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Phenotypic evaluation and molecular characterization of *alc*/vaibhav recombinant inbred population of tomato for yield, shelf life and fruit quality parameters

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Abstract

Tomato is second most important vegetable, having nutritional and aesthetic value. Due to high temperature and lack of proper storage facility most of the produce is wasted. This loss can be reduced by increasing shelf life of tomato, so that produce can be stored for long time. In the present investigation *alcobacca*, a ripening mutant of tomato has been crossed with tomato variety, Vaibhav. To dissect trait genetics, Genetic variability, Correlation and path coefficient analysis were conducted in the F_6 recombinant inbred population developed from *alc* x Vaibhav by single seed descent method. Highest shelf life of 85 days was observed with a mean of 51 days for RILs. Shelf life was significantly and positively associated with fruits per cluster (0.13@5%), fruit firmness (0.23 @ 1%, 5%), total soluble solutes (0.15@1%,5%), lycopene content (0.12@5%), yield per plant (0.30@1%,5%) and number of fruits per plant (0.38@1%,5%). Thirty eight polymorphic SSR markers have been screened for RILs. QTL detection was done using ANOVA and Linear regression using Minitab® 16.1.1. Three SSR markers *viz*., SSR146, LEaat007 and TGS2259 have been found to be linked to shelf life with R square value of 2.9, 4.1 and 3.5 respectively.

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Introduction

Tomato is most important vegetable crop (FAOSTAT, 2013). In India, tomato is grown in 880,000 ha with a production of 18,227,000 Mt and productivity of 19.5 mt ha^{-1} (Indian Horticulture Database, 2014). The tomato grown regions where supply of the tomato produce is very limited for a particular period there will be always fluctuation in the price of the product. Due to lack of storage facilities, precarious transportation oftenly results in loss of the produce. As a result, farmer has to sell his product at lower price. By increasing the shelf life, the quality of the fruit losses can be minimized.

Many studies have suggested the use of mutants for increasing shelf life of tomato (Mutschler *et al*., 1992; Dias *et al*., 2003; Faria *et al*., 2003; Garg *et al*., 2008; Garg and Cheema, 2011; Rodríguez *et al*. 2011; Casals *et al*., 2012; CVIKIC *et al*., 2012; Yogendra and Ramanjini, 2012 and Pech *et al*., 2013). Apart from many ripening mutants like *rin*, *nor*, *Nr* and *alc*, *S. pimpinellifolium* is also used as donor for extending the post harvest life of tomato (Costa *et al*., 2013). However alcobac(*alc*)¸ most extensively used ripening mutant in tomato (Kopeliovitch *et al*., 1981; Mutschler *et al*., 1992; Ara´ujo *et al*., 2002; Dhatt *et al*., 2003; Dias *et al*., 2003; Faria *et al.,* 2003; Garg *et al.,* 2008; Casals *et al.,* 2012; Yogendra and Ramanjini 2012). The fruits of alc/alc genotype are characterized by reduced carotenoid, firm fruits and increased shelf life (Faria *et al.,* 2003). At molecular level it is replacement of thymine by adenine at position 317 (Casals *et al.,* 2012).

Plant genetics and breeding research has been revolutionalised by molecular markers and marker assisted selection (MAS) technology (Foolad and Panthee, 2012; Girish *et al.,* 2006). Recombinant Inbred lines (RIL), Near Isogenic Lines (NIL), Backcross population, F2 population are used for mapping. RIL population provides immortal study material with ease of replication. To exploit the population for various traits, a study was envisaged with an intention of dissecting yield and yield related traits along with shelf life and other fruit quality parameters in recombinant inbred (RI) population derived from the cross between alc × Vaibhav. A cross was made between alc and Vaibhav, and F_2 plants were generated by selfing F_1s (Yogendra and Ramanjini, 2012). RILs were then generated by continuously selfing the progeny of individual members of an F2 population until homozygosity was achieved. Attempt has been made to study shelf life trait, focusing also on yield and other quality parameters. Molecular analysis was done to help the trait selection.

Material and methods

The F6 population (200 RILs) of the cross *alc* and Vaibhav was evaluated in a randomised complete block design (RCBD) during June 2012 in open field with two replications. Thirty-day-old seedlings were transplanted into the experimental plot with a spacing of 90 x 40 cm as per standard cultural recommendations. Healthy crop stand was maintained by following proper plant protection measures. Ten plants per replication were taken for observation.

Traits evaluated

Phenotypic data was recorded for various traits in RILs: plant height (cm), number of fruits per cluster, fruit length (cm), fruit width (cm), fruit firmness [kgs/cm2; measured using a fruit penetrometer (Wagner Instruments, New Delhi, India)], number of locules, fruit yield in grams per plant, number of fruits per plant and single fruit weight (g), total soluble solids [TSS (%); measured using a hand refractometer (Swastik Scientific Co., Mumbai, India)]. 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). Fruit yield in grams per plant, number of fruits per plant and single fruit weight (g).

To evaluate RILs for shelf life, we carried out eight harvests of fruits in brick red stage. Five fruits from each plant were stored at room temperature (25°C). In the postharvest period, fruits were visually inspected every 5 days for signs of infections and fruit deterioration. Fruits with visual defects were discarded and shelf life was recorded. At 88 days (considered the end of the consumer acceptance period), all remaining fruits were discarded and their shelf life was recorded as 88 days. Shelf life (in number of days) was calculated as mean number of days from harvest to discard for the fruits from each plant. From each replication five plants were chosen randomly to calculate mean values for each trait. Mean value was used for conducting further statistical analysis for characters under study.

The methods suggested by Lush (1949), Weber and Moorthy (1952), Burton and Devane (1953), Lush (1949), Hanson *et al.* (1956) and Johnson *et al.* (1955), was employed to compute genotypic and phenotypic coefficients of variation, for heritability and genetic advance, respectively. The formula of Webber and Moorthy (1952) was used to work out correlation coefficients and significance was checked at the probability levels of 0.05 and 0.01 by comparing with Snedecor (1961) 'r' values. Phenotypic correlation for each Path co-efficient analysis was conducted by method explained by Wright (1921) and illustrated by Dewey and Lu (1959).

Molecular characterization

Genomic DNA was extracted from the young leaves (30 days after transplanting) of F_6 progeny and parents using a modified cetyltrimethylammonium bromide method (CTAB). 38 SSR markers (Table 7) distributed on different chromosomes were selected from database (He *et al.,* 2003; Frary *et al.,* 2005; Yogendra, 2011 www.oardc.ohio-state.edu/tomato/SNPdata/SSRPrimerVMar05. xls; http://solgenomics.net/) which were found to be polymorphic for two parents were used for genotyping RILs. Single factor ANOVA and Linear regression were performed between the marker loci and relevant traits using the SMA option in the Minitab® 16.1.1.

Results

Analysis of variance for shelf life, yield traits and fruit quality related traits of RILs (F_6) is presented in Table 1. The analysis of variance showed that there was significant differences among the genotypes for all the traits. The estimates of genetic parameters observed are depicted in Table 2. In all, the genotypic coefficient of variation was smaller than the phenotypic coefficient of variation. TSS was ranging from 1.15 to 8 with mean value of 3.35.

Table 1. Analysis of variance for quality parameters, yield and shelf life traits for RILs (F_6 generation) of *alc* × Vaibhav *kharif* 2012.

Sl. no	Source of variation	df	Mean sum of squares											
			PHT	FRC	FL	FW	FF	NL	TSS	LC	SHL	YLD	NOF	SLW
1.	Replication	1	472.85	0.48	0.01	1.55	0.60	0.01	0.01	0.02	430.18	250796.90	246.12	15.02
2.	Treatments	199	355.46**	4.38**	1.29*	1.95**	7.40**	3.80**	1.44**	1.33*	487.12**	329869.39**	150.60**	1343.06**
3.	Error	199	11.41	0.15	0.57	0.52	0.81	0.36	0.25	0.02	33.52	64011.00	24.37	373.43
	SEm		2.39	0.27	0.53	0.51	0.64	0.42	0.36	0.11	4.09	178.90	3.49	13.66
	CD at 5%		6.62	0.75	1.48	1.41	1.77	1.17	0.99	0.30	11.35	495.89	9.68	37.88
	C V		4.36	8.27	17.77	15.99	24.72	17.40	15.04	18.22	11.86	25.33	26.71	20.23

* Significant at 5%

** Significant at 1%

PHT = Plant height (cm) FRC = Fruits per cluster FL = Fruit length (cm)

FW = Fruit width FF = Fruit Firmness (kg/cm²) NL= Number of locules

TSS = Total soluble solutes (brix) LC= lycopene content (mg/100mg) SHL = Shelf life (days)

YLD = yield per plant (g) NOF = Number of fruits/ plant SLW=Single fruit weight (g)

Table 2. Range, mean and estimates of genetic parameters in RILs of *alc* × Vaibhav during *kharif* 2012.

Character	Range		Mean	PCV	GCV	Heritability	GA as% mean
	Minimum	Maximum					
Plant height (cm)	57.50	114.20	73.65	17.49	16.94	93.78	33.79
Fruits per cluster	1.00	8.00	4.60	32.31	31.23	93.45	62.19
Fruit length (cm)	2.50	7.50	4.25	22.71	14.14	38.77	18.14
Fruit width (cm)	2.10	10.75	4.48	24.68	18.79	57.98	29.47
Fruit Firmness (kg/cm^2)	1.00	12.70	3.80	55.56	49.75	80.20	91.79
Number of locules	2.00	8.50	3.31	41.85	38.06	82.71	71.31
TSS (brix %)	1.15	8.00	3.35	27.48	22.99	70.03	39.64
Lycopene content (mg/100g)	0.05	4.87	0.73	48.85	45.16	76.60	64.72
Shelf life (days)	18.50	85.50	51.15	33.05	30.85	87.12	59.31
Yield per plant (g)	66.00	2269.00	802.63	51.22	42.08	67.50	71.22
Number of Fruits/ plant	1.50	39.50	14.70	59.06	50.17	72.15	87.78
Single Fruit Weight (g)	30.50	189.58	76.88	38.10	28.64	56.49	44.34

The salient feature of present investigation is evaluation of RIL (F_6) population for shelf life at room temperature (Fig. 1). Some of the top performing lines with high shelf life were identified. Some genotypes were observed to have no capacity to develop normal red colour whereas some lines were having capacity to develop normal colour over a period of time and uniform ripening. Some of the RILs were found get deterioted within short period.

Fig. 1. Performance of some of the RILs (F_6) of the cross *alc* × Vaibhav for shelf life.

Correlation

The results of the correlation analysis are presented in Table 3. Fruit shelf life was positively and significantly associated with total yield per plant (0.302, 0.298), number of fruits per plant (0.385, 0.363) but negatively associated with single fruit weight (-0.295, -0.207). Positive association was observed between total yield per plant and number of fruits per plant (0.836, 0.788) where as negative association was recorded between number of fruits per plant and single fruit weight (-0.378, -0.284).

Path coefficient analysis

In this analysis, fruit yield and shelf life were taken as dependent variable and the rest of the characters were considered as independable variables (Table 4 Table 5). Among the characters studied, number of fruits per plant had the highest positive direct effect of 0.9435 towards plant yield followed by fruit length (0.2171), single fruit weight (0.2168), fruit width (0.1715) and plant height (0.0194) whereas, fruits per cluster (-0.0877) had negative direct effect towards plant yield. Fruit firmness had the highest positive direct effect of 0.2553 towards Shelf life followed by total soluble solutes (0.1396), fruit width (0.1129) and lycopene content (0.0958) whereas, number of locules (-0.2908) and fruit length (-0.1217) had negative direct effect towards fruit Shelf life.

Table 3. Genotypic and Phenotypic correlation between plant growth, fruit quality, shelf life and yield attributing traits in RILs of *alc* x Vaibhav during *kharif* 2012.

	PHT	FRC	FL	FW	FF	NL	TSS	LC	SHL	YLD	NOF	SLW
PHT	1	0.083	0.096	0.039	-0.043	0.044	0.427**	-0.013	-0.093	0.019	-0.010	-0.054
	1	0.075	0.050	0.016	-0.036	0.035	0.378**	-0.010	-0.093	0.010	-0.018	-0.031
FRC		1	0.077	0.196**	0.018	-0.093	0.019	0.025	0.130*	0.114	0.219**	-0.260**
		1	0.047	0.154**	0.020	-0.083	0.029	0.025	0.112	0.100	0.192**	-0.190**
FL			1	-0.042	0.083	-0.184	-0.099	-0.020	-0.067	0.149**	-0.077	0.074
			1	0.182**	0.070	-0.078	0.015	-0.026	0.001	0.092	-0.043	0.049
FW				1	0.340**	0.423**	-0.054	0.097	0.083	0.269**	0.060	0.308**

	PHT	FRC	FL	FW	FF	NL	TSS	LC	SHL	YLD	NOF	SLW
				1	0.285**	0.416**	0.005	0.074	0.069	0.177**	0.033	0.255**
FF					1	0.141**	-0.043	0.011	0.238**	0.039	-0.007	0.014
					1	0.154**	-0.039	0.008	0.207**	0.020	-0.024	0.024
NL						1	-0.030	-0.014	-0.190**	-0.185**	-0.288**	0.264**
						1	0.007	-0.004	-0.166**	-0.114	-0.215**	0.240**
TSS							1	0.093	0.152**	0.039	-0.033	0.170**
							1	0.096	0.104	0.053	-0.011	0.084
LC								1	0.129*	0.078	0.111	-0.077
								1	0.124*	0.073	0.098	-0.055
SHL									1	0.302**	0.385**	-0.295**
									1	0.298**	0.363**	-0.207**
YLD										1	0.836**	-0.049
										1	0.788**	0.023
NOF											1	-0.378**
											1	-0.284**
SLW												1
												1

*, **: Significant at 0.05 and 0.01 probability, respectively

a Abbreviations for the traits are the same as in Table 1

Table 4. Path coefficients of component yield attributing traits on plant yield in RILs (F_6) of the cross *alc* x Vaibhav in tomato (*Solanum lycopersicum*).

	PHT	FRC	FL	FW	NOF	SLW
PHT	**0.0194**	-0.0073	0.0213	0.0067	-0.0094	-0.0117
FRC	0.0016	**-0.0877**	0.0164	0.0336	0.2068	-0.0563
FL	0.0019	-0.0066	**0.2171**	-0.0074	-0.0712	0.0162
FW	0.0008	-0.0172	-0.0093	**0.1715**	0.0571	0.0667
NOF	-0.0002	-0.0192	-0.0164	0.0104	**0.9435**	-0.0819
SLW	-0.0011	0.0228	0.0162	0.0528	-0.3563	**0.2168**

Table 5. Path coefficients of component fruit quality attributing traits on fruit shelf life in RILs (F_6) of the cross *alc* x Vaibhav in tomato (*Solanum lycopersicum*).

	FL	FW	FF	NL	TSS	LC
FL	**-0.1217**	-0.0047	0.0212	0.0534	-0.0138	-0.0019
FW	0.0051	**0.1129**	0.0867	-0.1231	-0.0075	0.0093
FF	-0.0101	0.0384	**0.2553**	-0.0410	-0.0060	0.0010
NL	0.0224	0.0478	0.0360	**-0.2908**	-0.0043	-0.0013
TSS	0.0120	-0.0061	-0.0110	0.0088	**0.1396**	0.0089
LC	0.0024	0.0110	0.0028	0.0041	0.0130	**0.0958**

QTL detection with SMA

In this study, 38 polymorphic SSR markers distributed on different chromosomes were used for screening RILs (F_6) of the cross *alc* x Vaibhav (Table 7). The scored data was used studying the association of marker with the trait. Single factor ANOVA was performed between the marker loci and relevant traits using the SMA option in the Minitab® 16.1.1. The results of single marker analysis revealed that markers were associated with all the traits studied in this investigation (Fig. 2a, 2b, 2c). Three markers LEAT013, LEGA006, LEtca001 are found to be linked to plant height at 0.035, 0.024, 0.028 probability explaining 2.5, 2.8, 2.8 percent variation respectively (Table 6). Highest R square value of 7.4 was estimated for number of fruits per plant. For single fruit weight SSR96 was found to be linked at 0.001 probability with R square value of 6.9.

Table 6. List of SSR markers showing association with different phenotypic characters in RILs (F_6 generation) of *alc* × Vaibhav.

Character	Marker	P value	R square
Plant Height	LEat013*	0.035293	2.5
	LEga006*	0.024613	2.8
	LEtca001*	0.028004	2.8
Fruits per cluster	LEat016*	0.042482	2.3
	LEga003*	0.018858	2.9
Fruit length	LEat016**	0.001807	5.4
	LEta20*	0.01523	3.9
Fruit width	SSR146*	0.023984	2.8
	TOM236*	0.042857	2.6
	SSR111*	0.027779	3.6
Fruit Firmness	LEtaa002*	0.015537	3.2
	LEat006*	0.027937	2.8
	LEta016**	0.008236	2.8
	SSR111*	0.025891	3.7
Number of locules	LEta20*	0.023848	3.4
	LEaat001*	0.036398	2.6
TSS	LEaat001*	0.012551	2.3
	LEga007*	0.012334	3.6
	TOM184**	0.000633	6.4
Lycopene	LEga005*	0.03458	2.6
	SSR146**	0.002839	4.8
	LEaat006*	0.017333	3.8
Shelf life	SSR146*	0.021488	2.9
	LEaat007**	0.007799	4.1
yield per plant	TGS2259*	0.011043	3.5
	TGS0385**	0.002508	4.9
	LEaat006*	0.025159	3.4
Number of fruits per plant	LEta007*	0.046507	2.3
	LEat20*	0.030858	3.1
	TGS0385**	0.000201	7.4
Single fruit weight	LEaat006*	0.04196	2.8
	SSR 96**	0.001267	6.9

* significant at 5 %

** significant at 1% probability

Table 7. List of simple sequence repeat markers used for marker analysis.

Primers	Sequence (5'-3')	Chromosome number	Expected size (bp)
LEaat001	F:GATGGACACCCTTCAATTTATGGT R:TCCAAGTATCAGGCACACCAGC	1	136
LEaat003	F:CTTGAGGTGGAAATATGAACAC R:AAGCAGGTGATGTTGATGAG	2	189
LEaat006	F:GCCACGTAGTCATGATATACATAG R:GCCTCGGACAATGAATTG	1	174
LEaat007	F:CAACAGCATAGTGGAGGAGG R:TACATTTCTCTCTCTCCCATGAG	5	100
LEaat008	F:GAGTCAACAGCATAGTGGAGGAGG R:CGTCGCAATTCTCAGGCATG	5	178
LEat006	F:CATAATCACAAGCTTCTTTCGCCA R:CATATCCGCTCGTTTCGTTATGTAAT	3	166
LEat012	F:CGGCAAAGGGACTCGAATTG R:GTGGCGGAGTAGAAACCTTAGGA	2	110
LEat013	F:ATCACAAGCTTCTTTCGCCACA R:ACCCATATCCGCTCGTTTCG	3	163
LEat016	F:CCCAAATGCTATGCAATACAC R:AGTTCAGGATTGGTTTAAGGG	4	184
LEat020	F:ACTGCCTCTCTTCAAAGATAAAGC R:ACGGAAAGTTCTCTCAAAGGAGTTG	5	212

Primers	Sequence (5'-3')	Chromosome number	Expected size (bp)
LEga003	F:TTCGGTTTATTCTGCCAACC R:GCCTGTAGGATTTTCGCCTA	-	241
LEga005	F:TTGGCCTAATCCTTTGTCAT R:AACAATGTGACGTCTTATAAGGG	-	314
LEga006	F:CCGTCCAGAAGACGATGTAA R:CAAAGTCTTGCCAACAATCC	-	248
LEga007	F:CCTTGCAGTTGAGGTGAATT R:TCAAGCACCTACAATCAATCA	-	193
LEtat002	F:ACGCTTGGCTGCCTCGGA R:AACTTTATTATTGCCACGTAGTCATGA	1	196
LEgata001	F:CTCTCTCAATGTTTGTCTTTC R:GCAAGGTAGGTAGCTAGGGA	-	335
LEta002	F:GCCTCCCACAACAATCATCTATACA R:TCCTCCGTACTTTGATCATCTTGTT	4	190
LEta003	F:GCTCTGTCCTTACAAATGATACCTCC R:CAATGCTGGGACAGAAGATTTAATG	1	111
LEta007	F:GCCGTTCTTGGTGGATTAG R:CCTCCTTTCGTGTCTTTGTC	1	291
LEta016	F:AGGTTGATGAAAGCTAAATCTGGC R:CAACCACCAATGTTCATTACAAGAC	2	174
LEta020	F:AACGGTGGAAACTATTGAAAGG R:CACCACCAAACCCATCGTC	4	275
LEtaa002	F:TGAGAGAGATCAACCAACTCC R:ACTACTCCTGCCTCTCTATATCC	2,11	133
LEtca001	F:TGCATGGCAACATTAAAGTC R:CGTGGATGCAACTTCATTG	-	176
SSR96	F:GGGTTATCAATGATGCAATGG R:CCTTTATGTCAGCCGGTGTT	2	210
SSR111	F:TTCTTCCCTTCCATCAGTTCT R:TTTGCTGCTATACTGCTGACA	3	190
SSR134	F:CCCTCTTGCCTAAACATCCA R:CGTTGCGAATTCAGATTAGTTG	1	175
SSR146	F:TATGGCCATGGCTGAACC R:CGAACGCCACCACTATACCT	4	220
SSR248	F:GCATTCGCTGTAGCTCGTTT R:GGGAGCTTCATCATAGTAACG	10	270
SSR310	F:GCGATGAGGATGACATTGAG R:TTTACAGGCTGTCGCTTCCT	4	175
SSR318	F:GCAGAGGATATTGCATTCGC R:CAAACCGAACTCATCAAGGG	10	180
TOM144	F:CTGTTTACTTCAAGAAGGCTG R:ACTTTAACTTTATTATTGCGACG	11	180
TOM152	F:ATTCAAGGAACTTTTAGCTCC R:TGCATTAAGGTTCATAAATGA	5	190
TOM184	F:CAACCCCTCTCCTATTCT R:CTGCTTTGTCGAGTTTGAA	4	180
TOM210	F:CGTTGGATTACTGAGAGGTTTA R:ACAAAAATTCACCCACATCG	4	205
TOM236	F:GTTTTTTCAACATCAAAGAGCT R:GGATAGGTTTCGTTAGTGAACT	9	200
TGS0385	F:ATGCCAAAAAGTGATCAGGG R:GGGACAAACGTGTAACACACA	10	163
TGS2259	F:ACGCAAGCTGAAGCCATAAT R:GTCTCCCTGCTGCTTACTGC	7	205

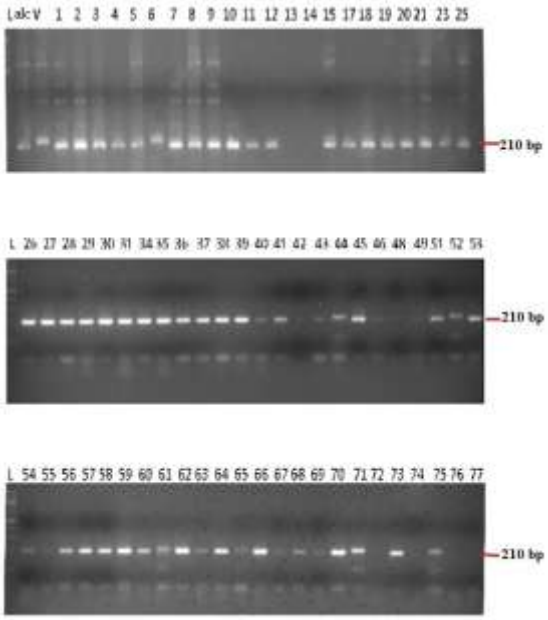

Fig. 2a. Agarose gel profile of SSR marker SSR96 showing the amplification of 200 RILs (F_6 generation) of the cross *alc* × Vaibhav (Legend L= 100bp ladder; 1 to 77= RILs).

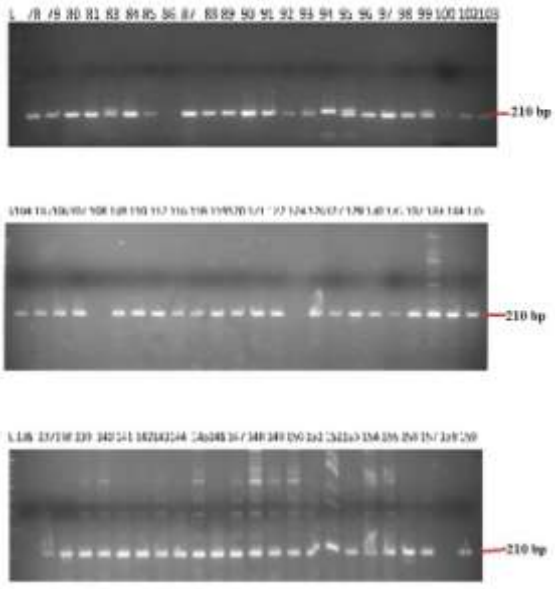

Fig. 2b. Agarose gel profile of SSR marker SSR96 showing the amplification of 200 RILs (F_6 generation) of the cross *alc* × Vaibhav (Legend L= 100bp ladder; 78 to 159 = RILs).

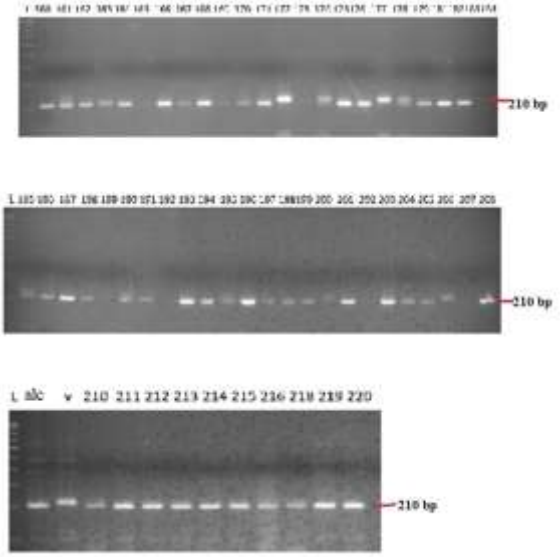

Fig. 2c. Agarose gel profile of SSR marker SSR96 showing the amplification of 200 RILs (F_6 generation) of the cross *alc* × Vaibhav (Legend L= 100 bp ladder; 160 to 220 = RILs).

Discussion

Significant differences were observed among all the characters under study for analysis of variance, which intern gives significance to wide variability with respect to traits under study. In all, the genotypic coefficient of variation was smaller than the phenotypic coefficient of variation, indicating some influence of environment on evaluation of traits under study. The tomato is a berry with 2–12 locules filled with many seeds. Processing tomatoes (such as cherry, plum, or pear tomatoes) have two locules. The locular area affects fruit firmness, flavour and weight. Here number of locules were ranging from 2-9 which shows that some genotypes have characteristics as that of processing varieties and some are like commercial cultivars. The shelf life was found to vary from 18 to 85 days.

Correlation Analysis

An over view of the results revealed that, in general, the genotypic and phenotypic correlations showed similar trend but genotypic correlation were at higher magnitude than phenotypic correlation in most of the cases. Very close values of genotypic and phenotypic correlation were also observed between some character combinations that might be due to

reduction in error (environmental) variance to minor proportions as reported by Dewey and Lu 1959 ; Manna and Paul (2012).

Path Coefficient analysis

In Path coefficient analysis, the correlation coefficients are divided into direct and indirect effects on single dependent characters. Direct effect traits are responsible for the performance of the trait whereas indirect traits affects the expression via other traits. The indirect association of traits becomes more complex when we include more variable to correlation analysis. In such cases, to estimate the effect of independent variable on dependent variable, path coefficient analysis is used. It gives specific magnitude of action to produce correlation and relative importance of each factor. Therefore it is necessary to decide the direct and indirect components of character under study. In this analysis, fruit yield and shelf life were taken as dependent variable and the rest of the characters were considered as independable variables.

For industrial purpose it is essential to pick fruits at right stage, to have longer shelf life. Here fruits were harvested at brick red stage and stored at room temperature to evaluate it for its shelf life. Path coefficients of component fruit quality attributing traits on fruit shelf life in RILs of the cross alc x Vaibhav was done. Among the characters studied, fruit firmness had the highest positive direct effect towards shelf life followed by total soluble solutes, fruit width and lycopene content whereas, number of locules and fruit length had negative direct effect towards fruit shelf life *Botrytis cinerea* is the major post harvest pathogen (Cantu *et al*., 2009; Zhang *et al*., 2013). In order to resist the infestation to pathogen plants produce many components, pigments etc.

Zhang *et al.,* 2013 reported that shelf life of tomato was extended by enrichment of anthocyanin. It alters the ROS burst during infection. In current study we estimated the lycopene content in ripening fruits. Lycopene ($C_{40}H_{56}$) imparts red colour to the fruit it is also an antioxidant with immuno-stimulatory properties and prevents human beings from atherosclerosis, cervical cancer and breast cancer (Kaur *et al.,* 2004). In present investigation the association between lycopene content and shelf life shows that lycopene being a strong antioxidant is reducing the speed of ripening and increasing the shelf life. TSS reflects dry matter content and is inversely proportionate to fruit size. TSS in large beefsteak tomatoes ranges from 3 to 5%, in medium-sized fruit from 5 to 7% and cherry tomato fruit from 9 to 15% (Beckles 2012; Gautier *et al.,* 2010; Georgelis *et al.,* 2004; Luengwilai *et al.,* 2010; Rick, 1974). Hence negative association was observed between TSS and fruit length and width. The residual effect for both the analysis was low, indicating right accountability of the characters in the study.

QTL detection with SMA

Three markers LEAT013, LEGA006, LEtca001 are found to be linked to plant height at 0.035, 0.024, 0.028 probability explaining 2.5, 2.8, 2.8 percent variation respectively. Highest R square value of 7.4 was estimated for number of fruits per plant. For single fruit weight SSR96 was found to be linked at 0.001 probability with R square value of 6.9.

In this experiment two markers were associated with firmness, both being detected on chromosome 3 which is in agreement with the finding of Tanksley *et al.* (1996) who found four QTLs for fruit firmness located on chromosome 3 verifying that the wild type alleles had significant and positive effect on this trait and also Costa *et al*., 2013 reported QTLs on chromosome no 3.

Conclusion

Shelf life trait has been addressed with genetic engineering and traditional breeding method. In many studies wild germplasms have been used as a donor for this trait. One of the most popular method is use of tomato ripening mutants. Alcobacca, a ripening mutant located on short arm of chromosome 10 is crossed with Vaibhav, a tomato variety released by UAS, Bangalore. RILs developed from the cross

revealed significant variability among the genotypes. Lycopene content, TSS and fruit firmness are the primary traits influencing shelf life of tomato fruit was revealed by Correlation and Path coefficient analysis studies. Three SSR markers were linked to shelf life of tomato. These markers can be used in backcross breeding to enhance the shelf life of commercial tomato varieties.

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