RESEARCH ARTICLE

Phenotypic and molecular characterization of selected tomato recombinant inbred lines derived from the cross *Solanum lycopersicum* × *S. pimpinellifolium*

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Abstract

An important trait defining fresh tomato marketability is fruit shelf life. Exotic germplasm of *Solanum pimpinellifolium* is able to prolong shelf life. Sixteen recombinant inbred lines with differing values of shelf life and fruit weight were derived by antagonistic-divergent selection from an interspecific cross involving *Solanum pimpinellifolium*. The objective of this study was to evaluate these recombinant inbred lines for many fruit quality traits such as diameter, height, size, acidity, colour, firmness, shelf life and weight, and to characterize them by amplified fragment length polymorphism markers. For most traits, a wide range of genetic variability was found and a wide range of molecular variation was also detected. Both sets of data allowed the identification of recombinant inbred lines by means of cluster analysis and principal component analysis. Genetic association among some amplified fragment length polymorphism markers and fruit quality traits, suggested by the principal component analysis, could be identified by single point analysis. Potential molecular markers underlying agronomical traits were detected in these recombinant inbred lines.

[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]

Introduction

Plant breeding applied to tomato (*Solanum lycopersicum*) has produced high-yielding varieties, though little attention has been paid to the fruit quality (Foolad 2007). Fruit shelf life (SL) is a ripening-associated trait influencing fresh tomato marketability (Schuelter *et al.* 2002). Tomato has been engineered to produce long SL fruits, but genetically modified crops are not yet well accepted by consumers. Several spontaneous ripening mutants were described in tomato, such as *rin* (*ripening inhibitor*), *nor* (*nonripening*) and *alc (alcobaca)* (Giovannoni 2004) that, however, also provoke pleiotropic effects on other ripening traits such as flavour, colour and texture, reducing fruit quality.

Wild species of *Lycopersicon* have been included in tomato breeding programmes as a source of disease resistance and environmental stress tolerance genes. Nevertheless, they could have valuable genetic variability for fruit quality traits (Zorzoli *et al.* 2000; Galiana-Balaguer *et al.* 2006; Rodriguez *et al.* 2006). Among them, *S. pimpinellifolium* produces small and high nourishing quality fruits, and easily crosses with the cultivated tomato (Zuriaga *et al.* 2009). Pratta *et al.* (1996) evaluated different *S. pimpinellifolium* accessions for the fruit SL, and selected one of them (LA722 from Tomato Genetic Resources Center, Davis, USA) because of its long SL. Zorzoli *et al.* (1998) crossed that accession to a standard Argentinian variety of *S. lycopersicum* (cv Caimanta, from Instituto Nacional de Tecnología Agropecuaria, Cerrillos, Salta, Argentina) and obtained a hybrid with a longer SL than the cultivated parent and other commercial varieties. This hybrid was similar to the wild parent and even to heterozygotes for the *nor* and *rin* genes, respectively, but its

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Keywords. plant breeding; plant genetic resources; AFLP; fruit shelf life; principal component analysis.

fruits had adequate quality attributes. So, exploiting these exotic genes could be an alternative to improve SL without diminishing fruit quality.

The goal of this research was to characterize 16 selected tomato RILs obtained through a breeding programme on the segregating generation of the cross (Caimanta × LA722) according to the genealogic method and selecting by both fruit SL and weight (W) (Zorzoli *et al.* 2000; Rodriguez *et al.* 2006). We were particularly interested in grouping these RILs according to their fruit quality traits and amplified fragment length polymorphism (AFLP) profiles, and to detect putative associations among the phenotypic and molecular data in this original genetic material. Also, we attempted to test the usefulness of multivariate analysis in identifying such associations.

Materials and methods

Phenotypic characterization

Sixteen tomato RILs (which represent the F_7 generation by selfing) derived from an interspecific hybrid between Caimanta (*S. lycopersicum*) and LA722 (*S. pimpinellifolium*) by five cycles of antagonistic and divergent selection for fruit SL and W (Zorzoli *et al.* 2000; Rodriguez *et al.* 2006) were characterized for a series of agronomic traits. The antagonistic-divergent selection began at the F_2 segregating generation and continued till F_6; in each cycle, plants having high W and long SL, low W and long SL, low W and short SL and high W and short SL were chosen to produce the next generation. Field assays were conducted at the Experimental Station 'José F. Villarino' (33°S lat. and 61°W long., Universidad Nacional de Rosario, Argentina). Seeds of the 16 RILs and their parents were germinated in seedling trays until end of June and transplanted to the field after a month in a completely randomized design.

The total number of plants was 360, with a mean of 20 plants per genotype (RILs and parents, which were the experimental testers). Mean number of flowers per cluster (FC) was evaluated on each plant as the average of flowers in the first three clusters. The total number of harvested fruits was 6072, approximately 17 fruits per plant. Fruit traits evaluated were: weight (W, in g), diameter (D, in cm) and height (H, in cm) at the moment of harvest in the breaker stage; SL, in days from harvest at the breaker stage to the beginning of fruit softening (Schuelter *et al.* 2002); shape index, as the H/D ratio; soluble solids content (SS, in Brix degrees), as the percentage of glucose and fructose in the homogenized juice from ripe fruits, measured with a hand refractometer SO-RH®; pH, in 100 g of the homogenized juice from ripe fruits diluted in 100 mL of distilled water; titrable acidity (TA, in g of citric acid per 100 g of the homogenized juice from ripe fruits); colour: the reflectance percentage (L) and the absorbency index (a/b ratio, where a is the absorbency at 540 nm and b at 675 nm) were assessed with a standard chromameter as the average of three measures per ripe fruit; firmness (F): measured with a Shore A (Durofel, Forges-Les-Eaux, France) equipment on two opposite sides of ripe fruits.

Molecular characterization

After transplanting to the field, young leaves were harvested from three random plants of each RIL and parents. Genomic DNA was extracted using a commercial kit. AFLP profiles were generated according to the standard protocol (Blears *et al.* 1998) with minor changes. DNA (0.375 μg) was digested with 1.25 units of *Eco*RI and 2 units of *Mse*I in a final volume of 12.5 μL incubated at 37°C for 2 h. Ligation of the digested fragments to the specific double strand sequences 5′-GACTGCGTACCAATTC-3′ (*Eco*RI adapter, final concentration 0.025 μM), and 5′-GATGAGTCCTGAGTAA-3′ (*Mse*I adapter, final concentration 0.25 μM) was achieved by incubation at 37°C for 2 h with 0.75 units of T4 ligase in a final volume of 15 μL. The digested–ligated solution was 1:5 diluted in sterile water. The preamplification samples were prepared using 5 μL of this dilution plus 75 ng of each primer+1 (*Eco*RI+1: 5′-GACTGCGTACCAATTCA-3′ and *Mse*I+1: 5′-GATGAGTCCTGAGTAAC-3′), 0.2 mM dNTPs and 1 unit of *Taq* polymerase in a final volume of 25 μL. The PCR conditions were 30 cycles of 30 s at 94°C, 1 min at 56°C and 1 min at 72°C each one. Once preamplified, the solution was 1:10 diluted in sterile water. The selective amplification samples were prepared using 5 μL of this dilution and the same components mentioned earlier. Three primer +3 combinations reported by Pratta *et al.* (2006) were used: A combination: 5′-GACTGCGTACCAATTCAGA-3′ / 5′-GATGAGTCCTGAGTAACTA-3′; B combination: 5′-GACTGCGTACCAATTCAGC-3′ / 5′-GATGAGTCCTGAGTAACAT-3′; N combination: 5′-GACTGCGTACCAATTCATC-3′ / 5′-GATGAGTCCTGAGTAACAT-3′.

Also, a touchdown PCR was used, starting with a cycle of 30 s at 94°C, 30 s at 65°C and 1 min at 72°C. During the next 10 cycles, the annealing temperature was reduced to 1°C per cycle, until reaching 56°C. Then, 23 cycles of 30 s at 94°C, 1 min at 56°C and 1 min at 72°C each were repeated. The amplified fragments were boiled for 3 min and loaded into a 6% poly-acrylamide denaturing gel. The same AFLP protocol, except by the selective amplification step, was applied to the DNA of λ phage, which was then loaded as a molecular weight marker. The gel was stained with the silver nitrate technique.

Data analysis

The normality of each trait distribution was verified by the Shapiro–Wilk's test (Shapiro and Wilk 1965). Mean values for all traits among genotypes (RILs and parents) were compared using Duncan test and the narrow sense heritability (h^2) was calculated from the mean square terms of the analysis of variance (ANOVA) including only 16 RILs (Kearsey and Pooni 1996). Quantitative phenotypic traits

were used to group RILs and parents on a dendrogram based on the Euclidean distances. The variables were also subjected to principal components analysis (PCA), with the aim of visualizing the magnitude and the structure of the phenotypic variation in the RILs set (Chatfield and Collins 1986).

For molecular characterization, the AFLP profiles were first compared among plants within genotypes (RILs and parents). For each primer combination, the total number of amplified fragments, number of polymorphic fragments and polymorphism percentage were calculated, the polymorphism being expressed as the presence or absence of a given fragment. Polymorphic fragments present in a given genotype were assigned 1 and those absent were assigned 0. This dichotomous variable was used to calculate the Jaccard's distance (Jaccard 1901) and to construct a dendrogram with the aim of evaluating the genetic relations among genotypes. The χ^2-test was used to verify 1:1 Mendelian segregation of each polymorphic AFLP, and those fragments showing a distorted segregation were discarded in posterior analysis (Kearsey and Pooni 1996). For each combination of pairs of fragments segregating 1:1, the χ^2 test was applied to prove the hypothesis of independent segregation 1:1:1:1. Fragments showing the expected segregation (1:1 and independent) were used in the PCA with the aim of visualizing the magnitude and structure of the genotypic variation in the RILs set (Roldán-Ruiz *et al.* 2001).

Associations among the polymorphic fragments showing the expected segregation and the phenotypic traits were detected by ANOVA single point analysis to identify molecular markers for the traits. The presence or absence of each polymorphic fragment was the source of variations and a significant level of $P < 0.01$ was considered to mark significant differences among the mean value of all traits (Dholakia *et al.* 2003). The R^2 value was used to evaluate the percentage of phenotypic variance for each trait that may be explained by the effect of the fragment. Then a factorial ANOVA considering all combinations of pairs of independent fragments associated to the same phenotypic trait was used to detect the putative interloci interactions among fragments that might underlie this trait. An epistasis effect was assumed when interaction was statistically significant while the lack of statistical significance was considered as an indicator of additivity. Fragments associated with more than one trait were accounted as an indicator of a pleiotropic effect (Hua *et al.* 2003).

Results and discussion

Phenotypic characterization

The mean values of all evaluated traits and their corresponding h^2 (narrow sense heritability) are provided in table 1. All traits have a normal distribution according to Shapiro–Wilk's test ($W > 0.95$, nonsignificant). Differences among genotypes were highly significant for all traits ($P < 0.01$).

Although none of the RILs had W similar to 'Caimanta', significant differences among them were found. Taking into account that the mean value of all RILs was W = 10.82 g, RILs 1, 3, 4, 14, 15, 16 and 18 were classified as high weighted and RILs 6, 7, 8, 9, 10, 11, 12 and 13 as low weighted. RIL 5 had an intermediate value, very close to that of F_1 (Caimanta × LA722), whose mean value across six cycles of evaluation was 9.16 g according to Rodriguez *et al.* (2006). With respect to SL, it was possible to identify three different groups: I- RILs 5, 6, 8 and 18 displayed values higher than both parents; II- RILs 1, 3, 4, 7, 9, 10, 11, 12, 13, 15 and 16 displayed SL values between parent values, and III- RIL 14, displayed values lower than both parents. The I and III groups clearly represent fixed transgressive variants in the sense reported by de Vicente and Tanksley (1993) because RILs 5, 6, 8 and 18 have SL mean values higher than LA722 (the tester of longest SL), and RIL 14 has a SL mean value shorter than the shortest tester 'Caimanta'. According to de Vicente and Tanksley (1993), transgression could be due to heterosis, which should not be probable in this case owing to the high level of homozygosity expected in the RILs, or to the presence in both parents of alleles increasing and decreasing the trait value that recombine during the selfing and selection cycles, which appears to be more probable in this case. The SL mean value of all RILs was 18.58 days, very similar to the mean value across six cycles of evaluation of F_1 generation, 19.34 days, as reported by Rodriguez *et al.* (2006). The remaining traits, that were not target of selection in the experiments reported by Rodriguez *et al.* (2006), also showed a wide range of genetic variability (see h^2 values in table 1), and RILs transgressive for TA, L, and F were obtained. In consequence, the antagonistic and divergent selection applied on these RILs resulted in a set of genotypes highly discrepant for W, SL and other agronomic traits. Some of them are highly promising for agronomic production, such as RILs 1 and 18, since they present favourable combinations of fruit quality traits.

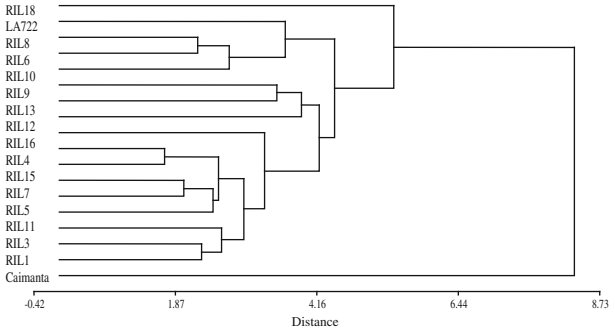
Figure 1a shows the cluster of all genotypes under analysis according to the phenotypic traits, where Caimanta is the most divergent one. The other tester, LA722, grouped in the opposite side of the cluster and most RILs were among both parents. Exception was RIL 18, which grouped alone probably because it was the only RIL having an ovate shape. There are many reports that in interspecific crosses, phenotypes of the recombinant generations are very similar to that of the wild parent (Bernacchi *et al.* 1998; Lecomte *et al.* 2004; Rodriguez *et al.* 2005). The multivariate analysis (table 2) showed that the first two principal components explained 62% of total phenotypic variability, PC1 and PC2 accounting for 41% and 21%, respectively. The traits having a main relative contribution to PC1 were W, D, H, TA and FC. Traits F, pH, and a/b had a moderate relative contribution. For PC2, traits H/D and L were the largest relative contribution. FC, pH and a/b had a moderate relative contribution. Traits SL and SS did not associate with any of these two principal components, which indicates that their contribution to the

Table 1. Mean values of 16 tomato RILs and their parents (cv. Caimanta and LA722, the experimental testers).

Genotype		Traits											
		W	SL	D	H	H/D	SS	pH	TA	L	a/b	F	FC
Caimanta		110.23^{a}	14.23^{de}	6.12^{a}	4.79^{a}	0.76^{e}	4.26^{e}	5.38^{a}	0.43^{f}	39.76^{g}	0.82^{c}	52.67^{d}	5.15^{h}
LA722		0.98^{h}	20.24^{bc}	1.01^{f}	0.90^{g}	0.90^{bc}	6.61^{a}	5.11^{c}	0.85^{c}	42.36^{d}	1.01^{ab}	53.45^{cd}	12.13^{a}
RILs	L1	27.24^{b}	17.81^{bcd}	3.76^{b}	3.19^{bc}	0.85^{d}	4.51^{d}	5.28^{b}	0.60^{e}	43.93^{b}	0.88^{bc}	56.11^{ab}	6,13^{g}
	L3	20.99^{c}	18.28^{bcd}	3.52^{b}	2.84^{bc}	0.82^{de}	4.86^{cd}	5.06^{cd}	0.55^{e}	42.86^{c}	1.02^{ab}	55.16^{c}	6,47^{g}
	L4	18.82^{c}	15.84^{cd}	3.24^{bc}	2.94^{bc}	0.91^{bc}	5.56^{b}	5.10^{c}	0.57^{e}	–	–	–	6.37^{g}
	L5	8.34^{d}	23.51^{b}	2.39^{d}	2.09^{d}	0.88^{cd}	5.11^{c}	5.13^{c}	0.74^{d}	42.38^{ad}	0.89^{bc}	50.25^{e}	7,00^{fg}
	L6	1.98^{g}	21.83^{b}	1.47^{e}	1.28^{f}	0.88^{cd}	4.96^{c}	4.85^{de}	0.93^{c}	41.82^{de}	1.07^{a}	54.50^{c}	9.96^{cd}
	L7	4.08^{e}	16.33^{cd}	1.84^{de}	1.64^{e}	0.90^{bc}	4.56^{d}	5.20^{bc}	0.54^{e}	41.63^{e}	0.95^{b}	56.06^{b}	7.08^{fg}
	L8	1.98^{g}	31.11^{a}	1.43^{e}	1.31^{f}	0.92^{b}	5.51^{b}	5.02^{d}	1.18^{b}	42.23^{d}	1.02^{ab}	49.70^{e}	9,87^{cd}
	L9	2.13^{g}	16.60^{cd}	1.47^{e}	1.34^{f}	0.92^{b}	3.79^{f}	5.14^{bc}	1.15^{b}	43.90^{b}	0.93^{ab}	47.64^{ef}	11.36^{b}
	L10	4.02^{e}	20.97^{bc}	1.83^{de}	1.63^{e}	0.90^{bc}	5.44^{bc}	5.17^{bc}	1.28^{a}	42.99^{c}	1.01^{ab}	52.59^{d}	9,52^{d}
	L11	3.20^{f}	18.04^{bcd}	1.73^{de}	1.51^{e}	0.87^{cd}	5.26^{bc}	4.95^{d}	0.72^{d}	42.01^{de}	1.05^{a}	56.85^{ab}	7.92^{ef}
	L12	3.23^{f}	16.29^{cd}	1.77^{de}	1.59^{e}	0.91^{bc}	5.26^{bc}	5.07^{cd}	0.74^{d}	44.47^{ab}	0.95^{b}	38.50^{g}	8.17^{ef}
	L13	3.26^{f}	14.47^{de}	1.69^{de}	1.58^{e}	0.94^{b}	5.11^{c}	5.24^{b}	0.80^{c}	41.96^{de}	1.13^{a}	49.00^{e}	10.50^{c}
	L14	25.77^{b}	11.42^{f}	3.81^{b}	2.95^{bc}	0.78^{e}	4.71^{cd}	5.24^{b}	0.57^{e}	42.41^{d}	1.05^{a}	54.54^{c}	7.48^{f}
	L15	19.15^{c}	14.95^{d}	3.33^{bc}	2.74^{c}	0.83^{de}	5.21^{bc}	5.34^{ab}	0.63^{e}	40.88^{f}	1.00^{ab}	50.41^{e}	6.38^{g}
	L16	16.96^{c}	16.96^{cde}	3.19^{bc}	2.68^{c}	0.85^{d}	4.81^{cd}	4.97^{d}	0.82^{c}	44.06^{ab}	0.78^{d}	57.32^{ab}	9.36^{d}
	L18	18.15^{c}	22.97^{b}	3.09^{c}	3.34^{b}	1.10^{a}	5.01^{c}	5.28^{b}	0.57^{e}	45.85^{a}	0.84^{c}	59.77^{a}	8.50^{e}
h^2		0.76	0.20	0.83	0.82	0.72	0.33	0.50	0.50	0.64	0.66	0.42	0.40

h^2, Narrow sense heritability; –, missing data. Different letters indicate significant differences ($P < 0.01$) among genotypes according to Duncan test. W, weight (in g); SL, shelf life (in days); D, diameter (in cm); H, height (in cm); H/D, shape (ratio); SS, soluble solids content (in Brix degrees); pH; TA, titrable acidity (in g of citric acid per 100 g of homogeneized juice); L, reflectance percentage; a/b, absorbency index; F, firmness; FC, number of flowers per cluster.

a. Euclidean distances calculated from mean values of quantitative traits.



b. Jaccard distances calculated from presence/absence of AFLP.

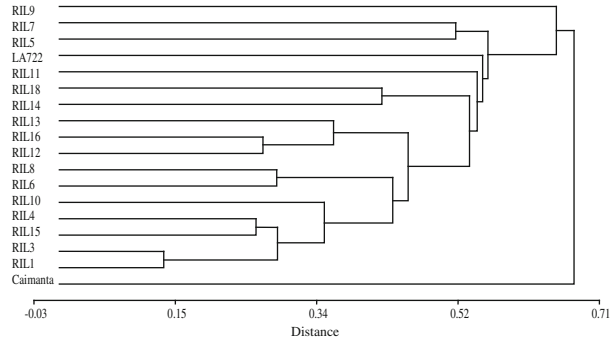


Figure 1. Associations of RILs and their parents according to the dendrograms constructed by the UPGMA method.

general phenotypic variability was small. This fact is also verified by the grouping of RILs and traits in the coordinates delimited by PC1 and PC2 (figure 2a), since the vectors corresponding to SL and SS are those of the shortest magnitude. On the other hand, there is a clear distribution of RILs in the direction of PC1 according to their W.

Molecular characterization

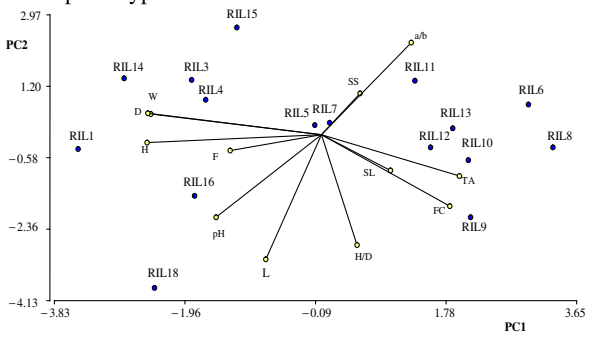
Amplifications of the three primer combinations revealed a total of 97 fragments, of which 70 (72%) were polymorphic among the characterized RILs. This percentage of polymorphism was larger to that obtained just among parents (about 21%). In fact, fragments that are not differing among parents were polymorphic among RILs, and also it was possible to detect new polymorphic fragments, which were absent in both parents. Many authors reported a *de novo* performance of DNA fragments in interspecific crosses. For instance, Shaked *et al.* (2001) found new AFLP markers in interspecific crosses of wheat, and Marfil *et al.* (2006) also detected new RAPD and AFLP markers in interspecific hybrids of potatoes, a crop that has a high degree of homosequentiality and is very close to tomatoes. A different degree of DNA methylation in the hybrid genome with respect to the parents was proposed as the possible cause of the *de novo* performance.

Each fragment was identified with a letter corresponding to primer combination and a number indicating the relative position in the gel. The number of amplified fragments per primer combination was 29 with A combination, 40 with B combination, and 28 with N combination. Within genotypes, as it was expected, there was no difference among the three plants assayed, so that they had high repeatability, the frequency of each polymorphic fragment being in fact 1 or 0 in coincidence with the dichotomous values that were assigned a *priori*. The dendrogram obtained using Jaccard's distances is shown in figure 1b. Jaccard's distances among RILs had a minimum value of 0.14 (distance between RIL1 and RIL3) and a maximum value of 0.69 (distances between RIL12 and RIL9, RIL13 and RIL9, and RIL15 and RIL9, respectively), the mean value being 0.51. RIL9 was in an average the most distant RIL with respect to the rest of the RILs, although the greatest distance (0.88) was that between parents. The dendrogram had a cophenetic correlation of 0.92, and it had a

Table 2. Coefficient of the first two principal components (PC1 and PC2), proportion of explained variance and correlation with the fruit traits.

Traits	PC1		PC2	
	Coefficient	Correlation	Coefficient	Correlation
Weight (W)	−0.42	−0.93	0.09	0.14
Shelf life (SL)	0.17	0.38	−0.16	−0.25
Diameter (D)	−0.43	−0.96	0.09	0.14
Height (H)	−0.43	−0.96	−0.04	−0.06
Shape (H/D)	0.09	0.20	−0.48	−0.76
Soluble solids content (SS)	0.09	0.20	0.18	0.28
pH	−0.26	−0.58	−0.36	−0.57
Titrable acidity (TA)	0.34	0.76	−0.18	−0.28
Firmness (F)	−0.23	−0.51	−0.07	−0.11
Reflectance percentage (L)	−0.14	−0.31	−0.54	−0.85
Absorbency index (a/b)	0.22	0.49	0.40	0.63
Number of flowers per cluster (FC)	0.32	0.71	−0.31	−0.49
λ (proportion of explained variance)	0.41		0.21	

a. Principal components calculated with mean values of phenotypic traits.



b. Principal components calculated with presence/absence of Mendelian segregant AFLP.

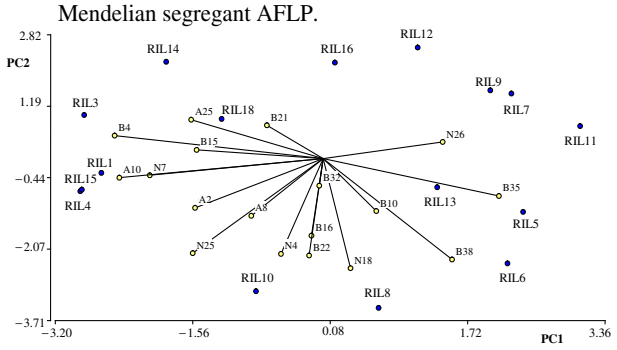


Figure 2. Association of 16 tomato RILs according to the plane defined by the two first principal components (PC1 and PC2). Fruit traits: W, weight (in g); SL, shelf life (in days); D, diameter (in cm); H, height (in cm); H/D, shape (ratio); SS, soluble solids content (in Brix degrees); pH; TA, titrable acidity (in g of citric acid per 100 g of homogeneized juice); L, reflectance percentage; a/b, absorbency index; F, firmness; FC, number of flowers per cluster.

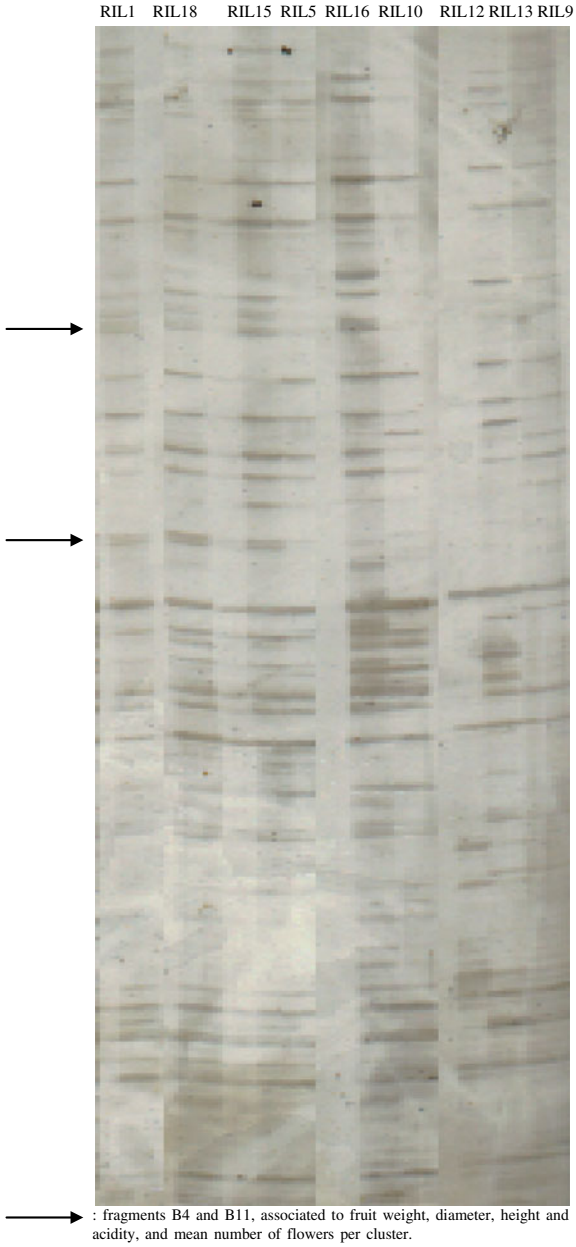


Figure 3. Section of a polyacrilamide gel showing the AFLP profiles of a set of tomato RILs with the primer combination B.

low similarity with the cluster of these genotypes according to phenotypic data. This finding could be due to the fact that different levels of genetic variability were measured in both analyses: the first dendrogram is based on phenotypic variation while the second is based on molecular variation. One of the few agreements between both clusters was that parents always grouped clearly separated and most RILs were located among them. All these findings suggest that these amplified fragments could be representing random regions at the whole RILs genome.

Thirty-nine (55.7%) of the 70 detected polymorphic fragments did not adjust to the expected proportion 1:1, which indicates a high frequency of distorted segregation. Many explanations were given by Kearsey and Pooni (1996) for such observations, including linkage to lethal factors and improper assignation of the molecular genotypes. Eight of the polymorphic fragments that adjusted to 1:1 (B6, N18, B36, B25, B38, B23, B39 and A10) did not show an independent segregation but conformed an important 'linkage group', which is a common fact when dealing with interspecific hybridizations (Lecomte *et al.* 2004). Fragments B36, B38 and B39 were carried by LA722, while N18 was carried by Caimanta. The concomitant presence of these four markers in many lines suggested that there was an early crossingover in the chromosome regions containing them. Other markers (B4, B11 and B35; B32, N2, N19 and A8; B32 and B30; A2 and A3; N7 and A11; A25 and A26) also cosegregated, but the number of fragments involved in each case was lower. Among them, B4, B11 (figure 3) and B35 were relevant. The first two fragments were carried by Caimanta and they were present just in RILs selected for high weight, while B35 was contributed by LA722 and mainly present in lines with low weight. Hence B4, B11 and B35 could be

marking a chromosome region tightly associated with fruit weight.

Four (N18, B36, B38 and A10) of the eight linked fragments were introduced into the PCA in order to avoid including data representing replicated information (Chatfield and Collins 1986). These four fragments segregated independently and showed linkage when other intermediate fragments were considered. Therefore, they were contributed by a different parent. With respect to the other smaller blocks of fragments, just one or two were randomly chosen for including in the analysis. In this PCA, the data matrix was composed by 1 and 0, these values reflecting the allele frequency of each polymorphic fragment in the RILs. So principal components obtained by this method should reflect the molecular variation among these genotypes and AFLP bands highly contributing to genotypic variability. The 59% of the total molecular variation was explained by the three first principal components (26% by the first, 20% by the second and 13% by the third, respectively). Hence, when the polymorphic fragments were analysed by this method, it was necessary to consider the three first principal components to explain the same percentage of total variation (about 60%) than that explained by the first two principal components at the phenotypic level. This result would be due to the greatest variation present at the molecular level. The composition of each of the three principal components is not shown due to the large number of data included, but the first one was highly associated to fragments B4, B35, N7 and A10. The second was highly associated to B22, B38, N4, N18 and N25 (the correlation coefficients were greater than 0.65 in all these cases). Figure 2b shows the associations of 16 RILs and fragments in the coordinates defined by the first (PC1) and the second (PC2) principal components. A clear discrimination among high fruit weight RILs (placed at the upper left quadrant) and middle-to-low fruit weight RILs (placed at the right quadrant) was detected. Fragment B4 was also placed at the left of the plane, while fragments B35 and B38 were placed to the right (together with the low fruit weight RILs). This observation also indicates that these fragments could be potential molecular markers of chromosome region associated with W, and suggests the usefulness of multivariate analysis in identifying such associations.

QTL detection

Eight (26%) of the 31 polymorphic fragments that adjusted to the expected segregation 1:1 showed association with at least one of the phenotypic traits (table 3). This proportion of markers with respect to the total number of detected fragment agrees to other reports on interspecific tomato

Table 3. Association among Mendelian segregant AFLP and quantitative traits.

Trait	Fragment	Significance	Origin	R^2	Fragment effect	
					Presence	Absence
W	B4	$P < 0.0001$	Caimanta	79.80%	21.69	4.92
	B35	$P = 0.0009$	LA722	55.64%	5.23	18.89
	B36	$P = 0.0082$	LA722	40.36%	3.76	15.68
	B38	$P = 0.0087$	LA722	39.89%	3.80	15.65
	A10	$P = 0.0015$	Caimanta	52.42%	17.01	3.75
SL	N18	$P = 0.0046$	Caimanta	44.72%	23.05	16.56
	A25	$P = 0.0067$	Caimanta	41.90%	16.33	22.34
D	B4	$P < 0.0001$	Caimanta	74.64%	3.46	1.88
	B35	$P = 0.0023$	LA722	46.32%	1.93	3.17
	B36	$P = 0.0067$	LA722	38.86%	1.74	2.91
	B38	$P = 0.0087$	LA722	36.27%	1.76	2.90
	A10	$P < 0.0021$	Caimanta	47.35%	3.02	1.77
H	B4	$P < 0.0001$	Caimanta	76.28%	3.00	1.67
	B35	$P = 0.0017$	LA722	48.34%	1.70	2.76
	B36	$P = 0.0060$	LA722	39.15%	1.55	2.54
	B38	$P = 0.0083$	LA722	36.48%	1.57	2.53
	A10	$P = 0.0020$	Caimanta	47.32%	2.62	1.58
TA	B4	$P = 0.0073$	Caimanta	41.29%	0.58	0.88
	B36	$P = 0.0071$	LA722	41.48%	0.96	0.66
FC	B4	$P = 0.0057$	Caimanta	43.14%	6.89	9.07
	A26	$P = 0.0059$	Caimanta	42.84%	7.20	9.31

R^2, Percentage of phenotypic variation explained by each associated fragment.
The effect of each fragment on the quantitatives traits was estimated as the mean value of two gropus of RILs: one defined by the presence of the fragment ond the other defined by its absence. Such mean values were compared by one-way ANOVA. For fruit traits see footnote of table 1.

crosses (Grandillo and Tanksley 1996; Bernacchi *et al.* 1998; Lippman and Tanksley 2001).

Two fragments associated with SL were detected (table 3). Interestingly, the shortest SL parent Caimanta contributed both N18 and A25, the first of them could explain the transgressive segregation fixed in some lines. According to de Vicente and Tanksley (1993), any parent having the lowest value for a given trait could carry alleles that enlarge it, but such genes would only express in an adequate genetic background when recombining with genes from the other parent. Two additional fragments contributed by LA722 (B36 and B38) were associated with SL, but they were not included in table 3 because their P values were 0.0306 and 0.0352, respectively; i.e., 10-fold greater than those finally shown, which are more statistically accurate. Nevertheless, the presence of both fragments increased SL (mean of group of RILs with presence of B36 = 21.73 days, mean of group of RILs with absence of B36 = 16.70 days, percentage of variance explained = 29.24%; mean of group of RILs with presence of B38 = 21.66 days, mean of group of RILs with absence of B38 = 16.74 days, percentage of variance explained = 27.964%).

W was the trait for which the greatest number (5) of associated fragments was detected. All fragments had the expected effect, i.e., those carried by Caimanta increased W while those carried by LA722 reduced W. The B4 fragment was present in all the high W lines, and absent in the low W lines (figure 3). It had a high R^2 value (greater than 0.70), explaining a high proportion on the phenotypic variance.

With respect to other traits, the five fragments marking *W* were also associated with D and H, two fragments were associated with TA (B4 among them), and two to FC (again B4 in this case). No association among any fragment with H/D, SS, pH, L, a/b and F was detected.

When pairs of fragments associated with the same trait were studied, N18 and A25, had significant effects on SL (F ratio = 9.38 and F = 7.46, $P < 0,01$), but interaction between them was not significant (F ratio = 1.01, nonsignificant). The two-way ANOVA explained a higher percentage of the phenotypic variation (67.41%) than the one-way ANOVA, indicating that both chromosome regions marked by these fragments interact additively to determine SL. With respect to W, B4 nullified the statistical significance of the other fragment associated with this trait and consequently the epistatic interaction was not significant. Instead, fragments B35 (F ratio = 6.89, $P < 0.01$) and B36 (F ratio = 8.43, $P < 0.01$) appeared to interact epistatically (F ratio = 4.56, $P < 0.01$). This two-way ANOVA explained by itself a higher percentage of the phenotypic variation (81.57%) than B4 per se. The two latest fragments were independently contributed by LA722, and were detected in low weighted RILs while B4 was contributed by Caimanta and present in high weighted RILs. Similar results were detected for D and H, and no other two-way ANOVA was significant for any traits. Consequently, the interlocus interaction was relatively important in determining tomato fruit quality among these selected genotypes. Some of these interactions were additive, such as those determining SL, while others were epistatic, such as those determining W, D and H.

A great number of fragments displaying pleiotropic effects were detected for W, D, H, TA and FC. Earlier, Rodriguez *et al.* (2005) had also reported a high genetic correlation among these traits.

Conclusions

A high level of phenotypic and molecular polymorphisms was detected among RILs, which clearly allows the characterization and grouping of this set of new genetic material according to their genetic distances. The multivariate analysis of principal components was effective in identifying RILs by either phenotypic or molecular polymorphisms and in detecting putative associations among both sets of data. Eight polymorphic fragments were associated with agronomically important quantitative traits in this set of selected tomato RILs. Associations among morphological fruit quality traits and molecular polymorphisms could be used as molecular markers in assisting tomato breeding programmes.

Acknowledgement

We thank CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) and ANPCyT (Agencia Nacional de Promoción Científica y Tecnológicas) for financially supporting this research.

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Received 30 October 2009, in revised form 10 September 2010; accepted 4 January 2011
Published on the Web: 19 August 2011