtained by SRAP markers and these

morphological traits chosen to assess fruit quality traits in this genetic background. Baranger *et al.* (2004) have suggested that when there is a high correlation between molecular and morphological diversity, it would be attributable to the linkage disequilibrium between molecular markers and those genes that control morphological traits. Hence, the SRAP detected here could be linked to some tomato fruit quality traits.

In conclusion, SRAP markers were suitable for detecting polymorphisms both preexisting and *de novo* in this novel genetic background. These preexisting bands persisted even in SCH. The GPA allowed verifying the consensus between the variability of phenotypic traits and molecular markers to characterize this germplasm. The most remarkable observation in this experiment is the substantial preservation of fragments from the wild genotype, which increased the variability in fruit quality traits in populations whose genetic diversity is limited.

References

Balzarini M. and Di Renzo J. 2003 Info-Gen: Software para análisis estadísticos de datos genéticos. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba. Argentina.

Baranger A., Aubert G., Arnau G., Lainé A. L., Deniot G., Potier J. *et al.* 2004 Genetic diversity within *Pisum sativum* using protein and PCR-based markers. *Theor. Appl. Genet.* **108**, 1309–1321.

Bramardi S. J., Bernet G. P., Asíns M. J. and Carbonell E. A. 2005 Simultaneous agronomic and molecular characterization of genotypes via the generalised procrustes analysis: an application to cucumber. *Crop Sci.* **45**, 1603–1609.

Bredemeijer G. M. M., Arens P., Wouters D., Visser D. and Vosman B. 1998 The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification. *Theor. Appl. Genet.* **97**, 584–590.

Cravero V., Martin E. and Cointry E. 2007 Genetic diversity in *Cynara cardunculus* determined by sequence-related amplified polymorphism markers. *J. Am. Soc. Hort. Sci.* **132**, 208–212.

Ferriol M., Picó B. and Nuez F. 2004 Morphological and molecular diversity of collection of genetic diversity of a collection of *Cucurbita maxima* landraces. *J. Am. Soc. Hort. Sci.* **129**, 60–69.

Foolad R. 2007 Genome mapping and molecular breeding of tomato. *Int. J. Plant Genome* **27**, 1–52.

Gower J. C. 1966 Some distance properties of latent root and vector methods in multivariate analysis. *Biometrika* **53**, 315–328.

Gower J. C. 1975 Generalised procrustes analysis. *Psychometrika* **40**, 33–51.

Grandillo S. and Tanksley S. D. 1996a QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium*. *Theor. Appl. Genet.* **92**, 935–951.

Grandillo S. and Tanksley S. D. 1996b Genetic analysis of RFLPs, GATA microsatellites and RAPDs in a cross between *L. esculentum* and *L. pimpinellifolium*. *Theor. Appl. Genet.* **92**, 957–965.

Griffing B. 1956 Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* **9**, 463–493.

Gulsen O., Suleyman K. and Kazim A. 2007 Diversity and relationships among *Turkish okra* germplasm by SRAP and phenotypic marker polymorphism. *Biol. Brat.* **62**, 41–45.

He C., Poysa V. and Yu K. 2003 Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. *Theor. Appl. Genet.* **106**, 363–373.

Jaccard P. 1901 Étude comparative de la distribution florale dans une portion des Alpes et du Jura. *Bull. Soc. Vaud. Sci. Nat.* **37**, 547–579.

Kearsey M. J. and Pooni H. S. 1996 *The genetical analysis of quantitative traits*. Chapman and Hall, London, UK.

Li G., Gao M., Yang B. and Quiros C. F. 2003 Gene for gene alignment between the *Brassica* and *Arabidopsis* genomes by direct transcriptome mapping. *Theor. Appl. Genet.* **107**, 168–180.

Li G. and Quiros C. F. 2001 Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor. Appl. Genet.* **103**, 455–461.

Marchionni Basté E., Liberatti D. R., Mahuad S. L., Rodriguez G. R., Pratta G. R., Zorzoli R. and Picardi L. A. 2010 Diallel analysis for fruit traits among tomato recombinant inbred lines derived from an interspecific cross *Solanum lycopersicum* × *S. pimpinellifolium*. *J. Appl. Hort.* **12**, 21–25.

Monforte A. J. and Tanksley S. D. 2000 Development of a set of near isogenic and backcross recombinant inbread lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: a tool for gene mapping and gene discovery. *Genome* **43**, 803–813.

Nuez F. 1991 La mejora genética de hortalizas. In *La horticultura española en la comunidad Europea* (ed. L. Rallo and F. Nuez), pp. 483–505. Sociedad Española de Ciencias Hortícolas, Madrid, Spain.

Pratta G. R., Rodriguez G. R., Zorzoli R., Valle E. M. and Picardi L. A. 2011 Phenotypic and molecular characterization of selected tomato recombinant inbred lines derived from the cross *Solanum lycopersicum* × *S. pimpinellifolium*. *J. Genet.* **90**, 229–237.

Rodriguez G., Pratta G. R., Zorzoli R. and Picardi L. A. 2006 Recombinant lines obtained from an interspecific cross between *Lycopersicon* species selected by fruit weight and fruit shelf life. *J. Am. Soc. Hort. Sci.* **131**, 561–656.

Ruiz J. J., García-Martínez S., Picó B., Gao M. and Quiros C. F. 2005 Genetic variability and relationship of closely related Spanish traditional varieties of tomato as detected by SRAP and SSR markers. *J. Am. Soc. Hort. Sci.* **130**, 88–94.

Schuelter A. R., Finger F. L., Casali V. W. D., Brommonschenkel S. H. and Otoni W. C. 2002 Inheritance and genetic linkage analysis of a firm-ripening tomato mutant. *Plant Breeding* **121**, 338–342.

Shapiro S. S. and Wilk M. B. 1965 An analysis of variance test for normality (complete samples). *Biometrika* **52**, 591–611.

Smulders M. J. M., Bredemeijer G., Rus-Kortekaas W., Arens P. and Vosman B. 1997 Use of short microsatellites from database sequences to generate polymorphisms among *L esculentum* cultivars and accessions of other *Lycopersicon* species. *Theor. Appl. Genet.* **97**, 264–272.

Sokal R. R. and Rohlf F. J. 1967 *Biometry*. W. H. Freeman, San Francisco, USA.

Tanksley S. D. 1993 Mapping polygenes. *Ann. Rev. Genet.* **27**, 205–233.

Ward J. H. 1963 Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* **58**, 236.

Received 30 May 2012, in final revised form 19 February 2013; accepted 22 February 2013
Published on the Web: 18 July 2013