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Tomato near isogenic lines to unravel the genetic diversity of *S. pimpinellifolium* LA0722 for fruit quality and shelf life breeding

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Abstract The introgression of genes from the wild species *S. pimpinellifolium* has the potential to improve tomato commercial varieties for numerous fruit quality traits. One of particular interest for fresh-market tomatoes is long shelf life (SL). Twenty-two near isogenic lines (NILs) were developed in three to four backcrosses using *S. pimpinellifolium* accession LA0722 as the donor parent, based on phenotypic selection for long SL and using a set of 28 SSRs for marker assisted selection. A total of 30 QTLs for fruit quality were found and 18 were subsequently validated in previous generations. A two year trial of the NILs homozygous for the LA0722 introgressions showed several novel QTLs. Fruit quality QTLs revealed an increased effect promoted by LA0722 alleles. A SL QTL was found on chromosome 9 and was consistent both years. A wild introgression on chromosome 3 showed a stable QTL for increased firmness and for yellow fruits. Therefore, the wild introgressions provide a source of variation with positive effects in fruit traits of commercial value. While obtaining new varieties, this strategy provided an effective method that integrates the QTL analysis and the prediction of positive alleles in wild germplasm.

Keywords *S. lycopersicum* · Advanced backcrosses · QTL analysis · Marker-assisted selection

Di Giacomo and Luciani did equal contribution to the manuscript.

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Introduction

Human selection and domestication of tomato (*Solanum lycopersicum* L.) have produced important genetic changes on this crop, mainly modifying the reproductive system and increasing fruit size. The modern tomato breeding programs were focused not

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only on biotic and abiotic stress resistances but also on increasing yield. However, fruit quality attributes have been disregarded. As a consequence, flavour degradation in modern fresh-market tomatoes has been compromised and this constitutes the main complaint by consumers nowadays (Klee and Tieman 2018). Recently, the study of the tomato pan-genome has revealed a substantial gene loss in modern varieties during domestication, thus contributing to narrow their genetic base compared to their wild relatives (Blanca et al. 2015; Gao et al. 2019). Since wild genotypes are valuable genetic resources, crossings them with cultivated tomato species will broaden the genetic diversity. Wild germplasm was first used as a source of resistance genes for many tomato diseases and pests (Hajjar and Hodgkin 2007). Since then, breeders have discovered new alleles in different wild species so as to improve cold, drought and salinity tolerance, yield and carotenoid content (Hobson and Grierson 1993; Eshed and Zamir 1995; Fridman et al. 2004; Semel et al. 2006; Ikeda et al. 2013; Soyk et al. 2017).

Interspecific crossings can reveal alleles left behind during tomato domestication (Bauchet and Causse 2012). Molecular markers have helped to a more efficient use of wild genetic resources by dissecting complex traits into quantitative trait *loci* (QTLs) and by identifying key alleles (Foolad and Sharma 2005). Although, many studies have reported methods based on molecular markers to detect, map and characterize QTLs, only a few have helped to create new varieties (Labate et al. 2007). This may be due to the consideration of QTLs analysis and the development of new varieties as separate processes that do not involve elite germplasm. However, both methods could be applied as an integrative process and by doing this, a gradual improvement of the elite varieties could be reached by the introgression of QTLs from wild parents, using backcrosses (BC) for this purpose (Priyadarshan 2019). Only three to four generations of BC are needed to find precursors of Near Isogenic Lines (NILs).

Wild species used as donor parents in the development of NILs have the potential not only to increase the genetic variation in an elite germplasm but also to study the contribution of wild genes or QTLs in an appropriate genetic background. A wild species of interest is *S. pimpinellifolium* L. which is characterized by its small size and high-quality red fruits (Peralta et al. 2008). *S. pimpinellifolium* has great potential to improve fruit quality traits; being shelf life (*SL*) one of particular interest for fresh-market tomatoes. In previous studies, we have demonstrated that the *S. pimpinellifolium* accession LA0722 carries long *SL* genes since the hybrids with the cultivated tomato had longer fruit *SL* and better fruit quality when compared to testers (Zorzoli et al. 2000; Pratta et al. 2003; Rodríguez et al. 2006; Cambiaso et al. 2019). Tomato NIL collections have already been obtained by introgressing chromosomal segments from *S. pimpinellifolium* for fruit-related traits and plant structure (Bernacchi et al. 1998; Doganlar et al. 2002; Barrantes et al. 2016; Nakano et al. 2016; Celik et al. 2017). However, collections developed by targeting *SL* with wild introgressions of the accession LA0722 have not been studied yet.

Breeding strategies to extend tomato *SL* rely on varieties that exhibit late or partial ripening. Breeding programs have recovered spontaneous mutations of ripening mutants. These include well-studied pleiotropic mutations on transcription factors, such as ripening-inhibitor (*rin*), non-ripening (*nor*) and Colorless non-ripening (*Cnr*) (Vrebalov et al. 2002; Manning et al. 2006; Wang et al. 2019). These mutations improve shelf life but have undesirable effects on fruit quality. Other approaches that delay fruit ripening have targeted cell wall modification proteins in transgenic tomatoes (Brummell and Harpster 2001; Meli et al. 2010; Uluisik et al. 2016; Yang et al. 2017); even long *SL* tomato lines were developed replacing alcobaca gene (*alc*) with CRISPR/Cas9 mutagenesis (Yu et al. 2017). Although these strategies have obtained good results, they lack consumer acceptance and have to deal with the legislations in different countries (Ishii and Araki 2016; Schmidt et al. 2020). Therefore, the introgression of wild alleles from *S. pimpinellifolium* LA0722 represents a favorable opportunity to develop long *SL* varieties that avoids genetic engineering or undesirable pleiotropic effects caused by mutant genes introgressions.

In this context, the aim of the study was to exploit the genetic diversity for fruit quality and *SL* from the *S. pimpinellifolium* accession LA0722 by developing a tomato NIL collection. This population will enable the identification of wild introgressions effects over fruit quality traits in the genetic background of *S. lycopersicum* cv. Caimanta. Moreover, this approach will release pre-breeding materials to study the genetic

bases of these traits that could also be introduced as fresh-market tomato varieties.

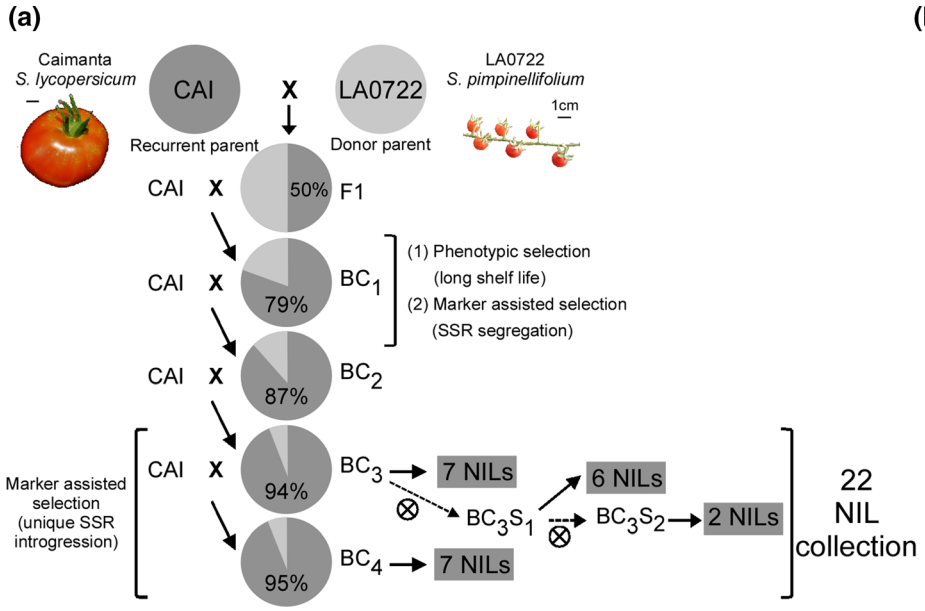
Materials and methods

Plant material

The NIL collection was obtained backcrossing the Argentinian cultivar Caimanta (CAI, *S. lycopersicum*) as the recurrent parent with the *S. pimpinellifolium* accession LA0722 (LA0722) as the donor of chromosomal segments for fruit quality traits. CAI has a determined growth and slightly flattened fruits (with higher values of diameter than height), with 98.5 ± 9.9 g of weight and a *SL* of 9.7 ± 0.9 days. LA0722 has an indeterminate growth, numerous flowers per inflorescence and round fruits of small size with 0.9 ± 0.1 g of weight and a long *SL* of 18.7 ± 0.4 days (Rodríguez et al. 2006). CAI was provided by EEA INTA Cerrillos (Salta, Argentina) and the wild accession LA0722 by the Tomato Genetic Resources Center, Department of Vegetable Crops, University of California (Davis, USA).

NIL collection development

A breeding scheme from the NIL collection development is shown in Fig. 1a. A single F_1 plant from the initial cross between CAI and LA0722 was backcrossed with the recurrent parent generating 74 BC_1 plants. Seven BC_1 plants were selected and backcrossed with CAI to obtain BC_2 generation. The selection criteria were both phenotypic and genotypic plant values. The phenotypic selection was based on long *SL*. For marker-assisted selection (MAS), a set of 28 simple sequence repeat (SSR) markers distributed throughout the tomato genome was used (Online Resource 1). Plants segregating for the 28 SSRs were selected. Forty-eight BC_2 plants were obtained and used to develop the BC_3. Henceforth, the selection criterion was followed by MAS based on plants carrying a single SSR introgression from LA0722. From a total of 274 BC_3 plants, seven were selected setting the first group of NILs in the 2011–2012 season (Fig. 1b, NIL598, NIL320, NIL115, NIL162, NIL286, NIL327 and NIL069). Different BC_3 plants carrying target LA0722 segments with less than two additional wild introgressions were self-pollinated (BC_3S_1),



(b)

NIL collection

NIL	Chr	Population
NIL095	1	BC_4
NIL220060	1	BC_4
NIL295	2	BC_4
NIL598	2	BC_3
NIL320	3	BC_3
NIL115	5	BC_3
NIL162	5	BC_3
NIL211044	6	BC_3S_1
NIL128	6	BC_3S_2
NIL286	7	BC_3
NIL276	7	BC_3S_1
NIL045	7	BC_3S_1
NIL327	8	BC_3
NIL063	8	BC_4
NIL069	9	BC_3
NIL070	9	BC_3S_2
NIL034	9	BC_3S_1
NIL596	10	BC_3S_1
NIL318	10	BC_3S_1
NIL080	11	BC_4
NILG036	11	BC_4
NIL1061	11	BC_4

Fig. 1 Breeding scheme **a** Development of the 22 NIL collection with introgressions from *S. pimpinellifolium* accession LA0722 in the genetic background of *S. lycopersicum* cv. Caimanta (CAI). Different tones of gray show the fraction of each parent's genome in every generation. The values correspond to the percentage of the recurrent parent genome recovered in the progeny. Representative fruit of the parental genotypes are shown. **b** NILs from the collection indicating their names, the chromosome (Chr) corresponding to the location of the SSR introgression and the population from which the NILs were obtained. NIL, near isogenic line; BC_N, backcross; BC_NS_N, self-pollinated backcross

giving place to six other NILs in the 2013–2014 season (Fig. 1b, NIL211044, NIL276, NIL045, NIL034, NIL596 and NIL318). Moreover, the analysis of BC_3 with two rounds of self-pollination (BC_3S_2) permitted to identify two additional NILs (Fig. 1b, NIL128 and NIL070). Other BC_3 plants with different target LA0722 segments that also carried more than two additional wild introgressions were backcrossed to obtain 210 BC_4 plants and seven additional NILs were derived in the 2013–2014 season (Fig. 1b, NIL095, NIL220060, NIL295, NIL063, NIL080, NILG036 and NIL1061). A total of 22 NILs were obtained containing LA0722 introgressions in heterozygous condition (NIL-CP). Each NIL-CP was self-pollinated in order to obtain plants carrying the homozygous introgression as the recurrent parent (NIL-CC), homozygous as *S. pimpinellifolium* LA0722 (NIL-PP) or heterozygous (NIL-CP). Here and after, segregating genotypes derived from each self-pollinated NIL-CP plant will be named as NIL family. At this point, the breeding process ended up with 22 NILs-PP that reached 96.43% of the cultivated background of *S. lycopersicum* cv. Caimanta (Fig. 1b).

Genotyping for marker-assisted selection

Genomic DNA was extracted from young leaves using a commercial kit (Wizard® Genomic DNA Purification Kit from Promega®). The extracted DNA was dissolved in buffer TE (10 mM Tris–Cl pH 8.00, 1 mM EDTA) and the final concentration was adjusted to 40 ng/μL. The 28 SSR markers used for MAS are described by the sequence of primers and chromosomal location on SL4.0 (Online Resource 1). These SSRs were chosen due to their codominant nature and their chromosome location, considering at least two by chromosome, one per each arm (Pereira da Costa et al. 2013). Standard PCR protocols were used to obtain amplification products from the DNA using the SSR markers. Electrophoresis of SSR markers was conducted on 6% w/v polyacrylamide gels. The visualization was performed by commercial silver staining kit (Silver SequenceTM Staining Regeants, Promega®).

Experimental design and phenotyping

Trials were conducted at the Estación Experimental José F. Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Argentina (33° 02′ S and 60° 53′ W), during spring–summer seasons. The plant material was sown in seedling trays and grown for 45 days before being transplanted to soil in a greenhouse under natural light. A completely randomized design was used. The distance between plants was 0.45 m and row spacing was 1 m. Plants were grown under drip irrigation and standard fertilizer regimes. All the pollinations to obtain the backcrosses were made by hand.

In every phenotypic evaluation, 10 fruits per plant were harvested at the breaker stage defined by Giovannoni (2004). The following fruit traits were evaluated: diameter (*D*, cm), height (*H*, cm), fruit shape (*FS*, H/D ratio), fruit weight (*FW*, g), pericarp thickness (*PT*, cm), locule number (*LN*) and *SL* (days). Fruit *SL* was measured considering the days from the harvesting until the first visual sign of deterioration and softening (Rodríguez et al. 2010). Fruits at breaker stage were stored on shelves at 25 ± 3 °C and were discarded at 48 h interval when considering commercially unacceptable due to wrinkling or excessive softening. Other fruits harvested at red ripe stage (Giovannoni 2004) were used to measure color indexes (*a*/*b* and *L*) with a CR-400 Chroma Meter of Minolta, and fruit firmness (*F*) with a Durofel DFT100. Afterwards, homogenized juice was obtained from red ripe fruits in order to evaluate soluble solids content (*SSC*, Brix), *pH* and titratable acidity (*TA*, g citric and malic acid/100 g of homogenized juice). *TA* was determined by titrating 10 g of juice dissolved in 100 mL of distilled water against 0.1 N sodium hydroxide (NaOH), using a pH 8.1 as final point.

QTL detection in selfed NILs-CP

The segregation obtained by selfing the NILs-CP was used for the QTL detection. In the 2014–2015 season, a total of 30 plants per NIL family were grown and the fruits were collected for phenotyping. The association between segregating SSR and phenotypic traits was determined by Single Point Analysis (Tanksley 1993). R^2 value served to estimate the percent of the phenotypic variation explained by each QTL (Liu 1998). A significance level of $p < 0.001$ was used. Nevertheless, we explore the presence of QTLs at a level of significance of $p < 0.05$ as a subsequent validation was made by comparing with QTLs

detected in previous generations of the breeding process (Collard et al. 2005; Pereira da Costa et al. 2013). A validated QTL was considered when the association was found in two or more generations at $p < 0.05$.

Performance evaluation of NILs containing wild introgressions from LA0722

A two year trial on spring–summer season 2015–2016 (year 1) and 2018–2019 (year 2) was conducted at the Estación Experimental José F. Villarino, Argentina (33° 02′ S and 60° 53′ W) under the same crop management, as described in the experimental design. Relative humidity and mean temperature were different between years (Online Resource 2). The phenotypic traits were measured on fruits collected from 10 plants of the 22 NILs-PP. Ten plants of the parental genotype were used as controls. Mean values and standard errors for the phenotypic traits were calculated and the normal distribution was verified according to Shapiro–Wilk test (1965). To identify pairwise differences between NILs and the recurrent parent within each year, the mean values were compared by a Dunnett test with an alpha level of 0.05. Genotype, environment and genotype × environment interaction effects on the phenotypic variation were investigated. The components of phenotypic variance were estimated from the ANOVA using the linear mixed effects function (*lmer*) of the *lme4* package (Bates et al. 2015). For all traits, the following model was applied:

$$Y_{ij} = \mu + g_i + y_j + gy_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the phenotypic trait, μ is the overall mean, g_i is the effect resulting from the ith genotype, y_j is the effect resulting from the jth year, gy_{ij} is the effect resulting from genotype by year interaction, and ε_{ij} is the residual error. All the effects were treated as random. Best Linear Unbiased Predictors (BLUPs) based on random models were estimated for each trait. The random effect function (*ranef*) in the *lme4* package was used to estimate BLUPs in the model (Bates et al. 2015). BLUPs for NILs across years were extracted and used for the principal component analysis (PCA) and the correlation analysis using *FactoExtra* and *ggcorrplot* packages of the R software (R Core Team 2017). To decide which traits were the most relevant in the NIL collection, Pearson correlation coefficients and eigenvector loadings from PCA were considered. The retained traits were used to perform a cluster analysis with Ward's minimum variance method. The optimal number of clusters was selected considering the total within-cluster sum of square (WSS). The significant differences among individual clusters were identified by an ANOVA and Tukey HSD test for pairwise comparison.

Results and discussion

Development of the NIL collection

In tomato breeding, higher efficiency in terms of time and amount of materials has been demonstrated through the application of MAS (Foolad and Sharma 2005). In our breeding process, we combined MAS with phenotypic selection for *SL*. This has allowed us to identify 22 lines almost isogenic to CAI with introgressed segments from LA0722 (Fig. 1). A total of 28 SSR markers were used initially for the MAS (Online Resource 1). Two markers (SSR009 and SSR344 on chromosome 1 and 8, respectively) were monomorphic in the BC_2 generation and two others (SSR288 and SSRH301 on chromosomes 1 and 12, respectively) in the BC_3 generation. These markers fixed the cultivated alleles in all the evaluated plants and therefore, NILs with wild introgressions for those chromosomal segments were not possible to develop. For the 24 remaining SSR markers, 22 NILs were obtained (Fig. 2). Twenty of them have a wild introgression in a single marker *locus* and the two others (NIL320 and NIL063) have an introgressed region that comprises two closely related marker *loci* (SSR320-SSR014 and SSR063-SSR038, respectively) that failed to recombine due to tight linkage. In our NIL collection, the number of NILs per chromosome ranged from one to three (Fig. 2). For chromosomes 7, 9 and 11 we were able to develop three NILs. For chromosomes 4 and 12, no NIL could be obtained.

NIL analysis: QTL approach

As part of a breeding program, the QTL analysis in interspecific crosses and backcross generations is a very useful tool to explore the contribution of wild germplasm for improving different traits. Phenotypic and molecular data allowed a trait-marker association

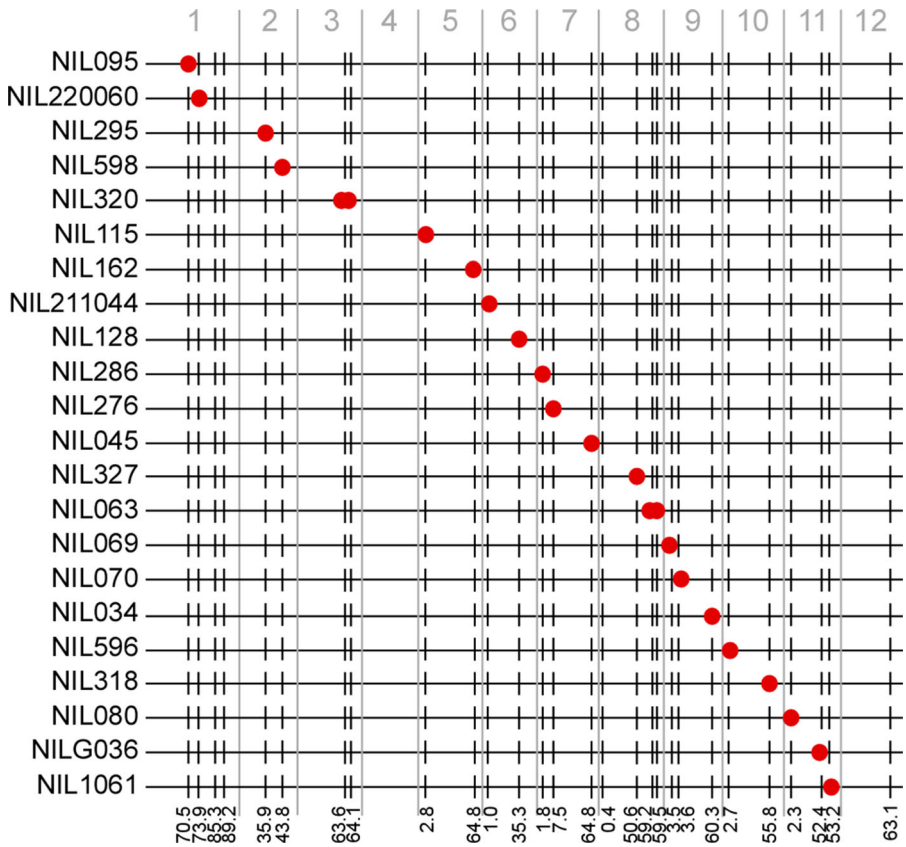


Fig. 2 Schematic representation of the wild introgressions in the 22 NILs across the 12 chromosomes. Points highlight SSRs where introgressions from *S. pimpinellifolium* accession LA0722 were fixed. Lines represent the SSRs used for marker assisted selection that fixed cultivated alleles from the recurrent parent *S. lycopersicum* cv. Caimanta. NIL names are presented on the first column. Chromosomes numbers are shown on the top and on the bottom, the SSR physical positions (Mb) on the tomato genome sequence reference version SL4.0

study in order to detect QTLs contributed by the wild introgression in each NIL family. A total of 30 QTLs associated with fruit quality traits were detected in 12 NILs (Table 1). The evidence of a QTL in some preceding generation of CAI × LA0722 cross were used to validate the association found in a derived NIL. From a total of 30 QTLs, 18 (60%) were found in some previous generation of the backcrosses (Online Resource 3). From these validated associations, 12 were observed in an additional generation and six, in two or more. For 10 of the 18 QTLs, the effect on the trait was consistent across generations. However, some QTLs showed discrepancies; particularly, two QTLs for color and one for *TA* showed opposite effects. These may suggest new epistatic interactions in different genetic contexts caused by advanced backcrosses and self-pollinations. These differences could also be attributed to the interaction QTL × environment.

The NILs development involved a positive phenotypic selection based on *SL*; therefore, the NILs were expected to show QTLs that improve this trait. A QTL detected for *SL* on chromosome 8 (SSR327) is quite promising since the presence of wild allele in homozygous state promotes an extension of *SL* without undesirable pleiotropic effects on others quality traits. QTLs for *SL* on chromosome 8 have been previously reported, where the presence of the wild allele improved the trait in a previous generation of the same cross (Pereira da Costa et al. 2013) as well as in an independent cross between *S. lycopersicum* cv. Rio Grande and *S. pimpinellifolium* LA1589 (Green et al. 2016). For NIL080, it was determined that the SSR080 region of chromosome 11 was

Table 1 QTLs for tomato quality traits identified in a collection of 22 NILs developed by wild introgressions from LA0722 of *S. pimpinellifolium* in a cultivated background of *S. lycopersicum*

Trait	Marker	NIL	Chr	R^2	*p* Value	CC			CP			PP		
						n	M ± SE		n	M ± SE		n	M ± SE	
Diameter (D)	SSR320	NIL320	3	0.21	0.0423	10	5.40 ± 0.32	**b**	13	5.61 ± 0.28	**b**	7	4.37 ± 0.38	**a**
	SSRG036	NILG036	11	0.23	0.0349	9	6.81 ± 0.30	**b**	15	6.52 ± 0.23	**b**	5	5.47 ± 0.40	**a**
	SSR1061	NIL1061	11	0.37	0.0018	5	6.54 ± 0.22	**b**	16	5.77 ± 0.12	**a**	9	5.46 ± 0.16	**a**
Height (H)	SSR211044	NIL211044	6	0.26	0.0351	5	5.76 ± 0.43	**ab**	11	6.09 ± 0.29	**b**	9	4.90 ± 0.32	**a**
Shape (FS)	SSR110	NIL034	9	0.45	0.0156	5	0.72 ± 0.03	**a**	10	0.70 ± 0.02	**a**	2	0.86 ± 0.04	**b**
	SSRG036	NILG036	11	0.59	**< 0.0001**	9	0.67 ± 0.02	**a**	15	0.76 ± 0.01	**b**	5	0.83 ± 0.02	**c**
	SSR1061	NIL1061	11	0.62	**< 0.0001**	5	0.71 ± 0.22	**a**	16	0.81 ± 0.01	**b**	9	0.86 ± 0.01	**c**
Weight (FW)	SSR211044	NIL211044	6	0.25	0.0398	5	136.06 ± 21.20	**ab**	11	148.19 ± 14.30	**b**	9	91.15 ± 15.80	**a**
	SSRG036	NILG036	11	0.22	0.0368	9	128.50 ± 10.03	**b**	15	113.78 ± 7.77	**b**	5	82.56 ± 13.45	**a**
	SSR1061	NIL1061	11	0.22	0.0366	5	116.33 ± 9.34	**b**	16	94.87 ± 5.22	**a**	9	94.87 ± 6.96	**a**
Pericarp thickness (PT)	SSR211044	NIL211044	6	0.37	0.0149	3	0.61 ± 0.08	**ab**	10	0.78 ± 0.05	**b**	8	0.56 ± 0.05	**a**
Locule number (LN)	SSR211044	NIL211044	6	0.36	0.0191	3	4.06 ± 0.36	**b**	10	2.81 ± 0.20	**a**	8	2.83 ± 0.22	**a**
	SSRG036	NILG036	11	0.43	0.0087	5	6.54 ± 0.63	**b**	11	4.18 ± 0.42	**a**	4	3.54 ± 0.70	**a**
	SSR1061	NIL1061	11	0.61	**0.0005**	2	8.00 ± 0.78	**b**	12	4.40 ± 0.32	**a**	5	3.40 ± 0.49	**a**
Shelf life (SL)	SSR115	NIL115	5	0.23	0.0371	13	21.51 ± 3.71	**a**	10	15.86 ± 4.23	**a**	5	35.93 ± 5.98	**b**
	SSR327	NIL327	8	0.26	0.0304	10	10.76 ± 1.10	**a**	13	14.00 ± 0.96	**ab**	3	16.44 ± 2.01	**b**
	SSR080	NIL080	11	0.31	0.0236	4	11.33 ± 3.03	**ab**	10	18.10 ± 1.91	**b**	9	10.09 ± 2.02	**a**
Color a/b	SSR115	NIL115	5	0.70	**< 0.0001**	11	0.76 ± 0.09	**b**	8	0.95 ± 0.10	**b**	5	− 0.15 ± 0.13	**a**
	SSR1061	NIL1061	11	0.34	0.0280	2	− 0.30 ± 0.38	**a**	13	0.44 ± 0.15	**ab**	5	1.00 ± 0.24	**b**
Color L	SSR220060	NIL220060	1	0.29	0.0329	6	43.04 ± 1.04	**b**	11	39.62 ± 0.77	**a**	6	42.13 ± 1.04	**ab**
	SSR598	NIL598	2	0.31	0.0193	12	40.17 ± 1.44	**a**	9	43.44 ± 1.67	**a**	3	49.95 ± 2.89	**b**
	SSR115	NIL115	5	0.59	**0.0001**	11	44.95 ± 1.61	**a**	8	41.80 ± 1.89	**a**	5	57.88 ± 2.39	**b**
	SSR063	NIL063	8	0.43	0.0360	1	41.51 ± 1.83	**a**	9	39.08 ± 0.61	**a**	5	42.04 ± 0.82	**a**
	SSR1061	NIL1061	11	0.33	0.0340	2	52.91 3.98	**b**	13	45.77 ± 1.56	**ab**	5	39.89 ± 2.52	**a**
Firmness (F)	SSR115	NIL115	5	0.44	0.0023	11	56.26 ± 2.75	**a**	8	54.48 ± 3.23	**a**	5	74.03 ± 4.08	**b**
	SSR045	NIL045	7	0.35	0.0302	6	48.82 ± 2.82	**a**	7	59.08 ± 2.62	**b**	6	49.93 ± 2.82	**a**
Soluble solids content (SSC)	SSR220060	NIL220060	1	0.57	**0.0002**	6	4.52 ± 0.28	**a**	11	5.87 ± 0.21	**b**	6	6.55 ± 0.28	**b**
pH	SSR115	NIL115	5	0.41	0.0041	11	4.63 ± 0.06	**a**	8	4.87 ± 0.05	**b**	5	4.45 ± 0.09	**a**
Titratable acidity (TA)	SSR220060	NIL220060	1	0.39	0.0071	6	0.35 ± 0.07	**a**	11	0.46 ± 0.05	**a**	6	0.68 ± 0.07	**b**

Table 1 continued

Trait	Marker	NIL	Chr	R^2	*p* Value	CC			CP			PP		
						n	M ± SE		n	M ± SE		n	M ± SE	
	SSR320	NIL320	3	0.24	0.0378	9	0.45 ± 0.03	**b**	11	0.35 ± 0.03	**a**	7	0.36 ± 0.03	**a**

Bold values indicate highly significant QTLs ($p < 0.001$)

Different letters indicate significant differences ($p < 0.05$)

[a]Explained phenotypic variation

[b]Number of individuals

[c]Mean value and standard error

[d]Homozygous Caimanta (*S. lycopersicum*) alleles

[e]Heterozygous

[f]Homozygous LA0722 (*S. pimpinellifolium*) alleles

associated with *SL* where the heterozygous condition increased *SL* compared to homozygous individuals for LA0722 allele. This QTL had been reported in some previous generations (BC_1S_1 and BC_2S_1), manifesting the same effect (Online Resource 3). The contribution of some genes affecting fruit texture could be postulated. Seymour et al. (2013) showed more than 50 genes involved in the modification of cell wall architecture highly expressed during fruit ripening. On the region of chromosome 8, a cellulose synthase gene (*CesA*, Solyc08g061100) was highly expressed during fruit development but subsequently decreased its expression during fruit ripening and, as a consequence, there was no evidence that this gene may be involved in this process (Song et al. 2019). On the other hand, on the region of chromosome 11, no gene involved in cell wall remodeling processes was found, revealing possible novel QTLs on these regions. On chromosome 5, NIL115 presented a strong association of the SSR115 with the fruit color. The presence of the wild allele in homozygous condition determined yellow fruits. In addition, this region was associated with *SL* and *F*, where the wild allele increased both traits. These properties suggest that some genes involved in the fruit maturity could be affected. Given the characteristics of NIL115, it could be proposed some alteration in the *rin locus.* In the short arm of chromosome 5 was described *rin*, which encodes a MADS-BOX transcription factor involved in climacteric respiration and ethylene biosynthesis related to maturation (Vrebalov et al. 2002). Mutants for this gene presented green fruits that later turn bright yellow with a delayed maturation. In silico analysis based on genomic sequence comparisons between the wild-type RIN gene with LA0722 and CAI showed 99.67% and 100% of similarity, respectively. Nevertheless, to refute the hypothesis of *rin* mutation, a gene expression analysis should be performed. Other reasons for this phenotype could correspond to carotenoid pathways genes. Razifard et al. (2020) have recently provided evidence of *S. pimpinellifolium* genes in the trajectory of tomato domestication that were involved in carotenoid biosynthesis but none of them were located on chromosome 5.

The association with *SSC* and *TA* of chromosome 1 (SSR220060) was detected in previous generations (BC_1S_1/BC_3 and BC_2, respectively). Several studies have found QTLs related to *SSC* on tomato chromosome 1 using crosses with different wild species,

including *S. pennellii*, *S. pimpinellifolium*, *S. habrochaites*, *S. chmielewskii* and *S. peruvianum* (Eshed and Zamir 1994; Fulton et al. 1997; Chen et al. 1999; Monforte and Tanksley 2000; Frary et al. 2003; Causse et al. 2004). More recently, Bauchet et al. (2017) have found an association on chromosome 1 (solcap_snp_sl_38210, SL4.0ch01:72138615) with pleiotropic effect for fructose and glucose content, two primary metabolites that showed positive correlation to *SSC*. This observation corresponded to variation of *ADP glucose pyrophosphorylase* (AGPase) large subunit *locus* (Schaffer et al. 2000). On the other hand, on this region of chromosome 1, Schauer et al. (2006) showed a QTL for malate, principal contributor to fruit acidity. In our study, the wild alleles from *S. pimpinellifolium* determined an increase for *SSC* and *TA*, representing a possible breeding target region to improve tomato flavor. Although flavor represents a complex polygenic trait that relies on sugars, acids and volatiles content, targeting positive flavor genes/QTLs impacting tomato fruit chemicals could make considerable improvement (Tieman et al. 2017). Instrumental measurements of fruit organoleptic quality only helped partially to predict perception of flavor. However, it has been demonstrated that *TA* and *SSC* were positively correlated to acidity and sweetness perceived by consumers (Causse et al. 2003; Casals et al. 2019) and therefore, they represent possible targets. These studies have also demonstrated that adding descriptive sensory analyses as another strategy in breeding would display significant improvement in flavor. Another *TA* QTL was found in NIL320 on chromosome 3 where the presence of wild alleles decreased the value. The SSR320 marker was also associated with *D*. Previous work with *S. pimpinellifolium* reported QTLs on chromosome 3 for *D* (Chen et al. 1999; Lippman and Tanksley 2001), and for *TA* using *S. pennellii* (Causse et al. 2004).

Regarding the size and morphology of the fruits, an important effect in the NILG036 was found through the generations (BC_1, BC_2S_1 and BC_3). The SSRG036 located on chromosome 11 was associated with *D*, *FS*, *FW* and *LN*. In this NIL, the QTL explained about 40% and 60% of the phenotypic variation in *LN* and *FS*, respectively. The wild alleles for this region determined a decrease in *LN*, *D* and *FW*, and an increase in shape index, as expected based on LA0722 phenotype. For NIL1061, the region of chromosome 11 was associated with the same size and morphology traits. The QTL explained about 60% of the variation for the *LN* and *FS* and the presence of the wild alleles showed the expected effect according to the parental phenotype. Given the relationship between these traits, it is more likely to attribute them to pleiotropic effects than to different QTLs in the same region. On this region of chromosome 11, a major QTL, *locus FAS*, has important effects on *LN* affecting both fruit *FS* and *FW* (Cong et al. 2008). Other associations with tomato fruit *FW* have been previously found on chromosome 11 (Lippman and Tanksley 2001; Cambiaso et al. 2019) where *FW11.3* was identified (Mu et al. 2017). For NIL211044, the SSR211044 on chromosome 6 was associated with traits related to fruit size such as *H*, *FW*, *LN* and *PT*. However, these associations were weak and had not been previously detected. These regions could be masked by others, manifested only by decreasing the proportion of the donor genome. Another cause could be new interactions in the genetic context that are changing when advancing in backcrosses. For *FS*, in the NIL034, a QTL was detected in the region of chromosome 9 corresponding to SSR110, where the presence of wild alleles increased the *FS* index resulting in more spherical fruits. This association had been previously found in several generations of these backcrosses (BC_1, BC_1S_1, BC_2S_2 and BC_3). In addition, QTLs related to *FS* on tomato chromosome 9 have been described by other authors using the wild species *S. peruvianum*, *S. habrochaites* and *S. pimpinellifolium* (Fulton et al. 1997; Bernacchi et al. 1998; Chen et al. 1999).

Performance of the NILs containing wild introgressions from LA0722

Phenotypic measures on replicated trials in several locations and years are possible due to the immortality of the NILs. This increases the statistical power in the evaluation of quantitative traits and makes it possible to estimate environmental interactions for traits of complex inheritance. The homozygous NILs for the LA0722 introgressed segments (NILs-PP) were evaluated in a two year trial with their recurrent parent CAI in order to determine their performance. The mean values and the standard errors of the 13 quality traits were calculated within each year (Online resource 4). Due to the high number of missing plants of the NIL598 and the NIL211044 in the second year, they

were not considered in this analysis. Several differences between the NILs-PP and the recurrent parent were observed within each year and the QTLs detected are shown in Fig. 3. Novel associations were found and they could be the result of new uncovered pleiotropic effects and epistatic interactions within different backgrounds or other fixed wild introgressions (Chaïb et al. 2006).

In the NILs-PP, QTLs for size and morphology traits were mostly found as they showed the strongest differences between the parental lines. The presence of LA0722 alleles decreased those trait values. Many of these associations could be attributed to a pleiotropic effect or a set of linked genes. For *D*, the QTLs in previous generations were also observed in at least one year. In addition, several new QTLs were observed; 13 of the 22 introgressions showed association and half of them were consistent both years. The QTLs for *H* were found in almost all the NILs (19/22) and six showed stability over the years. For *FS*, nine QTLs were observed towards more round fruits in the first year of evaluation. In the second year, this NILs showed the same tendency although values were not significantly different from the recurrent parent (Online Resource 4). The QTLs for *FW* and *LN* previously reported in several generations for SSRG036 and SSR1061 (chromosome 11) were also found with the same LA0722 allele effect. Many previously undetected associations for *FW* and *LN* were also observed (Fig. 3).

For quality traits, fewer QTLs were found probably as a consequence of a more complex inheritance and a smaller divergence between the parental genotypes. However, all traits showed significant differences with the recurrent parent ($p < 0.05$) except for *SSC* that presented low variations and no QTL was found. Disregarding some associations with *TA*, all the detected fruit quality QTLs displayed greater values compared to the cultivated parent (Fig. 3). The QTLs for *SL* showed values between two-fold and five-fold higher. Although most of them were only significant in one year, the increased values were consistent both years (Online Resource 4). The first year NIL115 presented yellow fruits and the SSR115 was associated to *SL* and *F*. Unexpectedly, the second year, red fruits and no significant associations for *SL* or *F* were found. Same results were observed in NIL128, NIL276 and NIL069. These phenotypic differences between years could be due to recombination of nearby genomic segments. Nevertheless, the interaction with the environment has important effects to the stability of the QTLs, particularly in a trait like *SL* that has demonstrated to be highly influenced by the environment (Pratta et al. 2011; Cambiaso et al. 2019). Despite the SSR327 was associated to *SL* in previous generations, NIL327 did not show QTLs for this trait. However, it showed a tendency to increase *SL* values both years. A novel QTL for *SL* in the NIL034 was found and the association was consistent both years.

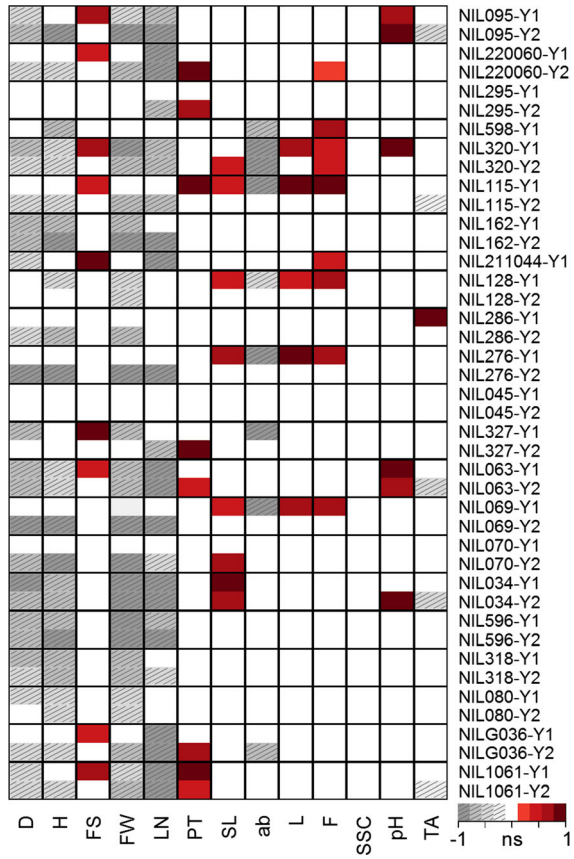


Fig. 3 QTL detection in the 22 NILs with homozygous *S. pimpinellifolium* LA0722 introgressions in a two year trial (Y1 and Y2). Heatmap representation of the differences between the recurrent parent *S. lycopersicum* cv. Caimanta (CAI) and the 22 NILs. Data was normalized to the minimum or maximum value in each trait. NIL598-Y2 and NIL211044-Y2 were not considered in the analysis. Significance level of 0.05 (Dunnett test). White blocks mean non-significant. Lined light color blocks indicate decreased LA0722 allele effect while full dark color blocks indicate increased LA0722 allele effect. NIL, near isogenic line; D, diameter; H, height; FS, shape; FW, weight; LN, locule number; PT, pericarp thickness; SL, shelf life; a/b and L, color indexes; F, firmness; SSC, soluble solids content; TA, titratable acidity

The *SL* values were increased 502% and 281% compared to the control in the first and second year, respectively. *LeBR1* (Solyc09g092520), a member of xyloglucan endotransglucosylase/hydrolases (*XTHs*) family, was described near the region of chromosome 9 (Koka et al. 2000). *XTHs* are cell wall-modifying enzymes and many of them are associated to fruit ripening since their activity occurs during softening (Saladié et al. 2006). However, *LeBR1* was related to fruit growth as its expression was higher during this process (Miedes and Lorences 2009). As a consequence, the NIL034 presented a promising region on chromosome 9 that improves *SL* without affecting other quality traits.

The color indexes showed novel QTLs on the NILs-PP. It could be noticed that the associations lead to yellow-fruited NILs, even though both parents have red fruits. This could be the result of recombination within the interspecific progeny that has uncovered transgressive variation. This could also apply for *PT* QTLs that showed increased values caused by the LA0722 alleles. These alleles in the wild germplasm could be identified in the cultivated background only after being isolated from other donor regions.

The QTLs for *F* were found with increased values caused by the LA0722 alleles. Particularly one, not previously detected, was observed for NIL320 and showed stability both years. NIL320 was also associated to color indexes *a/b*, showing yellow fruits. Near the region of SSR320 on chromosome 3, it was found a gene involved in carotenoid biosynthesis that encodes a *phytoene desaturase 1* (PDS1, Solyc03g123760). This gene showed signs of selection during tomato domestication from *S. pimpinellifolium* (Razifard et al. 2020). Some alteration of the gene may be involved causing this yellow-fruited phenotype. In addition, other genes of fruit softening within the region of SSR320 may be implicated. Well-characterized *pectate lyase* (PL) gene is located within this region of the chromosome (Yang et al. 2017).

Four and six QTLs were found for *pH* and *TA*, respectively. Two QTLs for *pH* on chromosomes 1 and 8 were consistent both years where LA0722 alleles increased the values. Same results in these regions of chromosome 1 and 8 were obtained in other *S. pimpinellifolium* inbred backcross lines (Celik et al. 2017). The *TA* QTLs only appeared in one year. In five of them, the LA0722 alleles showed a decreased effect on the trait while in the QTL found in NIL286, LA0722 allele highly increased the value.

Comprehensive analysis of the NIL collection

To understand the potential sources of phenotypic variation in the NIL collection, we tested the effects of genotype, environment (i.e. year) and genotype × environment interaction. The analysis of the variance components showed a highly significant genotype effect for all traits ($p < 0.0001$). However, the environment effect (for 6/13 traits) and the genotype × environment interaction (for 9/13 traits) were also significant ($p < 0.01$), indicating their high influence. Thus, BLUPs were estimated per NIL across environments. Using the BLUPs values, a correlation analysis and a PCA were conducted to determine which traits were the major sources of variation within the NIL collection (Fig. 4).

Pearson coefficients were calculated between each pair of traits using the estimated BLUPs (Fig. 4a). The most significant correlations were found for size traits (*D*, *H* and *FW*, $p < 0.0001$). This is consistent with the associations where pleiotropic effects for size traits were detected (Table 1 and Fig. 3). *SL*, *F* and color index *L* were positive correlated as well (all with $r = 0.58$). These represent highly important traits for fresh-market tomato breeding. Pleiotropic effects for *SL*, *F* and color indexes were also detected in our NIL collection (Table 1 and Fig. 3). On the other hand, there were some negative correlations, for example, *TA* and *pH* ($r = -0.78$). This is in accordance to Fulton et al. (2002) where *pH* was negatively correlated to citric and malic acid measured by *TA*. As citric acid was positive correlated to total acid concentrations, these authors have proposed that the selection of high content of citric acid would be an effective way for maximize the effect on both acidity levels and *pH*.

In the PCA (Fig. 4b), the first two PCs explained 59.9% of the variation. The PC1 accounted for 36.0% and was weighted towards size (*D*, *FW* and *LN*) and quality (*pH* and *TA*) traits, while the PC2 accounted for 23.9% and was weighted toward *F, SL* and color indexes *a/b* and *L*. In order to avoid redundancy, traits were selected based on their correlations and their high contributions to the first two PCs. The traits explaining the most variation for each PC were retained with the exceptions of *D* and *F* as they were highly correlated to *FW* and *SL*, respectively. *H* was not retained because

of its positive correlation to *FW* and its low loading to the first two PCs. *FS*, *PT* and *SSC* were retained because of their implications to consumers' perception despite of their low contribution to the PCs.

A cluster analysis was performed based on the estimated BLUPs from the retained phenotypic traits. The 22 NILs-PP segregated into four major clusters displayed in Fig. 4b. The NILs from cluster I and II grouped separately although they share many size and morphology characteristics (Online Resource 5). Cluster I was formed by five NILs in which fleshy fruits predominate (0.56 ± 0.02 cm of mean *PT*). These NILs are characterized by red round fruits of 77.0 ± 4.0 g, with low *LN* and *SSC*. On the other hand, cluster II was differentiated by NILs of an intense red color and higher *SSC*. This cluster showed the lowest *FW* (69.0 ± 5.0 g). Cluster III was formed by four NILs that grouped with CAI. It showed lines with slightly flattened fruits with the highest values for *D* (5.8 ± 0.2 cm), *FW* (97.0 ± 8.0 g) and *LN* (7.41 ± 0.48), characteristics recovered from the cultivated parent. The last cluster grouped eight NILs represented by tomatoes of 74.0 ± 4.0 g of mean *FW* and with good attributes recovered from its wild parent such as the longest *SL* (21 ± 2 days) and the highest *F*.

This analysis differentiates NILs-PP from the collection based on the size and fruit quality. These results indicate that the wild introgressions from LA0722 provide a source of variation with positive effects on fruit quality. The NILs from cluster IV displayed attributes that delay fruit ripening such us long *SL* and high *F*. Particularly, a *SL* QTL observed in NIL034 showed stability over the two years without any deleterious effect on other quality traits. The NIL320 showed a stable QTL for *F* and also presented yellow fruits both years. Besides, three NILs (NIL115, NIL276 and NIL069) showed *F* QTLs in the first year and they also displayed pleotropic effects for yellow color and long *SL*. Other desirable characteristic for fresh market tomatoes are fleshy fruits. Cluster I grouped NILs with the highest *PT*. A *PT* QTL in NIL1061 showed stability both years. The NILs from cluster III grouped with the recurrent parent mostly for shape and size characteristics (heavier and more flattened fruits). In this group, NIL080 showed large red fruits and a *SL* QTL in which the heterozygous condition delay fruit ripening.

Implication of the tomato NIL collection

Genetically improved seeds represent the main vehicle for adding value to agricultural primary products. A new collection of 22 improved tomato NILs was obtained from the interspecific cross between *S. lycopersicum* cv. Caimanta and the *S. pimpinellifolium* accession LA0722. The methodology applied for obtaining the NILs consisted in a QTL analysis coupled to the development of new varieties. The selection process was assisted by a set of SSR markers distributed along the tomato genome, considering one SSR *locus* segregating per NIL, and promising

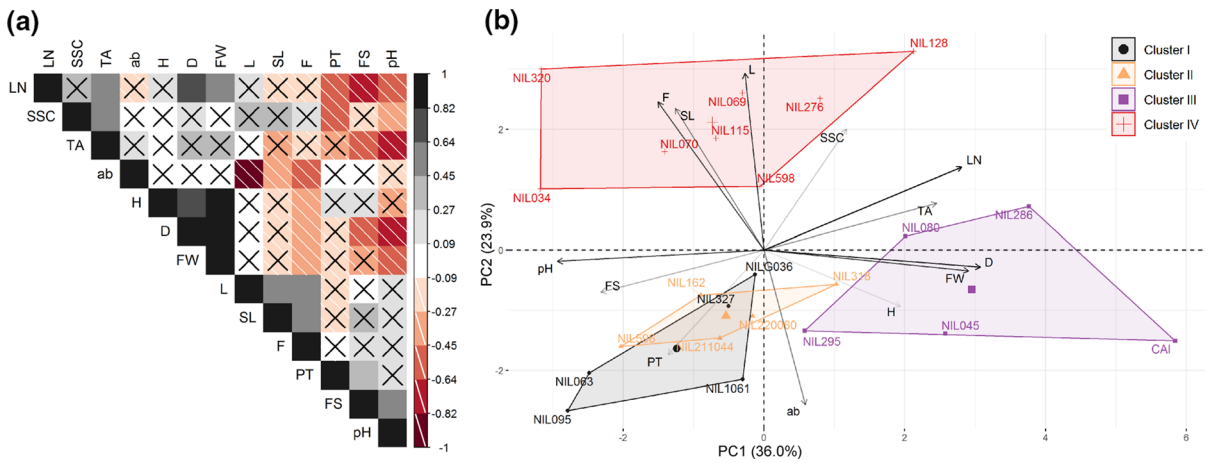


Fig. 4 Performance of the NILs with homozygous *S. pimpinellifolium* LA0722 introgressions **a** Correlation plot. Pearson's coefficients are detailed in the scale. Significance level of 0.05. The non-significant correlations are represented by a cross. **b** Principal component (PC) and cluster analysis based on the BLUPs. NILs of each cluster are differentiated in the PCA

phenotypic traits, particularly long *SL*. Thereby, new breeding lines were obtained in a relatively low number of generations (three or four BC and some self-pollination).

The NILs from the collection represent lines near isogenic to a cultivated tomato with positive attributes provided by the wild accession LA0722 from *S. pimpinellifolium*. New phenotypes were obtained due to combinations and interactions between donor alleles and the receptor background. This has allowed us to broaden the phenotypic and genetic variability. The tomato lines of our collection presented medium to large size, all differentiated from their wild parent LA0722 and some even reached the *FW* of CAI. We were also able to find several outstanding NILs with long *SL*, an essential trait for the reduction of postharvest losses. These results are promising to *SL* QTL pyramiding and would provide more insights in this trait. The NILs are extremely valuable genetic resources to study the genetic bases of fruit ripening and those components that define traits of agronomic importance and fruit quality. The 22 NILs of our collection constitute potential new cultivars with longer *SL* and improved fruit quality.

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Authors' contributions Melisa Di Giacomo and Marianela D. Luciani: first draft of the manuscript, plant material development, molecular and phenotype characterization, formal analysis; Vladimir Cambiaso: review and editing, molecular and phenotype characterization; Roxana Zorzoli: review and editing, conception and design, formal analysis; Gustavo R. Rodríguez: review and editing, conception and design, formal analysis, funding acquisition; Javier H. Pereira da Costa: review and editing, plant material development, conception and design, formal analysis, funding acquisition.

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Availability of data and material Data supporting the findings of this work are available within this published article and its Electronic Supplementary Material.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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