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Phenotypic and molecular characterization of a tomato (*Solanum lycopersicum* L.) F_2 population segregation for improving shelf life

K.N. Yogendra and P.H. Ramanjini Gowda

Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, India

Corresponding author: K.N. Yogendra
E-mail: yogendrakn@gmail.com

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ABSTRACT. Breeding for better quality fruits is a major focus for tomatoes, which are continuously subjected to post-harvest losses. Several methods have been used to improve the fruit shelf life of tomatoes, including the use of ripening gene mutants of *Solanum lycopersicum*. We developed extended shelf-life tomato hybrids with better quality fruits using ripening mutants. Nine tomato crosses were developed using 3 fruit ripening gene mutants of *S. lycopersicum* [alcobaca (*alc*), non-ripening, and ripening inhibitor] and 3 agronomically superior Indian cultivars ('Sankranti', 'Vaibhav', and 'Pusaruby') with short shelf life. The hybrid progenies developed from *alc* x 'Vaibhav' had the highest extended shelf life (up to 40 days) compared with that of other varieties and hybrids. Further, the F_2 progenies of *alc* x 'Vaibhav' were evaluated for fruit quality traits and yield parameters. A wide range of genetic variability was observed in shelf life (5-106 days) and fruit firmness (0.55-10.65 lbs/cm^2). The potential polymorphic simple sequence repeat markers underlying shelf life traits were identified in an F_2 mapping population. The marker association with fruit quality traits and yield was confirmed with single-marker analysis and composite

interval mapping. The genetic parameters analyzed in the parents and F_1 and F_2 populations indicated that the cross between the cultivar 'Vaibhav' and ripening gene mutant *alc* yielded fruit with long shelf life and good quality.

Key words: Composite interval mapping; Fruit firmness; Shelf life; Simple sequence repeats; Single marker analysis

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important crop in terms of nutrition, health, and economic value. It experiences post-harvest losses owing to natural perishability, precarious transportation, improper storage conditions, and inadequate packaging. Post-harvest losses of tomato in Southeast Asia account for 13-20% of all harvested tomatoes. In India, post-harvest losses during storage of tomato account for 30-35% of all harvested tomatoes (Kumar et al., 2004).

Post-harvest packaging methods, such as storage in perforated (0.25%) polythene bags under ambient conditions (temperature of 20°-25°C and relative humidity of 70-90%) (Nasrin et al., 2008) and the use of black perforated polythene bags (Rahman et al., 2010), treating fruits with chlorine and calcium chloride, and treatments of 0.1% gibberellic acid and 0.4 mM salicylic acid (Pila et al., 2010), have been shown to decrease fruit decay and weight loss. However, these methods are laborious, and chemically treated fruits are not readily accepted in the market. Tomato is a climacteric fruit, and ethylene is required for normal ripening (Alexander and Grierson, 2002). The advanced RNA interference technique is an efficient method for downregulating the genes involved in ethylene biosynthesis as well as enzymes that degrade the cell wall (Carrari et al., 2007) to extend the shelf life of tomato (Kramer and Redenbaugh, 1994; Xiong et al., 2005; Batra et al., 2010; Meli et al., 2010). However, genetically modified crops are not marketable worldwide owing to environmental safety issues and social acceptance (Qaim, 2009). Therefore, genetic enhancement of major fruit quality characteristics through exploration of genetic diversity within the available germplasm is a viable and environmentally safe option for improving shelf life. Kopeliovitch et al. (1979) have used several ripening gene mutants, such as alcobaca (*alc*), non-ripening (*nor*), never ripe, and ripening inhibitor (*rin*) to develop lines and cultivars with delayed ripening through disruption of the ethylene signaling pathway.

Integrating molecular approaches with conventional breeding to enhance fruit quality could significantly improve the post-harvest shelf life of tomatoes. The levels of genetic variation within the cultivated tomato detected by most molecular markers are very small, which is a burden for marker-assisted selection (MAS) with respect to a wide range of important fruit quality traits.

In this study, tomato hybrids with enhanced shelf life were developed using ripening mutants and agronomically superior Indian cultivars, and hybrids from all possible line x tester crosses were screened for shelf life, yield, and other fruit qualities. Furthermore, the F_2 population developed from the best-performing F_1 hybrid (*alc* x 'Vaibhav') was used to map quantitative trait loci (QTLs) associated with shelf life and fruit firmness using simple sequence repeat (SSR) markers.

MATERIAL AND METHODS

Plant materials and development of F_1 hybrids

The ripening gene mutants of *S. lycopersicum* - *rin* (green to lemon color fruits with little or no lycopene, *alc* (yellow to light red fruit), and *nor* (orange yellow fruit color without characteristic changes associated with ripening and fruit development) - were used as sources for enhanced shelf life and crossed with Indian cultivars ('Sankranti', 'Vaibhav', and 'Pusa-ruby') using a line x tester (3 x 3) design during Kharif 2009. 'Sankranti' and 'Vaibhav' are cultivars with high yield, resistance to leaf curl, and short shelf life released by the University of Agricultural Sciences, Bangalore, India. 'Pusaruby' is a short-shelf-life variety released by the Indian Agricultural Research Institute, New Delhi, India.

Thirty-day-old seedlings were transplanted into the experimental plot with a spacing of 90 x 40 cm per standard cultural recommendations for the area in a randomized complete block design. The parents and hybrids were evaluated for growth, yield, and fruit quality traits during Rabi 2009.

Traits evaluated

Phenotypic data were recorded for various traits in parents and hybrids: plant height (cm), number of branches per plant, number of fruits per cluster, total soluble solids [TSS (%); measured using a hand refractometer (Swastik Scientific Co., Mumbai, India)], and fruit firmness [lbs/cm^2; measured using a fruit penetrometer (Wagner Instruments, New Delhi, India)]. Fruit yield in grams per plant and lycopene content (mg/100 g) were estimated by blending tomato pulp in acetone (AR grade, Sigma-Aldrich, India) and dissolving it in petroleum ether 40-60 (AR, Sigma-Aldrich). The petroleum ether extract was decanted and the absorbance was measured in a spectrophotometer at 503 nm using petroleum ether as a blank (Ranganna, 1976).

Evaluation of shelf life

Five tomato fruits at the red ripening stage (breaker stage) were harvested and stored at 25° ± 1°C, and shelf life in days was assessed at weekly intervals. Shelf life was measured as the number of days elapsed between the harvest of fruits at the red ripening stage and the end of the consumption stage (first symptoms of deterioration and excessive softening).

Development of the F_2 mapping population

The *alc* x 'Vaibhav' cross was selected based on the mean performances of the hybrid and the parents. A mapping population of 210 F_2 individuals was developed by selfing the F_1 hybrids. The parental lines and F_2 population were evaluated for growth, yield, and fruit quality traits during Kharif 2010.

Genotyping F_2 mapping population

Genomic DNA was extracted from the young leaves (30 days after transplanting) of F_2 progeny and parents using a modified cetyltrimethylammonium bromide method (Saghai-Ma-

roof et al., 1984). In this study, 224 SSR markers distributed over all 12 tomato chromosomes were selected from the Sol Genomics Network database [He et al., 2003; http://solgenomics.net/ (accessed September 23, 2012)] and used to assess parental polymorphisms. Polymorphic SSR markers were used for single-marker analysis (SMA) using QTL Cartographer and to develop a genetic linkage map using MAPMAKER/ExP 3.0 with a threshold of 2.5 (Lander et al., 1987). The map was based on 45 polymorphic SSR marker loci (Table S1) using 210 F_2 plants of *alc x* 'Vaibhav'. The DNA markers linked to fruit quality traits and yield were identified using QTL Cartographer. The linkage map data were imported from MAPMAKER/ExP, and the composite interval mapping (CIM) was carried out using QTL Cartographer at a logarithmic odds likelihood ratio of 2.5. The QTL map was developed based on the CIM results using the Mapchart software, 2006 (Voorrips, 2002).

Statistical analysis

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability in the broad sense, and genetic advance were calculated using the methods described by Weber and Moorthy (1952) and Burton (1952). Normality for all traits recorded was tested using the Shapiro-Wilk W-test (Shapiro et al., 1968). Skewness and kurtosis were calculated for all traits using STASTICA (StatSoft India Pvt. Ltd., New Delhi, India).

RESULTS

Phenotypic characterization

In recent years, renewed interest has developed in the possibility of breeding not only for higher yielding but also better quality crops. One of the potential approaches is a combination of traditional breeding methods with spatial profiling and introgression breeding. The compositional approach to improve organoleptic properties, particularly in tomato, is driven by interest in nutritional attributes (increase in lycopene content and flavonoids), increased shelf life, and improved fruit quality. We developed tomato hybrids with extended shelf life and good quality by crossing the ripening gene mutants of *S. lycopersicum* (*rin*, *alc*, and *nor*) with agronomically superior Indian cultivars ('Sankranti', 'Vaibhav' and 'Pusaruby'). The mean values of all evaluated traits and their standard errors are provided in Table 1. The ripening gene mutants were significantly different from the Indian cultivars with respect to fruit quality traits. With respect to fruit shelf life, the mean number of 44 days in *alc* was significantly higher than that in the other ripening gene mutants *rin* (38 days) and *nor* (38.5 days). Indian cultivars 'Sankranti' and 'Vaibhav' had a mean number of 19 and 18.50 days, respectively, which was higher than that of 'Pusaruby' (14.5 days). The F_1 (*alc* x 'Vaibhav') had a mean fruit shelf life of 40.50 days, which was higher than the mean values of other hybrids (35.39 days; Figures 1 and 2). The hybrids developed using ripening gene mutants displayed a 2-fold increase in shelf life over those of the Indian cultivars. Similar results were obtained by Rodriguez et al. (2010) using mutant gene *nor*. They developed a cross *nor* x 'Ce' in which the F_1 had a shelf life of 64.1 days compared with those of *nor* (52.1 days) and 'Ce' (23.9 days). This difference occurs because the ripening gene mutants participate in ethylene-independent signaling and impart delayed ripening in tomato (Barry and Giovannoni, 2007).

Table 1. Mean performance of tomato parents and hybrids with respect to plant growth, fruit quality and yield traits.

	Plant height (cm)	No. of branches/plant	No. of fruits/ cluster	Lycopene (mg/100 g)	TSS (%)	Fruit firmness (lbs/cm²)	Shelf life (days)	Yield/plant (g)
Lines								
rin	95.00	24.50	4.50	0.04	19.5	7.88	38.00	2525.00
alc	103.50	18.50	4.75	0.73	29.00	7.56	44.00	3550.00
nor	109.50	23.00	4.00	0.15	18.50	8.44	38.50	3175.00
Mean ±	102.66	22.00	4.41	0.31	22.33	7.96	40.16	3083.33
SE	2.04	1.19	0.33	0.01	0.12	0.25	0.79	80.40
CD (P = 0.05)	4.39	2.56	0.72	0.03	0.27	0.54	1.7	172.88
Testers								
Sankranti	87.00	23.00	4.25	0.81	26.50	5.00	19.00	2925.00
Vaibhav	93.50	21.50	4.75	0.84	23.50	4.38	18.50	3125.00
Pusaruby	95.00	22.50	5.20	0.95	30.50	3.94	14.50	2475.00
Mean ±	91.83	22.33	4.73	0.86	26.83	4.44	17.33	2841.67
SE	2.04	1.19	0.33	0.01	0.12	0.25	0.79	80.40
CD (P = 0.05)	4.39	2.56	0.72	0.03	0.27	0.54	1.70	172.88
Hybrids								
rin x Sankranti	85.00	30.00	4.45	0.62	28.50	3.38	34.00	2575.00
rin x Vaibhav	67.50	26.50	4.50	0.48	30.50	4.66	35.00	2460.00
rin x Pusaruby	69.00	25.50	4.50	0.52	39.50	4.88	34.50	2675.00
alc x Sankranti	77.50	25.00	4.25	0.57	31.00	5.94	35.00	3475.00
alc x Vaibhav	62.00	21.00	4.25	0.61	31.50	5.72	40.50	3675.00
alc x Pusaruby	80.00	29.00	4.50	1.75	52.50	6.21	35.50	3675.00
nor x Sankranti	67.50	21.50	4.00	0.53	29.50	4.63	33.50	3175.00
nor x Vaibhav	64.00	24.00	4.00	0.63	29.00	4.38	35.00	3075.00
nor x Pusaruby	65.00	24.00	4.00	0.61	39.00	4.31	35.50	3250.00
Mean ±	70.83	25.17	4.27	0.70	34.60	4.90	35.39	3115.00
SE	1.52	0.82	0.12	0.01	0.16	0.19	0.54	59.94
CD (P = 0.05)	4.95	2.70	0.41	0.04	0.55	0.63	1.78	195.50

TSS = total soluble solids; SE = standard error; CD = critical difference.

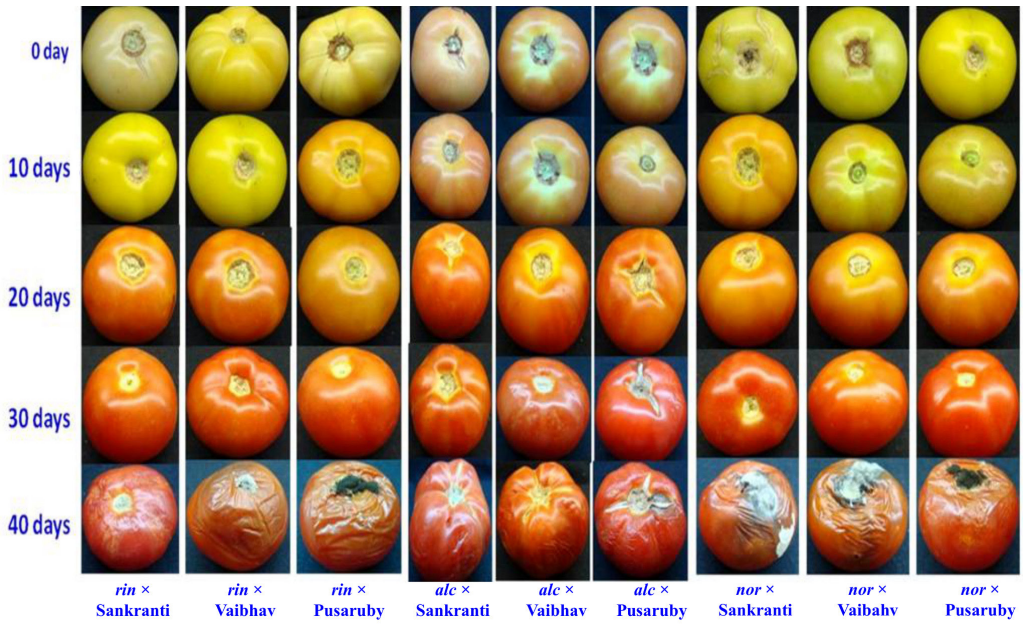

Figure 1. Fruit shelf life of 9 different tomato hybrids at 25°±1°C.

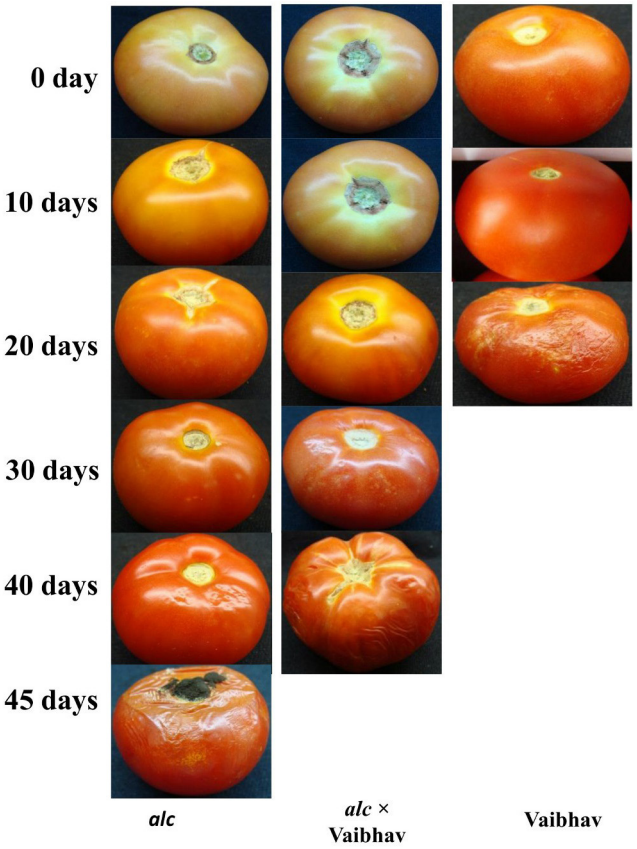

Figure 2. Fruit shelf life of tomato hybrid "*alc* x Vaibhav" and its parents at 25°±1°C.

For fruit firmness, *nor* recorded high values (8.44 lbs/cm^2) compared with those of the mutant genes *rin* (7.88 lbs/cm^2) and *alc* (7.56 lbs/cm^2). However, Indian cultivars 'Sankranti' (5.0 lbs/cm^2), 'Vaibhav' (4.38 lbs/cm^2), and 'Pusaruby' (3.94 lbs/cm^2) recorded low firm fruit values. The ripening mutants showed dominance F_1 crosses compared with Indian varieties. The F_1 *alc* x 'Pusaruby' (6.21 lbs/cm^2) showed intermediate firmness values between both parents. Fruit firmness is usually directly propositional to fruit shelf life, and these mutants have low pectolytic activity (Kopeliovitch et al., 1979). Rodriguez et al. (2010) have recorded similar findings in the F_1 cross ca x *nor*, and Dias et al. (2003) have found that the F_1 ('TOM591' x 'Mospomorist') *alc*$^+$/*alc* genotype improves the rate of fruit firmness to 8.6128 N/m^2 compared with that in the parents and contributes to an increase in post-harvest shelf life.

The Indian cultivars had significantly higher lycopene content than the ripening gene mutants. 'Pusaruby' displayed the highest lycopene content of 0.95 mg/100 g compared with those of 'Sankranti' (0.81 mg/100 g) and 'Vaibhav' (0.84 mg/100 g). The *rin* (0.04 mg/100 g) and *nor* (0.15 mg/100 g) mutants recorded low lycopene content, but *alc* (0.73 mg/100 g) displayed a significantly higher level similar to those of Indian cultivars, and Kopeliovitch et al. (1980) have reported a lycopene content in *alc* of 1.182 mg/100 g. The low lycopene

contents of the *nor* and *rin* ripening mutants agree with the results of others (Buescher et al., 1976; Tigchelaar et al., 1976). In both *nor* and *rin* mutants, the fruits develop normally before the mature green stage, but once mature, they fail to synthesize lycopene and other carotenoids (Cantu et al., 2009). However, the F_1 (*alc* x 'Pusaruby') showed significantly higher values (1.75 mg/100 g), whereas the values for other hybrids were similar to those of the Indian cultivars. This outcome may be due to heterotic vigor.

The maturity of tomato fruit is determined by its flavor. Flavor includes sugar and acid levels and aromatic volatiles (Jones and Scott, 1983). 'Pusaruby' displayed the highest lycopene content of 30.5%, followed by those of 'Sankranti' (26.5%) and 'Vaibhav' (23.5%). With respect to ripening, *alc* (29%) displayed a higher level of TSS compared to that in *rin* (19.5%) and *nor* (18.5%). In this study, TSS in the F_1 was higher than that in the parents. The cross *alc* x 'Pusaruby' recorded the highest TSS content of 52.5% compared with those of the other hybrids. This hybrid is significantly different from its parents. Conversely, Rodriguez et al. (2010) have reported an F_1 (*nor* x Ce) containing soluble solids of 4.8 °Brix, which is similar to that of the mutant genotype *nor* (4.9 °Brix).

Genetic variability analysis

The availability of genetic variation is a prerequisite for the initiation of a breeding program to facilitate selection in any crop. The analysis of variance recorded significant mean squares estimates for all the traits, indicating sufficient diversity among the F_2 genotypes (Table 2). Fruit yield, being a quantitative character, is influenced by a large number of genes that are greatly controlled by environmental factors. The variability observed is the sum total of the hereditary effects of involved genes as well as environmental influence. Hence the variability is partitioned into heritable and non-heritable components with suitable genetic parameters such as GCV, PCV, heritability, and genetic advance. The estimation of these variability parameters helps breeders achieve the required crop improvement through selection. Among all traits, GCV was smaller than PCV, indicating some influence of environment on the expression of these characters. The heritability estimates ranged from 19.02% in lycopene content to 98% in shelf life. The genetic advance as percent of the mean was 0.23 and 325.06% in lycopene content and fruit yield, respectively. The characters showing a wide range of variation provide ample scope for selecting the desirable types. The same trend in plant height, number of branches per plant, number of fruits per cluster, lycopene, TSS, fruit firmness, and shelf life has been reported by Ghosh et al. (2010) and Shashikanth et al. (2010), and fruit yield has been reported by Shashikanth et al. (2010) and Dar and Sharma (2011).

The shelf life of the fruits obtained from the selected F_2 breeding line varied from 5 to 106 days with a mean value of 53.56 days. However, Rodriguez et al. (2010) have indicated that the fruits obtained from the F_2 population of *nor* x Ce displayed a mean shelf life of 43.5 days. The mean and range in the number of days presented by several other researchers and the results obtained in the present study indicate the power of identifying the genomic regions that are significantly associated with quantitative traits in populations that segregate simultaneously for multiple QTLs scattered throughout the entire genome. The independent segregating loci can and are likely to mask the effect of one another through epistatic interactions. The actual variance estimated for the characters varies with the extent of variability in the breeding lines/populations. The coefficient of variation evaluated by considering the respective means shows higher values for the selected parameters, indicating wider diversity and vice versa.

Table 2. Mean and genetic parameters for plant growth, fruit quality and yield attributing traits in F_2 population of the cross '*alc* x Vaibhav'.

Trait	Mean	Range		PCV (%)	GCV (%)	H^2 (%)	GA (%) mean
		Minimum	Maximum				
Plant height (cm)	63.48	26.00	100.00	22.03	21.63	94.42	36.35
No. of branches/plant	11.20	2.00	25.00	39.92	38.22	91.67	11.06
No. of fruits/cluster	2.54	1.00	4.50	32.36	32.08	93.28	2.18
Lycopene (mg/100 g)	0.80	0.10	2.00	56.71	24.73	19.02	0.23
TSS (%)	32.00	10.00	59.00	37.07	37.07	95.50	3.20
Fruit firmness (lbs/cm^2)	4.95	0.55	10.65	30.27	30.10	95.00	4.00
Shelf life (days)	53.56	5.00	106.00	55.31	55.27	98.00	79.76
Yield/plant (g)	239.07	14.50	1115.00	78.35	62.59	63.81	325.06

TSS = total soluble solids; PCV = phenotypic coefficient of variation; GCV = genotypic coefficient of variation; H^2 = broad sense heritability; GA = genetic advance.

Test of normality

The Shapiro-Wilk W-test revealed that most of the traits in F_2 population of cross *alc* x 'Vaibhav' did not show a normal distribution pattern. Fruit firmness, number of fruits per cluster, lycopene content, and fruit yield were positively skewed, whereas fruit shelf life and total soluble solids were negatively skewed (Figure 3). The distribution curves for all traits were platykurtic except for fruit yield, which was leptokurtic with kurtosis more than 3. Inheritance of fruit shelf life and TSS involved a large number of dominant genes, the majority of them having increasing effects and duplicate epistasis owing to negatively skewed and platykurtic distribution. However, positively skewed and platykurtic distribution is evidence for the involvement of a moderate number of genes among which equal frequency of genes increases and decreases the effects with complementary types of epistasis present in expression of fruit firmness, lycopene level, and number of fruits per cluster. However, inheritance of fruit yield per plant was leptokurtic and positively skewed, involving dominance-based complementary interaction. The phenomena of linkage drag, linkage disequilibrium, natural selection, and meiotic distortion may explain some of the deviation from normal distribution of traits in the F_2 populations of this cross. These influences have also been noted in a recombinant inbred line population of rice ('IR50' x 'Moroberekan') exhibiting a departure from normal deviation under aerobic conditions (Girish et al., 2006).

Molecular characterization

Ripening is of interest to tomato breeders because it affects several quality traits, including color, flavor, TSS, and shelf life, which are important for fresh market tomatoes. Shelf life is quantitatively inherited, and improving such a trait requires molecular marker-based strategies. Although the tomato is completely sequenced, its genomic resources have not been fully exploited. Few studies have reported the detection of QTLs using SSR markers for fruit quality traits in tomato. In this study, 224 SSR markers were used to reveal genetic differences between 2 parents. Genetic polymorphism was scored among the F_2 mapping population of cross *alc* x 'Vaibhav' using 45 polymorphic SSR markers. The association of markers to fruit quality traits was detected using both SMA and CIM.

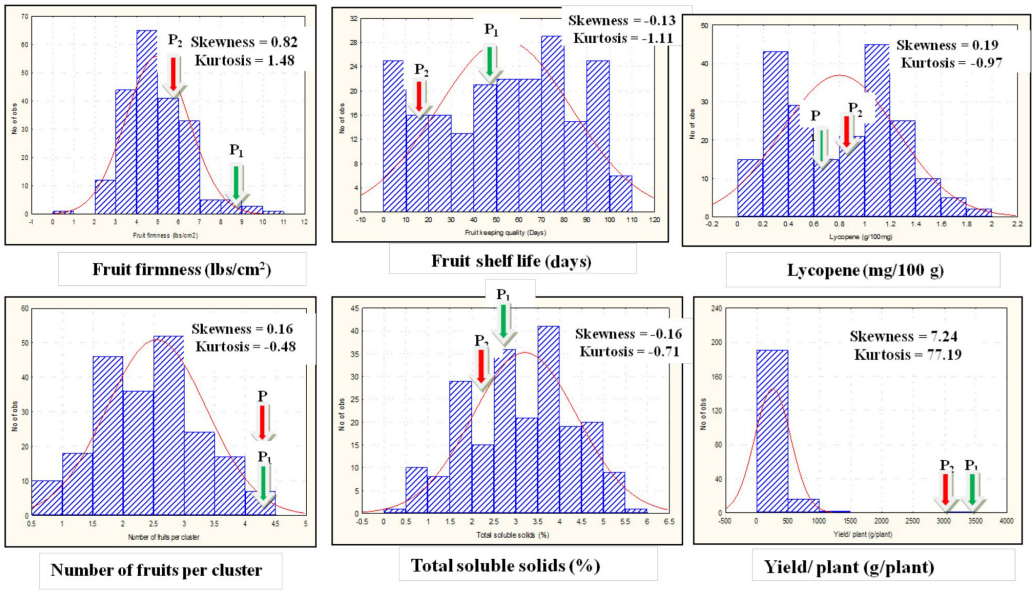

Figure 3. Frequency distribution for fruit quality and yield parameters among F_2 populations of the cross "*alc* x Vaibhav". P_1 = female parent "*alc*"; P_2 = male parent "Vaibhav".

The association of a single marker to a number of traits is important because it reveals the nature of the particular marker. The SMA revealed that SSR markers were associated with fruit quality traits. For shelf life, LEaat3 (P = 0.043), SSR310 (P = 0.031), and LEga7 (P = 0.047) showed significance at 5%. LEga7 (P = 0.007) and LEaat7 (P = 0.002) showed 1% significance with respect to fruit firmness, whereas LEga7 recorded 5% significance at P = 0.033 for TSS. Overlapping markers for TSS, fruit firmness, and shelf life were examined. The SSR marker LEga7 was associated with fruit quality traits such as TSS, fruit firmness, and shelf life, and 2 markers, LEaat3 and LEga7, were associated with fruit firmness and shelf life (Figure 4). Such examples have been well documented in tomato, in which SMA has associated markers with many traits. Kabelka et al. (2004) have detected 2 QTLs on chromosomes 4 and 11 for tomato color based on SMA in backcross line IBL2349. Similarly, Salari and Prasad (2010) conducted SMA that revealed that random amplified polymorphic DNA markers ($OPC4_{950}$ and $OPC4_{300}$) are highly correlated with lycopene content. This research demonstrates the pleiotropic nature of the markers, which can be used effectively in MAS programs or in trait introgression breeding.

QTL analysis

A genetic map containing 45 polymorphic SSR markers was used for CIM analysis. A total of 9 QTLs with significant effects were detected, distributed in 6 of 8 linkage groups (Table 3, Figure 5). These QTLs explaining phenotypic variations ranged from 4 to 11%, which confirms the complex genetic nature of these traits and the possible influence of environmental effects.

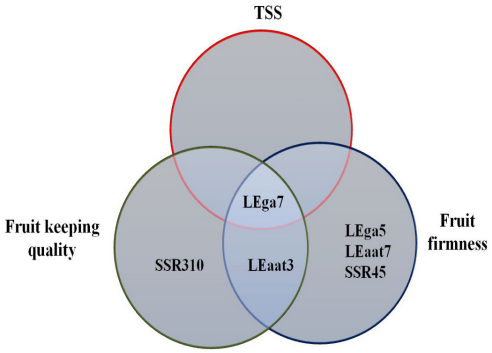

Figure 4. Overlapping markers between total soluble solids (TSS), fruit firmness and fruit keeping quality of single marker analysis in the F_2 population of the cross "*alc* x Vaibhav".

Table 3. Quantitative trait loci (QTLs) associated with fruit quality traits in the F_2 mapping population of the cross '*alc* x Vaibhav'.

Trait	Linkage group	QTL name	Marker intervals	LOD	R^2	Additive effect
Fruit firmness	1	*fr_fi*$_1$	LEga5-LEaat3	2.30	0.08	0.73
	3	*fr_fi*$_2$	LEat18-LEga7	2.60	0.10	0.72
	4	*fr_fi*$_3$	LEaat7-LEaat8	2.30	0.07	0.54
	8	*fr_fi*$_4$	SSR45-LEaat1	2.10	0.07	-0.47
Shelf life	6	*fr_ke_qlty*$_1$	LEgata2-LEta16	2.10	0.08	6.4
Fruit yield	1	*pl_yld*$_1$	LEga6-LEga5	64.18	0.08	0.05
	1	*pl_yld*$_2$	LEga5-LEaat3	59.77	0.08	0.01
	3	*pl_yld*$_3$	LEat18-LEga7	62.71	0.08	0.04
	8	*pl_yld*$_4$	SSR45-LEaat1	58.14	0.04	0.12

LOD = logarithm of the odds.

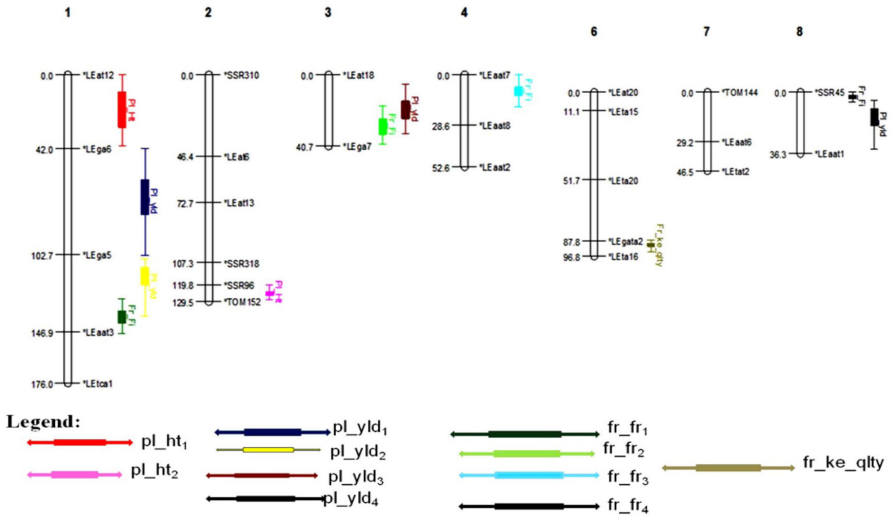

Figure 5. Quantitative trait locus (QTL) map showing location of the QTLs for plant height, fruit quality and yield traits in the F_2 population of the cross "*alc* x Vaibhav".

QTLs for fruit firmness

Four QTLs were identified for fruit firmness. QTL *fr_fi*$_1$ located on linkage group one was flanked by LEga5 and LEaat3. This QTL explained 8% of the phenotypic variation and displayed a positive additive effect of 0.73. Other QTLs, *fr_fi*$_2$ (LEat18-LEga7) and *fr_fi*$_3$ (LEaat7-LEaat8), explained 10 and 7% of the phenotypic variation at the test loci and had additive effects of 0.72 and 0.54, respectively. Another QTL named *fr_fi*$_4$, flanked by SSR45 and LEaat1, explained 7% of the total phenotypic variation. It had a negative effect of -0.47. QTLs for tomato fruit firmness have been detected in several segregating populations using various marker systems (Fulton et al., 1997, 2000; Frary et al., 2004; Walley and Seymour, 2006). Tanksley et al. (1996) have reported QTLs for fruit firmness on chromosomes 2, 3, 4, and 8 using a restriction fragment length polymorphism marker system. Likewise, QTLs for fruit firmness have been detected on chromosomes 1, 3, 4, 6, 9, and 11 (Fulton et al., 2000).

QTLs for shelf life

A single QTL was identified for shelf life on linkage group six. This QTL, *fr_ke_qlty*$_1$, was flanked by LEgata2 and LEta16 and recorded 8% of phenotypic variation and an additive effect of 6.40. Interestingly, markers LEaat3 and LEga5 flank a QTL for fruit firmness named *fr_fi*$_1$ that was also associated with fruit firmness when markers were associated with traits using an SMA approach (Table 4). Similarly, markers LEga7, LEaat7, and SSR45 flank QTLs for fruit firmness. The identified overlapping markers can be putatively used in crop improvement programs through MAS approaches.

Table 4. Markers linked to fruit firmness trait by both single marker analysis and composite interval mapping in F_2 populations of the cross *alc* x Vaibhav.

Trait	Composite interval mapping	Single marker analysis	
	Flanking markers of QTL	Marker	P
Fruit firmness	LEga5-LEaat3	LEga5	0.035*
	LEga5-LEaat3	LEaat3	0.013*
	LEat18-LEga7	LEga7	0.007**
	LEaat7-LEaat8	LEaat7	0.002*
	SSR45-LEaat1	SSR45	0.003**

*5% significance; **1% significance. QTL = quantitative trait loci.

Finally, our results demonstrate that one of the significant impacts of globalization on horticulture crops has been an increasing demand for quality improvement and the wider adoption of quality standards for fruits and vegetables. In self-pollinated crops such as tomato, genetic variability is lower, which results in low polymorphism. We have attempted to unravel the available variability for more important but less pursued traits such as fruit shelf life, fruit firmness, TSS, and lycopene content. The genetic parameters analyzed for the parents and F_1 and F_2 populations of the cross *alc* x 'Vaibhav' showed that the use of ripening gene mutants is a feasible way to improve shelf life through tomato breeding.

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Supplementary material

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