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ESTIMATION OF HETEROSIS AND COMBINING ABILITY IN TOMATO FOR FRUIT SHELF LIFE AND YIELD COMPONENT TRAITS USING LINE X TESTER METHOD

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ABSTRACT

Studies on tomato post-harvest losses are the main concern for its production, hence a study was conducted to evaluate 10 hybrids along with two checks for fruit shelf life and yield components. For improvement of shelf life of commercial varieties, we used four commercial commercially varieties and one germplasm were crossed with two testers in line x tester mating fashion to study combining ability effects and heterosis for plant height, number of branches, fruit per cluster, single fruit weight, total number of fruits per plant, fruit shelf life and total yield per plant during *summer* 2014 at UAS, GKVK, Bangalore, India. The analysis of variance revealed the variance due to lines effects and crosses were highly significant for fruit shelf life (days). In respect of GCA effects, Arka Alok observed highest for shelf life and L121 for total yield per plant. Among the crosses, Vaibhav X RIL-160 and L121 X RIL-108 were the most valuable combiners for fruit shelf life and total yield per plant. Thus, these two cross combinations revealed good potential to be used as a hybrid. The highest heterotic effect over mid parent was exhibited by the cross Vaibhav X RIL-160, Arka Alok X RIL-160 and Arka Alok X RIL-108 for both characters. We also observed the mean performance of fruit shelf life of all the crosses were significantly differed with lines. The RIL-160 and RIL-108 both testers can be recommended for improving the fruit shelf-life and cross can be forwarded to multilocation trial.

Keywords: tomato**,** fruit shelf-life, combining ability, Heterosis and F_1 Hybrids.

ISSN: 2455-6939

Volume:02, Issue:03

INTRODUCTION

Tomato (*Solanum lycopersicon* L.) is one of the most important vegetable crops grown throughout the world because of its wider adaptability, high yielding potential and suitability for variety of uses in fresh as well as processed food industries (FAOSTAT, 2013). It contains vitamins A, C, potassium, minerals and fibers so it is categorized as protective foods. In India, Post-harvest losses of tomato are major constraint, for increasing food production at the global level. It account from farm gate to consumer stage 13-26% of total harvested tomatoes (Kalidas and Akila 2014). Post-harvest losses are due to perishable nature of crop, method of harvesting, packaging and transportation etc. Exploration of genetic diversity within the available germplasm is a viable and environmentally safe option for improving shelf life. Many breeders have used the mutant germplasm for fruit shelf life and tried to 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 Gowda, 2013 and Pech *et al.,* 2013). In *alc/alc* genotype the carotenoid level is very less, fruit firmness is more and shelf life of fruit increased (Faria *et al.,* 2003) because one replacement of thymine base by adenine base at position 317 (Casals *et al.,* 2012).

The theory of specific combining ability (SCA) and general combining ability (GCA) established by Sprague and Tatum, (1942) have been used broadly in breeding of several economic species of crops. Combining ability analysis is one of the powerful tools available to estimate the combining ability effects and aids in selecting the desirable parents and crosses for the exploitation of heterosis (Sarker *et al*., 2002; Rashid *et al*., 2007). General combining ability (GCA) is attributed to additive gene effects and additive x additive epistasis and is theoretically fixable. On the other hand, specific combining ability attributable to non-additive gene action may be due to dominance or epistasis or both and is non-fixable. Line x Tester analysis is a useful tool for preliminary evaluation of genetic stock for use in hybridization programme with a view to identify good combiners. Considering this, an investigation was undertaken to identify the best parental combination having extended shelf life with a high yield per plant by using the line x tester crossing method and also estimated the percent of heterosis.

MATERIAL AND METHODS

Experimental materials

In this study we used two recombinant inbreed lines 160 and 108 which had shown very high shelf life more than 50 Days at room temperature. The tomato RILs 160 and 108 were derived from L121 (*alc* line) X Vaibhav cross and were selected by single seed descent method used as testers. We have selected four tomato cultivars Sankranti, Pusa Ruby, Vaibhav, Arka Alok and

ISSN: 2455-6939

Volume:02, Issue:03

one *alc* germplasm (L121) used as lines. The Sankranti and Vaibhav are high yielding varieties, released by University of Agricultural Sciences, Bangalore, India, the Pusa Ruby is low shelf life variety, released by the Indian Agricultural Research Institute (IARI) New Delhi, India. Arka Alok is high yielding and bacterial wilt resistance varieties, released by Indian institute of horticulture research, Bangalore, India. The L121 (*alc)* germplasm having high shelf-life produces yellow to light red fruits. The F_1 Hybrids developed using the line x tester (5 x 2) mating design during *rabi* 2013 (Table 1). The 25 days old seedlings of 10 F_1 hybrids along with parents and two standard checks (NS2535 and INDAM 1004) were transplanted at UAS, GKVK, Bangalore during *summer* 2014 in randomized block design, which was replicated thrice.

Table 1. Development of 10 hybrids 5 x 2 mating design

S. No.	Parental germplasm (lines)	Tester1(RIL-160)	Tester 2 (RIL-108)
1	Sankranti	Sankranti X Tester 1	Sankranti X Tester 2
2	Pusa Ruby	Pusa Ruby X Tester 1	Pusa Ruby X Tester 2
3	Vaibhav,	Vaibhav X Tester 1	Vaibhav X Tester 2
4	Arka Alok	Arka Alok X Tester 1	Arka Alok X Tester 2
5	L121	L121 X Tester 1	L121 X Tester 1

Traits evaluated

The observations were recorded on five randomly selected plants from each replication for viz., plant height (cm), number of branches, fruit per cluster, single fruit weight (g), total number of fruits per plant, fruit shelf life (days) and total yield per plant (g/plant). For fruit shelf life, five tomato fruits at the breaker stage were harvested and fruits were stored at 28°C and keeping quality were recorded at five days intervals and photographs were taken ten days interval. In the postharvest period, fruits were visually inspected for signs of infections and fruit deterioration and also parameters like fruit firmness and fruit weight were recommended, which are the indication of fruit shelf-life. Fruits with visual defects were discarded and shelf life was recorded in days.

Molecular characterization

Genomic DNA was extracted from the young leaves (20 days after transplanting) of F_1 hybrids, Lines and Tester using a modified cetyltrimethylammonium bromide method (CTAB) Saghi –

ISSN: 2455-6939

Volume:02, Issue:03

Maroof *et al.,* (1984). The true F_1 hybrids confirmed by SSR (simple sequence repeat) markers using Poly Acrylamide Gel Electrophoresis (PAGE).

Data Analysis

Data were recorded on five randomly selected plants from each replication of parents, hybrids and two checks. Combining ability analysis was done using line x tester method (Kempthorne, 1957). The variances for general combining ability and specific combining ability were tested against their respective error variances derived from ANOVA reduced to mean level. Midparent heterosis (MPH) and Standard heterosis (SH) were determined as outlined by Falconar and Mackay (1996). The data were subjected by the program Windostat 9.1 software version.

RESULTS AND DISCUSSION

Analysis of variance (table 2) for combining ability revealed that the variance due to lines effects were highly significant (@ P = 0.01) for fruit shelf life (days) whereas, mean squares due to testers were non-significant for all traits under study. Since both testers have been derived from the same parent and selected for high shelf life, fruit shape and single fruit weight. While the variance due to line x tester effects were highly significant (@P = 0.01) for plant height (cm), number of branches and fruit per cluster under study while there was no substantial genetic diversity observed for fruit shelf life, single fruit weight and total fruit yield because all the crosses recorded increased fruit shelf life and fruit yield. The mean performance of five lines, two testers, ten hybrids and two checks were analyzed by multiple Duncan range test. The mean performance of lines for shelf life is 24.6 Days, tester 58 Days and hybrids 44 days (table 6). The same letter indicates there is no significant variation and different letter indicate the variation. Among lines, most of the lines shown significant variation for fruit shelf life except Vaibhav and Arka Alok. The testers significantly differ for all characters except total number of fruits. None of the ten hybrids superior for all the characters studied, for both of the characters fruit shelf life (50 Days) and yield per plant (2638 g) observed maximum in cross L121 X RIL-108.

Table 2. Analysis of variance for combining ability analysis of Line x Tester

Source	df	Mean Sum of Squares						
		Shelf life(days)	Plant Height(cm)	Fruit Per Cluster	Total No. of Fruits	Total Yield(g)	Single Fruit Weight(g)	Number of Branches
Replicates	2	30.83	6.3	0.30	204.633	41851.60	140.23	4.43 **
Crosses	9	93.43 *	405.47***	0.96 ***	263.941	382576.40	307.39	7.44***
Line Effect	4	196.67 **	324.63	1.08	276.117	415657.30	193.13	5.53

ISSN: 2455-6939

Volume:02, Issue:03

Tester Effect	1	7.50	346.80	1.20	418.133	622368.10	172.80	0.83
Line X Tester Eff.	4	11.67	500.97***	0.78 **	213.217	289547.50	455.30	11.00 ***
Error	18	28.98	66.30	0.15	154.152	290483.0	198.27	0.62
Total	29	49.11	167.42	0.41	191.706	301916.80	228.13	2.99

*Significant at 5% level, ** Significant at 1% level and ***Significant at 0.1% level

General combining ability

General combining ability refers to the average performance of a line in a series of cross combinations and it is attributable to additive (fixable) gene action The estimates of GCA effects provides a measure of general combining ability of each genotype, thus aids in selection of superior ones as parents for breeding programmes. The line Arka Alok registered highest GCA effect for fruit shelf life, fruit per cluster and plant height while Pusa ruby recorded lowest GCA effect for fruit shelf-life (Table 3). Among testers, RIL 108 registered positive GCA effect for fruit shelf life, plant height and fruit per cluster, whereas RIL 160 exhibited positive GCA effects for total number of fruits, total yield per plant, single fruit weight and number of branches. None of the testers were found to be good general combiner for all the characters studied. Comprehensive assessment of parents by considering GCA effects for all the characters studied has resulted into identification of lines Arka Alok and Vaibhav as good general combiners for overall characters. Among testers, RIL-108 for shelf life and RIL-160 for yield per plant are good general combiners. Hence, these can be utilized in commercial breeding programme as good donors for yield and fruit shelf life. In another study, Yogendra and Gowda, (2013) observed *alc* is good general combiner with Vaibhav for fruit firmness and fruit shelf-life. The results were in conformity with Nguyen *et al*. (1997), Laxman (2001), Birader *et al*.(2004) and Talekar *et al*.,(2010).

Table 3. Estimates of General combining ability (GCA) effects of lines and testers for fruit shelf life and yield associated traits in tomato

Lines	Shelf life(days)	Plant Height(cm)	Fruit Per Cluster	Total No. of Fruits	Total Yield(g)	Single Fruit Weight(g)	Number of Branches
Sankranti	0.17	5.57	-0.50 **	-3.70	-158.67	0.10	0.80 **
Pusa Ruby	-9.83 ***	7.73		6.13	-339.33	-8.57	-0.53
Vaibhav,	1.83	-11.10 *	0.33	-0.53	-19.33	2.10	1.13***

ISSN: 2455-6939

Volume:02, Issue:03

Arka Alok	4.33 *	-1.10	0.50 **	-9.03 *	222.67	-0.73	-1.20 ***
L121	3.50	-1.10	-0.33	7.13	294.67	7.10	-0.20
SE	1.73	4.93	0.16	4.29	171.00	5.36	0.28
Tester							
(RIL-160)	-0.50	3.40	-0.20	3.73	144.03	2.40	0.17
(RIL-108)	0.50	-3.40	0.20	-3.73	-144.03	-2.40	-0.17
SE	1.09	3.12	0.10	2.71	108.15	3.39	0.17

Specific combining ability

The specific combining ability (SCA) reveals the best cross combination among the genotypes which can be useful for developing hybrids with high vigour for the traits. Results revealed that there was no significant SCA effect for fruit shelf life and total yield per plant. All the crosses recorded higher fruit shelf life and total yield per plant hence there is no much significance observed between the crosses. There are four cross combinations *viz.*, Pusa Ruby X RIL-160, Vaibhav X RIL-160, Arka Alok X RIL-108 and L121 X 2 RIL-108 have positive value for both characters of fruit shelf life and total yield (Table 4). Cross Vaibhav X RIL-108 for plant height, cross Sankranti X RIL-108, cross Pusa Ruby X RIL-160 Tester for number of branches, cross L121 X RIL-160, for fruit per cluster observed significant SCA effect. This results are conformity with Mahendrakar (2004), Dhaliwal *et al*. (2004), Premalakshme *et al.* (2006), and Duhan *et al.* (2005), Hannan *et al.* (2007), Saleem *et al.* (2009), Singh *et al*. (2010).

Table 4. Estimates of specific combining ability (sca) of lines and testers for fruit shelf life and yield associated traits in tomato

S. No.	Crosses	Shelf life(days)	Plant Height(cm)	Fruit Per Cluster	Total No. of Fruits	Total Yield(g)	Single Fruit Weight(g)	Number of Branches
1	1 Line X 1 Tester	-1.17	-0.90	-0.30	-2.56	161.80	6.10	-2.33 ***
2	1 Line X 2 Tester	1.17	0.90	0.30	2.56	-161.80	-6.10	2.33 ***
3	2 Line X 1 Tester	0.50	-3.07	0.20	5.60	91.46	6.76	1.00 *

ISSN: 2455-6939

Volume:02, Issue:03

4	2 Line X 2 Tester	-0.50	3.07	-0.20	-5.60	-91.46	-6.76	-1.00 *
5	3 Line X 1 Tester	2.17	15.76 *	-0.13	5.26	201.13	-2.23	0
6	3 Line X 2 Tester	-2.17	-15.76 *	0.13	-5.26	-201.13	2.23	0
7	4 Line X 1 Tester	-0.33	-7.57	-0.30	0.43	-141.20	3.60	0.66
8	4 Line X 2 Tester	0.33	7.57	0.30	-0.43	141.20	-3.60	-0.66
9	5 Line X 1 Tester	-1.17	-4.23	0.53 *	-8.73	-313.20	-14.23	0.66
10	5 Line X 2 Tester	1.17	4.23	-0.53 *	8.73	313.20	14.23	-0.66
	CD 95% SCA	5.14	14.65	0.49	12.73	508.07	15.94	0.82

1 line = Sankranti, 2 line = Pusa Ruby, 3 line = Vaibhav, 4 line = Arka Alok,

5 line = L121, Tester 1 = RIL-160 and Tester 2= RIL-108

Evaluation of heterosis

Heterosis is usually expressed in the form of percentage performance of cross, relative to average performance of parent and standard checks performance. Heterosis dependent on the contribution of many component characters (Garg and Cheema, 2011). For comparison of standard heterosis two commercial hybrids NS-2535 and INDAM-1004 were used. All the crosses recorded significantly positive heterosis as well as standard heterosis for fruit shelf life except cross Pusa Ruby X RIL-160 and cross Pusa Ruby X RIL-108. For total yield only cross, L121 X RIL-108 has recorded significantly higher yield compared to standard check1. For single fruit weight cross Sankranti X RIL-160 and cross L121 X RIL108 observed significantly higher to standard hybrids. Cross Pusa Ruby X RIL-160 recorded significant performance for total number of fruits from standard check 1. None of the crosses observed better parent heterosis for fruit shelf life whereas cross Sankranti X RIL-160, cross Pusa Ruby X RIL-160, cross Vaibhav X RIL-160, cross Arka Alok X RIL160, cross Arka Alok X RIL-108, cross L121 X RIL160, cross Arka Alok X RIL-108, estimated significant heterosis over average parent. Here our goal of this research is to improve the fruit shelf life of lines without compromising on yield, so here we have succeed in the improvement of fruit shelf life of line without compromising yield. All the crosses heterosis performance significantly higher to lines for fruit shelf life (Table 5). Significant heterosis over average parent is observed in the majority of the crosses for many traits indicates involvement of additive gene action in the genetic control of that trait. This was in agreement with previous

ISSN: 2455-6939

Volume:02, Issue:03

findings of Sharma *et al*. (1996), Padma *et al.* (2002), Patgonkar *et al.* (2003), Premlakshmi *et al*. (2006), Sharma *et al*. (2006), Kumar *et al*. (2009) and Kumari and Sharma (2011), Yogendra and Gowda, 2012. Keeping in view mean performance, heterosis, and standard heterosis for both characters fruit shelf life and total yield per plant, three crosses, 3 Line x 1 Tester, 4 Line x 1 Tester and 4 Line x 2 Tester are recommended for heterosis breeding.

Table 5. Mid Parent Heterosis (MPH) and Standard Heterosis SH1 (NS2535), SH2 (INDAM 1004) Estimates (%) in tomato crosses.

S. No.		Shelf life			Total yield			Single fruit weight			Total number of fruits		
	Crosses	MPH	SH1	SH2	MPH	SH1	SH2	MPH	SH1	SH2	MPH	SH1	SH2
1	Sankranti X 1 Tester	2.00	23.81 *	13.00	128.36 **	22.19	12.34	27.17	46.67 *	41.94	62.43 *	1.38	-10.91
2	Sankranti X 2 Tester	5.70	33.33 **	21.74 *	83.25 *	-10.00	-17.26	9.03	12.67	9.03	57.30 *	-3.45	-15.15
3	Pusa Ruby X 1 Tester	-1.90	0.00	-8.70	103.67 **	8.98	0.19	26.45	30.67	26.45	69.62 **	38.62 *	21.82
4	Pusa Ruby X 2 Tester	-6.30	0.00	-8.70	71.42 *	-15.81	-22.6	2.92	-6.00	-9.03	23.93	0	-12.12
5	Vaibhav X 1 Tester	26.09 **	38.10 **	26.09 **	138.13 **	31.6	20.98	50.00 *	34.00	29.68	55.84 **	24.14	9.09
6	Vaibhav X 2 Tester	12.50	28.57 **	17.40	87.27 *	-4.74	-12.42	72.41 **	33.33	29.03	10.53	-13.1	-23.64
7	Arka Alok X 1 Tester	23.40 **	38.10 **	26.09 **	179.94 **	26.32	16.13	28.44	40.00	35.48	59.09 *	-3.45	-15.15
8	Arka Alok X 2 Tester	22.45 **	42.86 **	30.43 **	209.41 **	26.02	15.85	19.59	16.00	12.26	32.95	-20.69	-30.3
9	L121 X 1 Tester	-1.80	33.33 **	21.74 *	142.11 **	21.05	11.29	14.29	20.00	16.13	55.56 *	11.03	-2.42
10	L121 X 2 Tester	1.70	42.86 **	30.43 **	204.48 **	38.86 *	27.66	79.93 **	67.33 **	61.94 **	87.25 **	31.72	15.76

*Significant at 5% level, ** Significant at 1% level and ***Significant at 0.1% level

ISSN: 2455-6939

Volume:02, Issue:03

Table 6. Means of the estimated traits for seven parents and 10 F_1 hybrids in tomato

Lines	Plant height (cm)	No. of Branches	Fruit per cluster	Single fruit weight (g)	Total number of fruits	Shelf life	Total yield per plant (g)
Sankranti	104.0b	5.0e	5.0ab	60.3abcd	26.7e	28.3cde	1033.3cdef
Pusa Ruby	147.0a	6.0bcde	4.0bcd	48.3bcd	45.3abcde	14.7e	1033.3cdef
Vaibhav,	93.3bc	6.0bcde	5.0ab	34.3d	43.3abcde	20.0de	1100.0cdef
Arka Alok	106.0b	6.0bcde	2.5d	54.0bcd	25.0e	21.6de	714.7f
L121	95.0bc	8.0abcd	3.0cd	50.0bcd	35.3de	38.3abcd	900.0def
Mean	109.067	6.200	3.9	49.400	35.133	24.60	956.27
Tester							
RIL-160	61.7c	7.0bcde	4.0bcd	55.0abcd	33.7de	56.7ab	100.0cdef
RIL-108	86.0bc	8.0abcd	6.0a	43.0cd	32.7de	60.0a	833.0ef
Mean	73.833	7.5	5.0	49.0	33.167	58.333	916.50
Hybrid							
Line 1 x Tester 1	101.7b	6.0bcde	3.0cd	73.3ab	49.0abcde	43.3abc	2321.7ab
Line 1 x Tester 2	96.7bc	10.3a	4.0bcd	56.3abcd	46.7abcde	46.7abc	1710.0abcde
Line 2 x Tester 1	101.7b	8.0abcd	4.0bcd	65.3abc	67.0a	35.0bcde	2070.7ab
Line 2 x Tester 2	101.0b	5.7cde	4.0bcd	47.0bcd	48.3abcde	35.0bcde	1599.7bcdef
Line 3 x Tester 1	101.7b	8.7a	4.0bcd	67.0abc	60.0abc	48.3abc	2500.3ab
Line 3 x Tester 2	63.3c	8.3abc	4.7abc	66.7abc	42.0bcde	45.0abc	1810.0abcd
Line 4 x Tester 1	88.3bc	7.0bcde	4.0bcd	70.0abc	46.7abcde	48.3abc	2400.0ab
Line 4 x Tester 2	96.7bc	5.3de	5.0ab	58.0abcd	38.3cde	50.0abc	2394.3ab
Line 5 x Tester 1	91.7bc	8.0abcd	4.0bcd	60.0abcd	53.7abcd	46.6abc	2300.0ab
Line 5 x Tester 2	93.3bc	6.3bcde	3.3bcd	83.6a	63.7ab	50.0abc	2638.3a
NS2535	103.3b	6.3bcde	5.0ab	50.0bcd	48.3abcde	35.0bcde	1900.0abc

ISSN: 2455-6939

Volume:02, Issue:03

INDAM 1004	105.0b	7.0bcde	6.0a	51.67bcd	55.0abcd	38.3abcd	2067.0ab
Mean	93.600	7.367	4.000	64.733	51.533	44.8	2174.500

Hybrid confirmation

True hybridity was confirmed by morphological as well as SSR Markers. The morphological markers used were plant texture, serration of leaf, flower anatomy and fruit shape. At the DNA level, also we have identified the true hybrids with the help of SSR markers. All germplasms did not show diverse at the level of DNA so polymorphism is very less at parental level. We used 40 SSR markers out of the nine SSR markers is polymorphic between parents (Fig 1 & 2). With the help of nine polymorphic markers we have indentified the ten true hybrids (table 7).

Fig. 1 PAGE gel profile of five hybrids along with five parents and tester 1

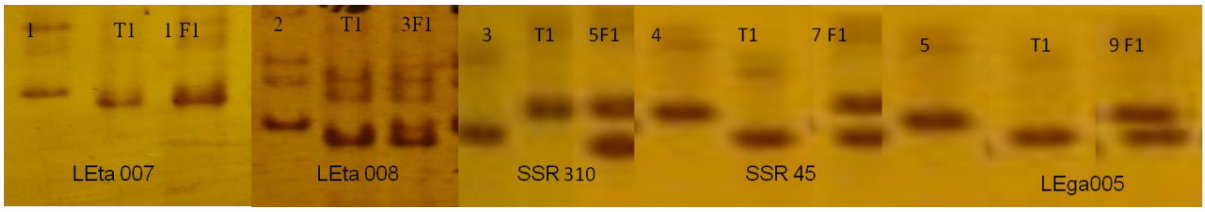
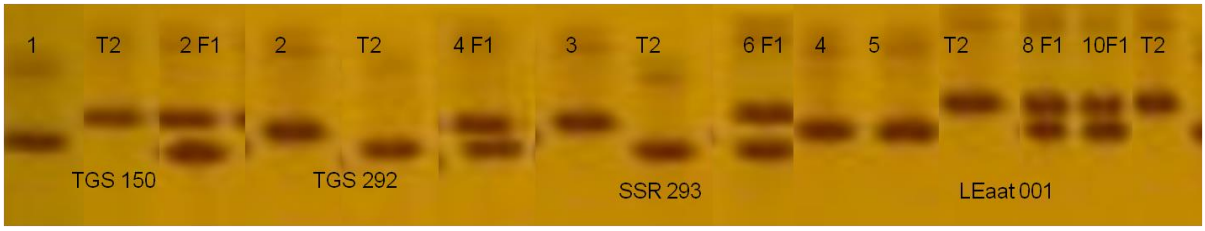


Fig. 2 PAGE gel profile of five hybrids along with five parents and tester 2



1-Sankranti, 2- Pusa Ruby, 3- Vaibhav, 4- Arka Alok, 5- L121,

T1- RIL-160, T2- RIL-108, 1F1- Sankranti x RIL160, 2F1- Sankranti x RIL-108,

3F1- Pusa Ruby x RIL-160, 4F1- Pusa Ruby x RIL-108, 5F1- Vaibhav x RIL-160,

6 F1- Vaibhav x RIL-108, 7F1-Arka Alok x RIL-160, 8F1-Arka Alok x RIL-108,

9F1- L121 X RIL-160 and 10 F1- L121 X RIL-108

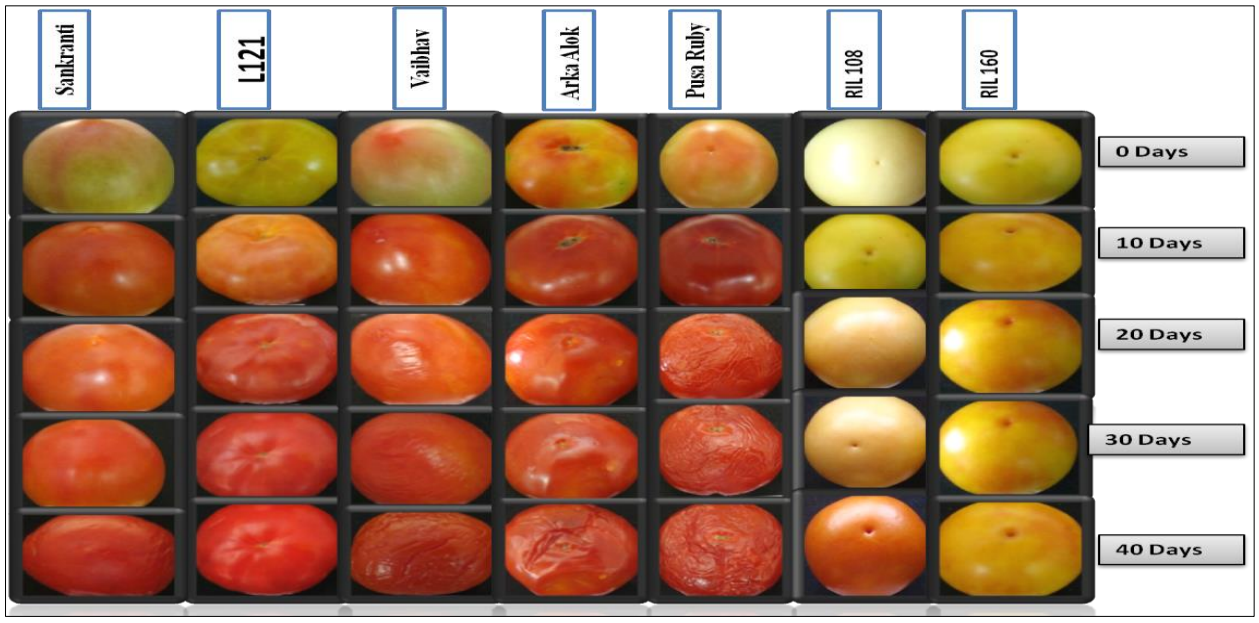
ISSN: 2455-6939

Volume:02, Issue:03

Table7. List of nine SSR polymorphic Markers used for identification of true hybrids.

S. No.	SSR Markers	Annealing temperature	Amplicon Size(bp)
1	LEta007	48	291
2	LEta008	45	168
3	SSR 310	45	175
4	SSR 45	50	260
5	LEga005	55	314
6	TGS 150	55	184
7	TGS 292	60	234
8	SSR 293	47	129
9	LEaat001	60	136

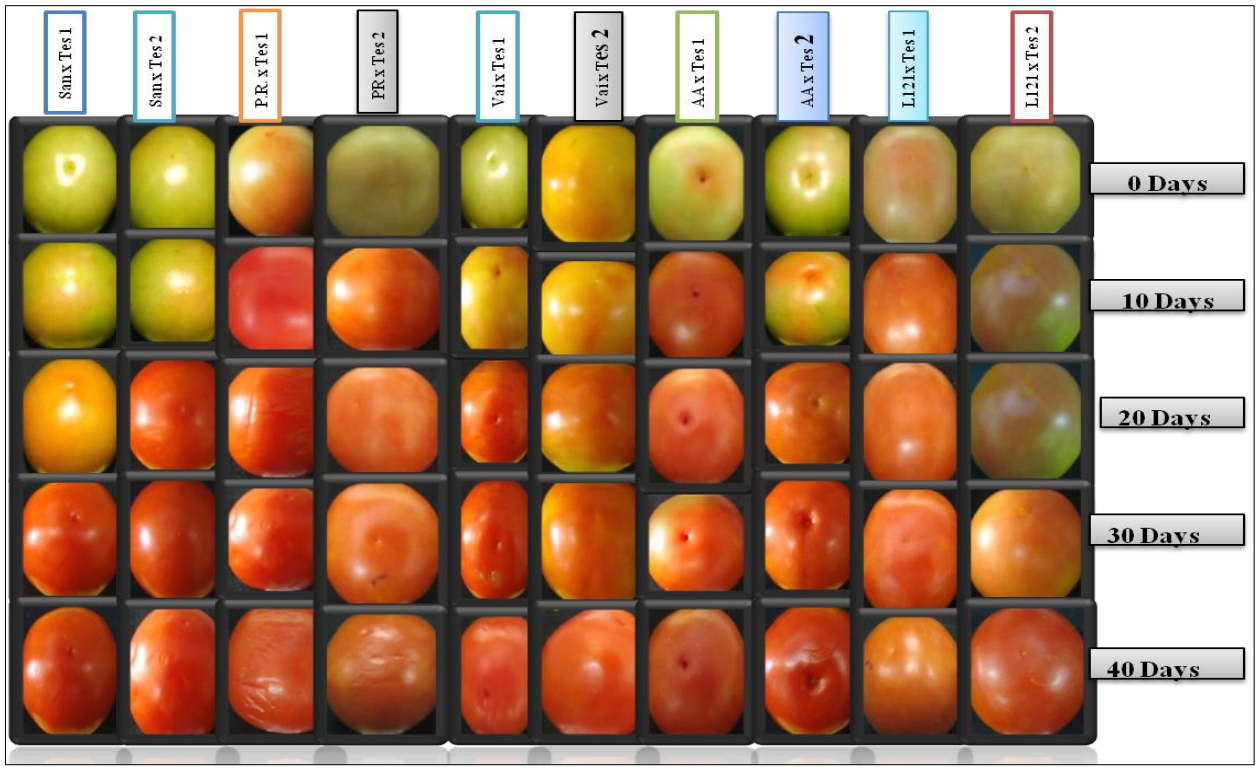
Figure 3: Five lines with two testers for high shelf-life at different post-harvest stages



ISSN: 2455-6939

Volume:02, Issue:03

Figure 4: Ten hybrids for high shelf-life at different post-harvest stages



CONCLUSION

Our goal of this research is to improve the shelf life of commercial varieties without compromise the other desirable traits. In this research we have improved the shelf-life of commercial varieties without any compromise on yield by exploiting the *alc* derived testers. Heterosis breeding is the only technically feasible method to exploit hybrid vigor for effective improvement in yield potential.

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ISSN: 2455-6939

Volume:02, Issue:03

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