EVALUATION AND MOLECULAR MARKER ANALYSIS IN BACKCROSS PROGENIES (BC_2F_1) OF TOMATO FOR SHELF LIFE

LAVANYA, N.

PALB-4241

DEPARTMENT OF PLANT BIOTECHNOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES

GKVK, BANGALORE-560065

2016

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Thesis Submitted to the
UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE
in partial fulfillment of the requirements
for the award of the degree of

MASTER OF SCIENCE (Agriculture)

IN

PLANT BIOTECHNOLOGY

BANGALORE JULY, 2016


Affectionately Dedicated
to My Beloved Parents,
Blessed Brother

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BANGALORE

CERTIFICATE

This is to certify that the thesis entitled, **"EVALUATION AND MOLECULAR MARKER ANALYSIS IN BACKCROSS PROGENIES (BC_2F_1) OF TOMATO FOR SHELF LIFE"** submitted in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in **PLANT BIOTECHNOLOGY** to the University of Agricultural Sciences, GKVK, Bangalore is a bonafide record of research work done by **Ms. LAVANYA, N., ID. No. PALB 4241**, during the period of her study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
July, 2016

(P. H. RAMANJINI GOWDA)
Major advisor

Approved by:

Chairman : _____
(P. H. RAMANJINI GOWDA)

Members : 1. _____
(H. E. SHASHIDHAR)

2. _____
(R. L. RAVIKUMAR)

3. _____
(P. CHANADRASHEKAR REDDY)

ACKNOWLEDGEMENT

I feel extremely honoured for the opportunity bestowed by Almighty upon me to work under the versatile guidance of ***Dr. P. H. RAMANJINI GOWDA,*** *Professor, Dept. of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore-65, Chairman of Advisory Committee for his excellent guidance and encouragement in completion of this thesis. It is my privilege to record a deep sense of gratitude for the invaluable guidance, constant inspiration and help, kind and constructive criticism, unfailing interest, meticulous planning right from suggesting the problem till the completion of this thesis.*

I am very much grateful to my advisory committee members, ***Dr. H. E. Shashidhar,*** *Professor, Dept. of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore,* ***Dr. Chandrashekhar Reddy*** *Professor, Dept. of Crop Physiology, University of Agricultural Sciences, GKVK,, Bangalore-65, and* ***Dr. R. L. Ravikumar,*** *Dept. of plant biotechnology, University of Agricultural Sciences, GKVK, Bangalore for their constant supervision, invaluable guidance and all the facilities extended during the course of this investigation.*

I express my sincere thanks to ***Dr. D. Dayal Doss*** *Professor & Head of the Dept. of Plant Biotechnology for his invaluable administrative support during course of investigation.*

I am thankful to ***Dr. Vijaykumar Swamy*** *former HOD of our department for his immense support during my MSc degree. I wish to place my sincere thanks to all the staff members of the Department of Plant Biotechnology* ***Dr. T. H. Ashok, Dr. Theertha Prasad, Dr. Anitha Peter, Dr. Harini Kumar, Dr. Shyamalamma, Dr. Veena*** *and* ***Poornima*** *for their excellent teaching and cooperation extended to me during the course of my study. I am thankful to* ***Dr. Mallikarjun*** *Professor & HOD Dept. of Agricultural Statistics for his immense support in solving the problems related to the research work.*

I owe thanks from depth of my heart to Lab Mates ***Ms. Neha Guleria, Mr. Mallikarjun N. M., Mr. Sujeet Kumar S., Ms. Bhavya K,, Mrs. Shruthi, Ms. Parvathi., Mrs. Hamsa, Mr. Ragavendra., Ms. Shipika, Mr. Shivkumar and Mr. Ravishanker*** *for their constant encouragement and cooperation throughout the study.*

It seems one uses the choicest words for kind help during the course of investigation to measure the boundless love and fearless sacrifice of someone, I find no such measure in adequate quantity to express my heartfelt gratitude to my father ***Mr. Narayana Swamy N****, my mother* ***Mrs. Nagaveni,*** *who always inspired me with love and affection, which enabled me to withstand all types of stresses and strains to reach this milestone in my life.*

This thesis would be incomplete if I do not reckon the sacrifice, love, affection and support of my brother, ***Mr. Venu****. Without his efforts, love and care, it was not possible for me to complete my degree programme.*

My special thanks to my Grandfather ***Mr. Narayana Swamy, Mr. Kempanna,*** *Uncle* ***Mr. Byre Gowda*** *for their encouragement and help throughout my academics. I thank my brother-in-law's* ***Mr. venkatesh****,* ***Mr. Anjinappa*** *and* ***Mr. Nagesh****, I also thank my co-brothers* ***Mr. Balanna****,* ***Mr. Ramesh*** *and* ***Mr. venu*** *for their support and encouragement during my degree programme.*

I also thank ***Dr. Murtuza Khan*** *for his help in solving research related problems. I thank* ***Mr. Mahanthesh*** *for his help during my degree programme.*

Man needs an offbeat to relieve him of tension that it was here my friends came in so handy, who is source of my strength and corners of spirit. I owe thanks from depth of my heart to ***all of my classmates*** *for their kind and genuine support throughout my academics till date.*

I would like to say special thanks to my extremely ***talented seniors*** *for their valuable and unforgettable help. I also thank my* ***lovely juniors*** *for their cooperation throughout the study.*

I greatly acknowledge the ***Department of Biotechnology (DBT), New Delhi and Department of Plant Biotechnology, UAS, GKVK, Bangalore,*** *for providing essential facility to carry out my research work.*

Any omission in this acknowledgment does not mean lack of gratitude.

Bangalore

July, 2016 ***(LAVANYA, N.)***

EVALUATION AND MOLECULAR MARKER ANALYSIS IN BACKCROSS PROGENIES (BC_2F_1) OF TOMATO FOR SHELF LIFE

LAVANYA, N.

Thesis Abstract

Tomato is second most important vegetable crop grown throughout the world. It experiences great post-harvest losses by its natural perishability, precarious transportation and storage conditions. In India upto 40 per cent of the fruit losses occur due to excessive fruit softening. Hence the present investigation was aimed to introgress the high shelf life character into commercially well accepted variety which has low shelf life by backcross breeding method. Two backcross populations (BC_2F_1) of cross Pusa Ruby/L121//Pusa Ruby and Vaibhav/RIL126//Vaibhav were developed and grown in the field condition. These were evaluated for morphological, yield, shelf life and fruit quality parameters in augmented design. All characters studied were significant among backcross progenies and between checks and progenies except number of fruits per cluster in both populations. The shelf life has positive significant correlation with number of fruits per cluster (0.22), rind thickness (0.45) and lycopene (0.35) in the population of the cross Pusa Ruby/L121//Pusa Ruby. In the other population of the cross Vaibhav/RIL126//Vaibhav shelf life have significant positive correlation with number of fruits per cluster (0.21) and lycopene content (0.21) whereas, rind thickness has no significant correlation (0.15). The SSR marker TGS 293 used to screen the 40 backcross progenies of the cross Pusa Ruby/L121//Pusa Ruby. Out of these 21 progenies showed heterozygous bands and 19 progenies showed recurrent parental type of band which shows the approximate 1:1 ratio and the heterozygous progenies was association with high shelf life character. Hence this marker is linked to shelf life.

July, 2016
Dept. of Plant Biotechnology
UAS, GKVK, Bangalore

Major Advisor
(P.H. Ramanjini Gowda)

ಟೊಮ್ಯಾಟೋ ಹಣ್ಣಿನ ಜೀವಿತಾವಧಿಗೆ ಹಿಂಸಂಕರಣ ತಳಿಗಳ (BC_2F_1) ಮೌಲ್ಯಮಾಪನ ಮತ್ತು ಮಾರ್ಕರ್ ವಿಶ್ಲೇಷಣೆ

ಲಾವಣ್ಯ, ಎನ್.

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಟೊಮ್ಯಾಟೋವು ವಿಶ್ವದಾದ್ಯಂತ ಎರಡನೇ ಪ್ರಮುಖ ತರಕಾರಿ ಬೆಳೆಯಾಗಿದ್ದು, ಭಾರತೀಯ ಪರಿಸರದಲ್ಲಿ, ಅನಿಶ್ಚಿತವಾದ ಸಾಗಾಣಿಕೆಯಿಂದ, ಸರಿಯಾದ ಶೇಖರಣೆಯ ಕೊರತೆ ಇರುವುದರಿಂದ, ಕೊಯ್ಲಿನ ನಂತರದಲ್ಲಿ ಟೊಮ್ಯಾಟೋ ಹಣ್ಣುಗಳು ಕೊಳೆತುಹೋಗುತ್ತವೆ. ಇದರಿಂದ ರೈತರು ಹೆಚ್ಚಿನ ನಷ್ಟವನ್ನು ಅನುಭವಿಸುತ್ತಿದ್ದಾರೆ. ಟೊಮ್ಯಾಟೋ ಹಣ್ಣಿನ ಅತಿಯಾದ ಮೃದುತ್ವದಿಂದ ಭಾರದಲ್ಲಿ ಶೇಕಡಾ ೪೦ ರಷ್ಟು ಇಳುವರಿ ನಷ್ಟವಾಗುತ್ತಿದೆ. ಆದ ಕಾರಣ ಕೊಯ್ಲಿನ ನಂತರದ ನಷ್ಟ ಕಡಿಮೆ ಮಾಡಲು ಹಿಂಸಂಕರಣ ತಳಿ ಸಂವರ್ದನೆಯು ಅತ್ಯಂತ ಸೂಕ್ತವಾಗಿದೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪೂಸರೂಬಿ/ಎಲ್೧೨೧//ಪೂಸರೂಬಿ ಮತ್ತು ವೈಭವ್/ರಿಲ್೧೨೬//ವೈಭವ್ ಹಿಂಸಂಕರಣ ಗುಂಪುಗಳನ್ನು ಹೊಲದಲ್ಲಿ ಬೆಳೆದು ಅವುಗಳ ರೂಪುರಚನೆ, ಇಳುವರಿ ಮತ್ತು ಹಣ್ಣಿನ ಗುಣಮಟ್ಟದ ನಿಯತಾಂಕಗಳ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗಿದೆ. ಹಿಂಸಂಕರಣ ಗುಂಪುಗಳಲ್ಲಿ ಈ ಎಲ್ಲಾ ಗುಣಗಳು ಗಮನಾರ್ಹ ವ್ಯತ್ಯಾಸಗಳನ್ನು ಹೊಂದಿದೆ. ಟೊಮ್ಯಾಟೋ ಹಣ್ಣಿನ ಜೀವಿತಾವಧಿ ಮತ್ತು ಅದರ ಹಣ್ಣಿನ ತೊಗಟೆಯ ದಪ್ಪವು ಪರಸ್ಪರ ಸಂಬಂಧವನ್ನು ಹೊಂದಿದೆ. ಅಧ್ಯಯನ ನಡೆಸಿದ ಎಲ್ಲಾ ಗುಣಗಳು ಹಿಂಸಂಕರಣ ಸಂತತಿಗಳ ನಡುವೆ, ಚೆಕ್ ತಳಿಗಳು ಮತ್ತು ಹಿಂಸಂಕರಣ ಸಂತತಿಗಳ ನಡುವೆ ಗಮನಾರ್ಹ ವ್ಯತ್ಯಾಸ ಕಂಡುಬಂದಿರುತ್ತದೆ. ಈ ಎರಡೂ ಹಿಂಸಂಕರಣದ ಪೀಳಿಗೆಗಳ ನಡುವೆ ಹಣ್ಣಿನ ಗೊಂಚಲು ಸಂಖ್ಯೆಯಲ್ಲಿ ಯಾವುದೇ ವ್ಯತ್ಯಾಸ ಕಂಡು ಬಂದಿರುವುದಿಲ್ಲ. ಪೂಸರೂಬಿ/ಎಲ್೧೨೧//ಪೂಸರೂಬಿ ಹಿಂಸಂಕರಣ ಪೀಳಿಗೆಯಲ್ಲಿ ಹಣ್ಣಿನ ಜೀವಿತಾವಧಿಯು, ಹಣ್ಣಿನ ಗೊಂಚಲು (೦.೨೨) ಹಣ್ಣಿನ ತೊಗಟೆಯ ದಪ್ಪ (೦.೪೫) ಮತ್ತು ಲೈಕೊಪೀನ್ ಅಂಶಕ್ಕೆ (೦.೩೫) ಗಮನಾರ್ಹ ಸಕಾರಾತ್ಮಕ ಸಂಬಂಧವಿದೆ. ಮತ್ತೊಂದು ವೈಭವ್/ರಿಲ್೧೨೬//ವೈಭವ್ ಪೀಳಿಗೆಯಲ್ಲಿ ಹಣ್ಣಿನ ಬಾಳಿಕೆ ಗುಣವು, ಹಣ್ಣಿನ ಗೊಂಚಲು (೦.೨೧) ಮತ್ತು ಲೈಕೊಪೀನ್ (೦.೨೧) ಅಂಶಕ್ಕೆ ಗಮನಾರ್ಹ ಸಕಾರಾತ್ಮಕ ಸಂಬಂಧವಿರುತ್ತದೆ. ಆದರೆ ಹಣ್ಣಿನ ತೊಗಟೆಯ ದಪ್ಪಕ್ಕು (೦.೧೫) ಹಣ್ಣಿನ ಬಾಳಿಕೆಯ ಗುಣಕ್ಕು ಯಾವುದೇ ಗಮನಾರ್ಹ ಸಂಬಂಧವಿರುವುದಿಲ್ಲ. ಪೂಸರೂಬಿ/ಎಲ್೧೨೧//ಪೂಸರೂಬಿ ಹಿಂಸಂಕರಣದಿಂದ ಬಂದ ೪೦ ಸಸ್ಯಗಳನ್ನು ಎಸ್.ಎಸ್.ಆರ್ ಗುರುತು ಟಿ.ಜಿ.ಎಸ್. ೨೯೩ ಯಿಂದ ಪರೀಕ್ಷಿಸಲಾಗಿದೆ. ಈ ೪೦ ಸಸ್ಯಗಳಲ್ಲಿ, ೨೧ ಸಸ್ಯಗಳು ಭಿನ್ನಯಗ್ಮಕ ಗುರುತನ್ನು ತೋರುತ್ತವೆ. ಈ ೪೦ ಸಸ್ಯಗಳು ೧:೧ ಅನುಪಾತವನ್ನು ತೋರಿರುತ್ತವೆ. ಅದರಲ್ಲಿ ಭಿನ್ನಯಗ್ಮಕ ಸಸ್ಯಗಳು ಅತಿ ಹೆಚ್ಚು ಬಾಳಿಕೆ ಗುಣವನ್ನು ಹೊಂದಿರುತ್ತವೆ. ಇದರಿಂದ ಕಂಡುಬರುವುದೆಂದರೆ ಈ ಟಿ.ಜಿ.ಎಸ್ ೨೯೩ ಗುರುತು ಹಣ್ಣಿನ ಜೀವಿತಾವಧಿ ಗುಣಕ್ಕೆ ಹೊಂದಿಕೊಂಡಿರುತ್ತದೆ.

ಜುಲೈ, ೨೦೧೬
ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ವಿಭಾಗ
ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾಲಯ,
ಜಿ.ಕೆ.ವಿ.ಕೆ, ಬೆಂಗಳೂರು

ಮುಖ್ಯ ಸಲಹೆಗಾರರ ಸಹಿ
(ಪಿ. ಹೆಚ್. ರಾಮಾಂಜಿನಿ ಗೌಡ)

Evaluation and Molecular marker analysis in Backcross progenies BC_2F_1 of Tomato for shelf life

Lavanya, N. and Ramanjini Gowda, P. H.

Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore.

INTRODUCTION

- Tomato is second most vegetable crop in the world after potato. www.faostat.org.com (2013).
- India ranks second in terms of area and production. It is cultivated in an area of 8,79,600 Ha with a production of 182,26,600 mt. The average productivity is 20.7 mt ha^{-1}. indiastat.com (2015).
- Tomato experiences great post harvest losses by its natural perishability, precarious transportation and storage conditions and inadequate packaging.
- In India up to 40 percent of produce are lost because of excessive fruit softening, with lower shelf life Kalidas and Akila (2014).
- Thus, farmers are facing the tomato glut in the market during peak season of production and results in huge losses.
- Therefore, the present study was conducted to develop extended shelf life tomato lines by using marker assisted backcross breeding.

OBJECTIVES

- To assess the genetic characteristics of parents, F_1 hybrids and backcross population.
- Validation of SSR markers linked to high shelf-life and other characters in backcross population.

MATERIAL AND METHODS

(Pusa Ruby) P1 X P2 (L121)
(Vaibhav) (RIL126)
(Low shelf-life) (High shelf-life)

F_1 X (Pusa Ruby)
(Vaibhav)

BC_1F_1 X (Pusa Ruby)
(Vaibhav)

BC_2F_1 (1,2,3,……,162)

- Experiments were conducted as per the Augmemented RCBD statistical design.
- Four parents, two F_1 hybrids and two commercial varieties i.e. Arka Alok and Sankranthi were used as checks.
- Total 4 blocks, 162 treatments (progenies) were considered for the experimental analysis.
- Analysis was done by using Windostat software version 8.5.

RESULTS

- ✓ There is increase in Shelf life in most of the progenies in Backcross population in comparison to their recurrent parent. Among the 162 progenies the shelf life varied between 10 to 60 days. The parent Pusa Ruby recorded 10 days of shelf life (Fig. 1)
- ✓ The phenotypic and genotypic coefficients of variability were high for yield, total number of fruits per plant, number of locules per fruit and lycopene content.
- ✓ SSR markers TGS 293 and TGS 450 were identified as polymorphic markers for shelf life (Fig. 2 and 3).

Table 1: Estimates of mean and components of variability for important characters in backcross population (BC_2F_1).

Character	Mean	Minimum	Maximum	Skweness	Kurtosis
PHT	21.96	27.00	69.00	0.910	-1.000
NFC	30.33	23.63	45.00	1.560	4.900
YLD	520.0	94.00	1284	0.609	0.280
TNF	15.0	3.00	43.0	1.11	1.38
SFW	51.10	21.00	85.00	-0.041	0.021
FL	3.02	2.40	4.50	1.58	4.95
FW	4.37	3.50	6.00	0.75	0.78
LOC	6.00	3.00	9.00	0.03	-0.48
RT	3.28	2.17	5.50	0.28	0.95
TSS	4.73	3.70	7.33	1.25	4.21
LYC	0.98	0.24	2.28	1.57	3.53
SL	35.00	10.00	60.00	-0.231	-0.895

PHT-Plant height, NFC-Number of fruits per cluster, YLD-Yield, TNF-Total number of fruits per plant, SFW-Single fruit weight, FL-Fruit length, FW-Fruit width, LOC-Number of locules per fruit, RT-Rind thickness, TSS-Total soluble solids, LYC-lycopene, SL-Shelf life

DISCUSSION

- ✓ In the present investigation, there is significant increase in the shelf life of tomato fruits in backcross progenies. This indicates the transfer of some favorable alleles from donor parent.
- ✓ GR Pratta *et al*., (2011) also reported the presence of favorable alleles for fruit keeping quality in the cultivated genotypes of tomato.
- ✓ In the recent years, the scientists observed heterozygous individuals of Backcross population have longer shelf life than homozygous individuals which indicate the heterotic effect. Coasta *et al.,* (2013).
- ✓ In our studies, the highest heritability 99 % and 72 % was observed for TSS and shelf life respectively. This indicates that there is greater scope for selection of these traits.
- ✓ G. R. Pratta *et al*., (2011) showed the evidence for prolonged shelf life is due to genes carried by both parents (the shortest and the longest shelf life genotypes), We have also observed the transgressive segregation in BC_2F_1 for shelf-life.

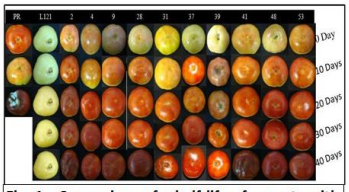

Fig. 1: Comparison of shelf life of parents with backcross progeny (BC_2F_1) of the cross PR/L121//PR (2,4,9,28,31,37,39,41,53).

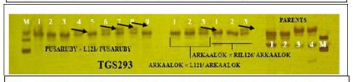

Fig. 2: Identification of true backcross progeny using polymorphic primer TGS 450. 1. Vaibhav 2. Pusa Ruby 3. Arka Alok 4. L121 5. RIL126 single banding pattern indicate parental type, double banding pattern indicate true backcross progeny.

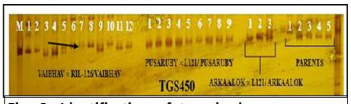

Fig. 3: Identification of true backcross progeny using polymorphic primer TGS 450. 1. Vaibhav 2. Pusa Ruby 3. Arka Alok 4. L121 5. RIL126. single banding pattern indicate parental type, double banding pattern indicate true backcross progeny.

SUMMARY

- ✓ This present investigation was carried out to transfer the high shelf life character to commercially accepted varieties (Pus Ruby and Vaibhav) with low shelf life.
- ✓ All the checks and progenies shown considerable amount of differences in their mean performance with respect to all the characters studied, this indicates genotypes under study were genetically variable.
- ✓ SSR markers TGS 293 and TGS 450 were found to be polymorphic for shelf-life between the parent and also identify the heterozygous plants which are used in further crossing.
- ✓ This study indicates, the shelf life trait can be introduced by back cross breeding using molecular markers.

REFERENCES

- Coasta *et al.,* 2013. *Sci Hort.,* 156: 47–53.
- GR Pratta *et al.,* 2011. *Crop Breed Appl Biotechnol.,* 11: 157-164.
- Kalidas and Akila 2014. *J Stored Prod Post harvest Res.,* 5:1-7.

ADVISORY COMMITTEE

Chairperson : Dr. Ramanjini Gowda,P.H.
Members : Dr. Shashidhar, H.E.
Dr. Ravikumar, R.L.
Dr. Chandrashekar Reddy, P.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols	Abbreviations
%	Per cent
@	At
ºC	Degree Celsius
cm	Centimeter
nm	Nanometer
cv.	Cultivar
et al.	Et allii (and other)
g	Gram
hr	Hour
µl	Microlitre
kg	Kilogram
mM	Milimolar
mg	Milligram
ml	Millilitre
mm	Millimeter
ppm	Parts per million
viz.	As follows
t	tons

I INTRODUCTION

Tomato (*Solanum lycopercicum,* formerly *Lycopercicum esculentum*) is the most important vegetable crop in India and is an important source of essential nutrients worldwide. Tomato belongs to the Solanaceae family, which includes >3000 species origins in Central and South America (Knapp, 2002). It diversified first in Peru, Mexico. *Solanum lycopersicum* CV. *cerasiforme* is thought to be the ancestor of cultivated tomato, based on its wide presence in Central America and the presence of a short style length in the flower (Cox, 2000). However, recent genetic investigations have shown that the plants known as '*cerasiforme*' are a mixture of wild and cultivated tomatoes rather than being 'ancestral' to the cultivated tomatoes (Nesbitt and Tanksley, 2002).

Total area under tomato in the world is 4582.438 m ha with production of 150,513,813 Mt and with productivity of 32.8 t ha^{-1}. The major tomato growing countries are China, USA, Italy, Turkey, India and Egypt (FAOSTAT, 2013).India ranks second in area under tomato cultivation (8,79,600 ha) and also production(18,226.6 Mt) with average productivity of 20.7 Mt ha^{-1}. In India Andra Pradesh is the leading state with an area of 260,910 ha and production of 5,218,100 Mt. Karnataka takes the first position in terms of productivity (33.2 Mt ha^{-1}). (www.indiastat.com, 2015).

Tomato is an ideal model plant for studying climacteric fruit ripening. Several tomato gene banks have been established and more than 75,000 accession of tomato are maintained. Several mutants affected in fruit size, shape, development, and ripening have been isolated. Recently the genome of *Solanum lycopecicum* has been fully sequenced and made publically available.With its short generation time and availability of routine transformation technology, mapping populations and microarrays of mapped DNA markers, tomato is highly tractable experimental system for improving the fruit quality (Alba *et al.,* 2005).

Fruit quality in the cultivated tomato is very important attribute to choice the appropriate cultivar by producers and also by consumers. The consumers reporting tomato use on any given day for fresh fruits are 28 per cent, processed 60 per cent, sauce 28 per cent, paste 8 per cent, total 18 per cent, and ketchup 15 per cent (Anonymous, 2015). Markets are today interested in differentiating products by attributes related to taste, aroma, acidity, sugar content and vitamins (Causse *et al*., 2002). Tomato is one of the most important supplementary sources of minerals and vitamins in the human diet. It acts as a blood purifier, improves skin texture and colour, protects liver from cirrhosis, prevents haemorrhages and is a rich source of vitamin K. It contains a pigment called lycopene, which is a powerful antioxidant and can fight cancer cells (Mukeshkumar *et al*., 2007). High levels of lycopene have been found in the serum, testes and adrenal glands of human body. Several recent studies have indicated that the dietary intake of tomato and its products containing lycopene is associated with a decreased risk of chronic diseases, such as cancer and cardiovascular diseases.

Lycopene is an acyclic carotene with 11 conjugated double bonds found in plants and also in plasma. The long chromophore in the polyene chain accounts for the red

colour of lycopene and also for its powerful antioxidant activity. It is able to react with singlet oxygen and various radical cations and has the highest TEAC (Trolox-equivalent antioxidant capacity) value of all carotenoids (Miller and Rice-Evans, 1997). A daily intake of 25-30 mg dietary lycopene is recommended to prevent diseases like cancer, cardiovascular diseases etc.Minimising the loss of fruit quality throughout the production process, storage and increasing shelf life is important. However, degradation of lycopene and colour loss of processed tomato products are affected by a number of factors such as high temperature, long processing time, light, oxygen, acids, and some metal ions. Compositional variation of lycopene in tomato occurs as a consequence of varietal differences, climatic conditions, agricultural variables, stage of maturity, harvesting and postharvest handling conditions during storage, and transportation (Kader, 2005).

The postharvest losses of fruits and vegetables in the developing countries account for almost 50 per cent of the produce. In India losses of up to 40 per cent of produce occur because of excessive fruit softening. Tomato experiences great postharvest losses because of its natural perishability, precarious transportation and storage conditions, and inadequate packaging. These losses can be minimised by increasing the shelf life of the tomato.

The maintenance of good organoleptic characters for an extended period of time after fruit harvest is defined as shelf life. A long shelf life is a desirable trait for fresh market tomatoes. Several postharvest packaging methods and treatments are available such as modified atmosphere packaging used in the storage of fresh fruits and vegetables i.e. storage in plastic films, which restrict the transmission of respiratory gases. This results in the accumulation of carbon dioxide and depletion of oxygen around the fruits, which may increase their storage life (Kader, 2005). Tomato treated with chlorine, packed in perforated (0.25 %) polyethylene bag and kept at ambient (Temperature 20-25 °C and Relative humidity 70-90 %) conditions showed substantial reduction in losses caused by decay and weight loss (Nasrin *et al.,* 2008). The maximum shelf life was noticed in 1 per cent calcium chloride treated fruits (16.50 days) followed by (0.75 %) calcium chloride treated fruits (16.17 days) (Bhattarai and Gautam, 2006). But, these methods are expensive and quite labour intensive.

Shelf life of fruit can also be increased through biotechnological approach (gene silencing & genetic engineering) but in India genetically modified food crops are not easily permitted because of many reasons. Several spontaneous ripening mutants were described in tomato, such as *rin* (*ripening inhibitor*), *nor* (*nonripening*) and *alc* (*alcobaca*) (Giovannoni, 2006). Among, these genes *alc* have been found to be located on the short arm of the chromosome number 10 of Tomato. While fruits of *alc* mutant gene confer uniform ripening in tomato, fruits of *nor* and *rin* fail to ripen and do not exhibit any climacteric rise. Introgression of these genes in local cultivar results in increase in shelf life of fruit. *Solanum pimpinellifolium* produces small and high nourishing quality fruits, and easily crosses with the cultivated tomato. Zuriaga *et al*. (2009) evaluated different *S. Pimpinellifolium* accessions for the fruit Shelf life, and selected one of them (LA722 from Tomato Genetic Resources Center, Davis, USA) because of its long Shelf Life. Zarzoli *et al.* (2000) crossed that accession to a standard Argentinian variety of *S.*

lycopersicum cv. *Caimanta*, and obtained a hybrid with a longer shelf life than the cultivated parent and other commercial varieties.

Several authors Pereira da costa *et al*. (2009) suggested that why tomato species are alternative genetic resources to develop long shelf life varieties without negative pleiotropic effects on other fruit quality traits. Several wild species were used in tomato quality breeding such as *Solanum pennelii* (Causse *et al.,* 2004), *Solanum peruvianum* and *Solanum hirsutum* (Fulton *et al*., 2002). *Solanum pimpemilifolium* is characterized by small size and high quality fruits (Stevens and Rick,1986) and previous studies have shown that this wild species and its hybrids with cultivated tomato had longer fruit shelf life with better fruit quality while compared to commercial cultivars (Rodriguez *et al*., 2011).

But production of the hybrids with high shelf life has a major disadvantage such as; some of the undesirable genes that are linked to this trait decrease the fruit quality. This type of demerits can be eliminated by marker assisted selection. The microsatellite or SSR markers are PCR-based, genetically co-dominant, robust, reproducible, hyper variable, informative and reasonably easy-to-use (Powell *et al.,* 2012). These can be widely used in the various studies like marker assisted selection, gene introgression studies etc. Backcross breeding is widely used for the introgression of several desirable traits into the well adopted variety for which it is deficient. Backcross population developed by crossing F_1 hybrid to one of its parents, are the simplest of mapping populations developed for self-pollinating species. They are very easy to develop in short time (Mc Couch and Doerge, 1995; Paterson, 1996).

Keeping the above points in the view, present investigation was conducted to introgress the high shelf life trait into commercially well accepted variety. We have developed two backcross populations (BC_2F_1) of the crosses Pusa Ruby/L121//Pusa Ruby and Vaibhav/RIL126//Vaibhav and these were evaluated to achieve the following objectives:

1) To assess the genetic characteristics of parents, F_1 generation and backcross population.

2) Validation of SSR markers linked to high shelf life and other characters in backcross population.

II REVIEW OF LITERATURE

In this chapter an attempt has been made to review the relevant literature on different aspects of the present study under the following sub-heads:

2.1 Classification and Phylogeny of Tomato

The Tomato belongs to the family solanaceae, division Magnoliophyta, class Magnoliopsida, subclass Asteridae, order Solanales, suborder Solanineae, genus *Lycopersicon*, subfamily Solanoideae and tribe Solaneae. The taxonomic classification of the tomato is still debated. In 1753, the Swedish botanist Linnaeus named it *Solanum lycopersicon*, but 15 years later, Philip Miller replaced the Linnaean name with *Lycopersicon esculantum* (Taylor, 1986). Although taxonomists have recently reintroduced its original name, *Solanum lycopersicon* (Heiser and Anderson, 1999), the commonly accepted and still valid name is *Lycopersicon esculantum.*

Tomatoes were introduced into Europe from the America and eventually came in to the focus of botanist, aged back at the middle of the sixteenth century, The scientific naming of tomatoes, including wild species, has been linked to concepts of diversity in *Solanum lycopersicum*, the cultivated species. All related wild species of tomato are native to the Andean region that includes parts of Chile, Colombia, Ecuador, Bolivia and Peru (Sims, 1980). The first domesticated tomato might have been a little yellow fruit, similar in size to cherry tomato, grown in Central Mexico (Smith and Andrew, 1994). Based on morphological characters, phylogenetic relationships, and geographic distribution, 13 species of wild tomatoes, including the cultivated tomato (*Solanum lycopersicum* L.) and four closely related species have been recognized as part of the tomato clade. Four species have been segregated from the highly polymorphic green-fruited species *S. peruvianum*, *S. arcanum*, *S. huaylasense*, *S. peruvianum* and *S. corneliomulleri*.

The first two have been described as new species from Peru and other two had already been named by Linnaeus, (1753) and Mc Bride, (1962), respectively, (Peralta and Spooner, 2005). Another yellow to orange-fruited species, *S. galapagense*, was segregated from *S. cheesmaniae*; both of which are endemic to the Galapagos Islands (Darwin *et al.*, 2003; Knapp and Darwin, 2007). (Peralta and Spooner, 2007) had included these 13 species, in addition other four closely related species (*S. juglandifolium*, *S. lycopersicoides*, *S. ochranthum*, *S. sitiens*) in the taxonomic series Systematic Botany Monographs.

A wide variety of studies, based upon several morphological and molecular characters such as chloroplast and mitochondrial restriction sites, nuclear RFLPs, isozymes, internal transcribed spacer (ITS) and granule bound starch synthase (GBSSI or waxy) sequences, simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs) have been carried out to determine the wild tomato phylogeny (Peralta and Spooner, 2005). Although evolutionary relationships among the known species of tomato have not been completely resolved, consensus is emerging on several aspects of the group.

2.2 Genome characterization

All the known tomato species are diploid (2n=2x=24) having equal chromosome number and similar chromosome structure, the *Lycopersicon* genus was initially divided into two major subgenera regarding the color of the fruits: *Eulycopersicon* (for color-fruited species) and *Eriopersicon* (for green-fruited species) (Muller,1940). A more comprehensive and objective division was proposed by (Rick, 1976), which included two major complexes: (1) the *esculentum* complex, for the species that cross easily with the commercial tomato; and (2) the *peruvianum* complex, for the species that could not be easily crossed with the commercial tomato.

2.3 Importance of tomato

2.3.1 As a commercial crop

2.3.2 As a nutritional source

2.3.3 As a model system

2.3.1 As a commercial crop

Tomato is one of the most popular vegetable crops at National, International, and State level. India ranks second in tomato production and also in area under tomato cultivation where area under tomato cultivation in India is 8,79,600 ha and total production is 18,226.6 Mt with average productivity of 20.7 Mt ha^{-1}. Tomatoes are second largest vegetable crop in India after Potato. The most important production states are Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and Assam. In Karnataka it is cultivated in an area of 64.25 ha and production is 2034.37 t and productivity is 33.9 Mt ha^{-1}.

In Karnataka the main season in which tomato crop is planted during end of November to early December and fruit availability period from this crop is very short that is from mid of April to May ending. Excess production of tomato during this short period of time leads to glut coupled with low prices and this period is followed by a period of scarcity and high prices in the market. Hence there is a need to make availability of produced tomato for long time. A few mutant genes interfering with ripening process of tomato have been reported. These genes include slow ripening *alcobac* (*alc*), *ripening inhibitor* (*rin*) and *non-ripening* (*nor*) mutants (Almeida, 1961). These alleles are helpful in extending fruit availability period of tomato by first delaying the ripening of the fruits on the plants (Kopeliovitech *et al.,* 1980 and Dhatt, 2001) and then by storing them at room temperature or in controlled conditions (Agar *et al.,* 1994; Ignatova *et al.,* 1999 and Kitagawa *et al.,* 2005).

Beside to extend the fruit availability period, another crop may be planted in first fort-night of March. Although the fruit quality components like TSS, total acidity, pH are better under late planting conditions than in main crop but there is decline in productivity due to inadequate vegetative growth and low fruit under high temperature conditions (Naveen *et al*., 2008). To break all these barriers and to evolve varieties having a built in

desirable character with high potential, hybridization, selection are successful to considerable extent. Nowadays marker assisted selection made this work easier.

Despite of tomatoes used as vegetables they are also used as a desert purpose for salads. In processing industries for sauce, ketchup, jam, pickle preparations. These processed tomato products has role in commercialization of grown tomatoes.

To meet these commercial demands fruit quality on the cultivated tomato is a very important attribute to choice the appropriate cultivars by producers and also by consumers. Markets are today interested in differentiating products by attributes related to taste, aroma, acidity, sugar content and vitamins (Causse *et al*., 2002 and Powell *et al*., 2012). Quality is a combination of visual stimuli such as size, shape, color, firmness and sensory factors like Sugars, acidity and taste. The maintenance of good organoleptic characteristics for an extended period of time after fruit harvest is defined as shelf life. A long shelf life is a desired character for fresh market tomatoes.

2.3.2 As a nutritional benefit

Besides all these aspects consumers are paying ever increasing attention to the health and nutritional aspects of horticultural products such as vitamin contents, mineral elements and antioxidants (Causse *et al*., 2010). Fresh fruits and vegetables have long been regards as having considerable health benefits, in particular to their antioxidant content, which can protect the human body against cellular events, cancer and other age related degenerative diseases (Adams and Adams, 2000). Tomatoes are large reservoir of diverse antioxidant molecules, such as ascorbic acid, vitamin E, carotenoids, flavonoids and phenolic acids. It is also rich in vitamins and minerals. These represent a major contribution to nutrition worldwide. Hence it is called as "protective food". (Naveen *et al*., 2008; Sacco *et al*., 2012) which play important role in human health.

These carotenoids including lycopene, flavonoids, vitamin C, and vitamin E have potential to prevent chronic diseases including epithelial cancer, cardiovascular diseases, digestive disorders and immune deficiency (Fooland, 2007). In this regards carotenoids have attracted significant attention, particularly Beta-carotenoid as provitamin A is very important for retinal health, preserving eyesight, night blindness and protection against cataract. Carotenoids like lycopene have also been implicated in reducing low density lipoprotein (LPL) improving immune system and preventing neuron degenerative diseases such as Alzheimer's, Parkinson's and vascular dementia. Flavonoids also have anti-oxidant properties reduces the risk of age related diseases, heart ailments and cancer.

The potential of phytonutrients in protecting against or delaying in incidence of fatal diseases is believed largely to occur via their antioxidants and free radical scavenging properties by protecting cellular macromolecules from oxidative damage induced by pro-oxidants, especially during aging. Therefore many researchers have been made conducted to enhance the nutritional quality of the commercial tomato varieties.

2.3.3 As a model system

In addition to obtain in depth knowledge of genetic regulation of nutritional content compounds in fruits, tomato also represent an excellent model system for both basic and applied research for many reasons including care of growth is a wide range of environment, a short lifecycle and well developed genetic and genomic tools (Fooland, 2007; Barone *et al.,* 2005). It has proven a highly useful model system for fruit development and ripening and in the system in which the role of ethylene during fruit ripening has been most thoroughly studied review (Cara and Giovannoni, 2008). It is an ideal model plant for studying climacteric fruit ripening several gene banks have established and more than 75,000 accesion of tomato are maintained. In addition several mutants affected in fruit size, shape, diversity and ripening have been isolated (Tanksley, 2004 and Rodriguez *et al*., 2011).

Recently the genome of *Solanum lycopericon* cv. "*Heinz 1706*" has been fully sequenced and made publically available. The predicted size of its diploid genome is approximately 900 mega bases (Mb), distributed on 12 chromosomes, more than 75 per cent of which is heterochromatin and largely devoid of genes. Around 33,000 genes have been predicted and some 5,000 genes are preferentially expressed in fruits. Tomato with its short generation time and the availability of a routine transformation technology, mapping population and micro array of mapping DNA markers, tomato is a highly tractable experimental system. Several "omics" tools (transcriptomics, proteomics and metabolomics) have been used to explore fruit formation and development (Alba *et al*., 2005 and Moore *et al*., 2005) leading to genetic characterization of several important traits that have been selected during tomato domestication (Pereira da Costa *et al*., 2013).

2.4 Shelf life

The maintenance of good organoleptic characteristics for an extended period of time after fruit harvest is defined as shelf life. A long shelf life is desirable trait for fresh market tomatoes (Pereira da Costa *et al*., 2013). Tomato is a product that experience great postharvest losses because of its natural perishability, precarious transportation, storage conditions and in adequate packaging. It is considered to be a perishable commodity its water content pertains to its poor postharvest life unless appropriate handling or storage technique is adopted. It is estimated about $1/3^{rd}$ of the harvested fruits do not reach the consumers as a result of these losses. In India about 40 per cent of produce is loss because of excessive softening. In order to minimize these losses it is important to study and understand the biological and environmental factors leading to postharvest loss in tomato (Kader, 2005).

2.4.1 Influence of gene regulation on shelf life

Tomato is a climacteric fruit and its ripening is dependent on an ethylene burst. Conversely in several tomato mutants in which ripening is delayed (*rin, nor, nr*) ethylene production is compromised. Synthesis of ethylene depends on the action of two enzymes ACC synthase (ACS) and ACC oxidase (ACO). ACS convert (s-adenosylmethionine into aminocyclopropane-1-carboxylate) which is subsequently transformed into ethylene by ACO. ACC synthases in tomato are encoded by a multigene family, but only *LeACS2* and

LeACS4 are upregulated during climacteric fruit ripening (Barry *et al.,* 1996; Giovannoni, 2006) down regulation of *LeACS2* and *LeACO2* delays ripening and the transgenic tomato fruits increases their shelf life.

2.4.2 Spontaneous ripening mutants of tomato

2.4.2.1 Never ripe (*nr*)

Never ripe (*nr*) is a semi dominant mutant that affects one of the seven ethylene receptors (*LeETR1-7*) present in tomato genome. Out of these seven genes, only *LeETR4*, *LeETR6* and *Nr LeETR3* are strongly expressed during fruit ripening, *nr* is insensitive to ethylene (Lanahan *et al.,* 1994; Wilkinson *et al.,* 1995) showed that *nr* was the outcome caused by a mutation member of the tomato ethylene receptor gene family. The *nr* mutant contains a mutation in the ethylene binding site conferring ethylene insensitivity. Analysis of *nr* showed a number of pleiotropic effects indicative of ethylene insensitivity throughout the plant (Lanahan *et al.,* 1994). The mutant only produces approximately 50 per cent of the normal level of ethylene and 20 per cent of the normal level of red carotenoid.

2.4.2.2 *Ripening inhibitor* (*rin*) mutant

Ripening inhibitor (*rin*), *Nonripening* (*nor*) participate in ethylene independent regulatory cascade during the early stages of fruit ripening. These neither produce autocatalytic ethylene nor ripen in the presence of exogenous ethylene. They display signs of ethylene insensitivity and ethylene inducible expression of several genes (Vrebalov *et al*., 2002) *LeMADS-RIN* encodes a member of MADS-box family of transcription factor. The *rin* mutant fruit fail to synthesize climacteric ethylene or accumulate lycopene in addition to being deficient in softening and remaining resistant to microbial infection. By manipulating these genes breeder are able to show the ripening process and these mutations are already being used commercially to extend shelf life. (Giovannoni *et al.,* 2008)

2.4.2.3 *Colourless non ripening mutant* (*cnr*)

It is a rare dominant mutation in a tomato gene that results in non-ripening phenotype that has firm fruit with reduced cell adhesion and complete absence of carotenoid synthesis in the pericarp (Giovannoni, 2006). The CNR promoter is progressively demethylated during ripening, but *cnr* mutants the promoter remain hyper methylated, which prevents RIN-MADS from binding to it (Zhong *et al*., 2013). The methylation states of several RIN-MADS targets change during ripening, indicating that progressive demethylation is necessary for RIN-MADS binding which lead to development of colourless, less softened fruits.

2.4.2.4 *Green ripening* (*gr*)

It is dominant non ripening mutant, whose phenotype is due to misexpression of *gr* gene in developing fruits and organs, where it is normally not active. In addition, a subset of ethylene responses associated with floral senescence, abscission and root

elongation are also impacted in mutant plants, but to lesser extent (Barry and Giovannoni, 2008).

2.4.2.5 *Alcobac* (*alc*)

It was discovered by Leal in 1973. It is an abnormally ripening mutant of tomato, as having an exceptionally long shelf life. Mutant is characterized by its extended shelf life and the yellow colour of its ripe fruits (Leal, 1973; Leal and Tabin, 1974). The long shelf life of the mutant facilitates its use for commercial breeding (Kopeiiovitch *et al.,* 1980). Alcobac containing the recessive alleles (*alc*) induces slow ripening, with a reduced level of ethylene production and have prolonged shelf life. This mutant is controlled by a single gene (*alc*) located on short arm of chromosome 10 (Kinzer *et al*., 1990). The use of these mutant alleles that interfere with the fruits natural ripening process is an attempt to minimize these problems (Mutschler *et al*., 1992). Postharvest shelf life of tomato may be increased by delaying mutant alleles which affect the natural ripening process by a favourable genotypic background. Among the several ripening mutant genes *alc* has proved to be highly efficient in increasing shelf life of commercial tomato fruits, especially in heterozygosis. The utilization of the *alc* mutant is heterozygosity (*alc+/alc*) decreased fruit firmness loss in tomato plants (Dias *et al.,* 2003).

The cultivation of tomatoes of the extra firm and long shelf life types showed increased quality of the fruits above 50 per cent. This increase happened because of improvement in the quality of fruit firm, which is suitable for consumption for an extended period of time after being harvested. These material bear genetic traits which interfere with the activity of enzymes that act directly upon the physiological process can be altered by mutant alleles which, in heterozygosity, extend the postharvest life of fruits. This is reflected as a higher resistance to transportation and a longer shelf life, without loss of their organoleptic characteristics (Dias *et al.,* 2003).

2.4.3 Recommendations for Maintaining Postharvest Quality

2.4.3.1 Maturity Indices

A. Standard Tomatoes

Minimum harvest maturity (Mature Green 2) is defined by internal fruit structure indices. Seeds are fully developed and are not cut upon slicing the fruit. Gel formation is advanced in at least one locule and jelly like material is forming in other locules.

B. Extended Shelf Life Tomatoes

Off-vine ripening is severely affected if fruits are harvested at the MG2 stage. Minimum harvest maturity is better defined as equivalent to ripeness class Pink (USDA Color Stage 4 more than 30 per cent but not more than 60 per cent of the fruit surface, overall, shows a pink-red color).

2.4.3.2 Quality Indices

Standard tomato quality is primarily based on uniform shape and freedom from growth or handling defects. Size is not considered as a factor for grade quality but may strongly influence commercial quality expectations.

Shape - well-formed for type (round, globe, flattened globe, aroma)

Color - Uniform color (orange-red to deep red; light yellow). No green shoulders.

Appearance - Smooth and small blossom-end scar and stem-end scar.

Firmness - Yields to firm hand pressure. Not soft and easily deformed due to an over ripe condition.

2.5 Different approaches to improve postharvest shelf life of Tomato

Tomato is one of the most important supplementary sources of minerals and vitamins in the human diet. It is highly perishable and encounters several problems during its transportation, storage and marketing. Owing to the lack of information on appropriate postharvest treatments, packaging, temperature etc., the fruits not only lose their quality but also encounter a substantial postharvest loss. In a commercial context, the control of fruit ripening is often achieved through early harvest, by controlling the postharvest storage atmosphere and by genetic selection for slow or late ripening varieties. However, an increasing understanding of the metabolic changes underlying fruit development and ripening may help in developing new strategies to improve shelf life and postharvest quality of fruit (Matas *et al.,* 2009).

2.5.1 Postharvest treatments:

(a) Effect of Polyethylene Packaging on Fruit

Tomatoes harvested at breaker stage and packed in 300 gauge polyethylene bags with three vents recorded minimum changes in moisture, total soluble solids, acidity and sugars than the control fruits. The organoleptic score was high in above acceptable limits for these fruits with a shelf life of 42 days (Naik *et al.*, 1993). Minimum weight loss of 77.5 g was recorded for tomatoes packed in black polyethylene bags. While, a maximum weight loss of 224.16 g occurred for unpacked fruits. The best colour retention and firmness was noted in black polyethylene bags (Badshah *et al.*, 1997). Tomato treated with chlorine; packed in perforated (0.25 %) polyethylene bag and kept at ambient (Temperature 20-25 °C and Relative humidity 70-90 %) condition resulted in substantial reduction in losses caused by decay and weight loss. This treatment combination considerably delayed compositional changes in total soluble solids (TSS), total sugar, reducing sugar, vitamin-C, β-carotene etc. Under this condition, shelf life of tomato had extended up to 17 days as compared to the non-treated tomatoes kept in ambient condition without packaging or packed in a gunny bag for 7 days (Nasrin *et al.*, 2008).

(b) Effect of Heat Treatment on Tomato Fruit

Matured tomatoes immersed in water at 40 °C for 15 min and stored for 21 days at 5 °C followed by 12 days at 20 °C exhibited lowest incidence of chilling injury and were firmer. Those fruits ripened normally at 20 °C characterized by climacteric ripening. Complete disappearance of chlorophyll followed by lycopene synthesis was observed after 9 days at 20 °C indicating normal ripening (Nagetey *et al.*, 1999). Hot water dips (39 °C for 90 min) of mature green cherry tomato fruits and subsequent storage in MAP substantially delayed the colour development (Ali *et al.*, 2004). 'Rapsody' tomato fruit exposed to 34 °C for 24 hr in air and stored at 10 °C for 30 days developed the best colour when ripened and had the least chlorophyll and highest lycopene content (Yahia *et al.*, 2003).

(c) Effect of calcium chloride on Tomato Fruit

Tomato fruits were dipped in 0, 0.5, 1.0 and 1.5 per cent of calcium chloride. Then the fruits were placed on foam tray and wrapped with PVC film. The fruits were kept at 5 ^{o}C, 85 per cent RH. It was found that vitamin C content and respiration were increased, while firmness, total solids and total acids were decreased. Bhattarai and Gautam, (2006) conducted experiment to find out the effect of harvesting method and calcium chloride treatment on postharvest physiology of tomato. Tomato fruits with stalk and without stalk were harvested at breaker stage and dipped in distilled water and different concentrations of calcium chloride i.e. 0.25 per cent, 0.50 per cent, 0.75 per cent and 1 per cent for fifteen minutes. Fruits were then air dried and stored at ambient conditions (24 ± 3 ^{o}C and 70 ± 5 % RH). Among the tested treatments the least cumulative physiological weight loss (12.14 %) was exhibited by 1 per cent calcium chloride.

The maximum shelf life was noticed in 1per cent calcium chloride treated fruits. Low O_2 and high CO_2 atmospheres (3 % O_2 + 20 % CO_2) prevented the total carotenoid and lycopene biosynthesis and as well as β-galactosidase activity in tomatoes. The chlorophyll degradation and loss of firmness was slowed down (Gabriel *et al.*, 1999). Modified atmosphere packaging provided good quality tomato slices with a shelf life of 2 weeks at 5 °C (Hong and Gross, 2001). Mature green tomatoes packed in MAP had a built in atmosphere of 4 per cent O_2 and 5 per cent CO_2 and delayed the fruit ripening. These fruits had a low rate of physiological loss in weight and better overall quality than control (Onwuzulu *et al.*, 1995). Tomatoes packed with several polyvinylchloride (PVC) films or with K-resin had a slow rate of colour change than control, but continued to ripe normally after the packs were perforated and transferred to 20 °C. The aroma, flavour and texture of these fruits were slightly better than control fruits (Geeson *et al.*, 1985). A parallel delay in fruit softening was attributed to an inhibition of polygalacturonase and pectin methyl esterase, and the most effective treatment was that of 20 ml ethanol per 2.5 kg fruit (Thakur *et al.*, 2000).

2.5.2 Genetic engineering approaches

The manipulation of tomato fruit quality through genetic engineering is reasonably well advanced. Many important processes in plants are regulated by ethylene,

especially those involved with postharvest processes. There are several methods available to manipulate ethylene responses. First, by blocking ethylene synthesis: with antisense RNA for ACC synthase or ACC oxidase, using gene silencing or co-suppression, by metabolic interference, diverting SAM or ACC away from ethylene synthesis. Secondly, by blocking the expression of genes that are induced in response to ethylene by antisense RNA for PME (pectin methylesterase) or PG (polygalacturonase). Third, the expression of a dominant mutant ethylene receptor blocks the perception of ethylene. To identify the function of genes and their role in the ripening process, an antisense RNA strategy has been used by several research groups and several transgenic plants that showed reduced expression of ripening related genes (Gray *et al.*, 1994; Stearns and Glick, 2003). The understanding of the biology of fruit ripening has improved, has boosted the ability to improve the organoleptic and nutritional qualities of fruits through crop management, breeding or biotechnology.

One important target for modification of tomato fruit ripening to decelerate is the softening of the fruit. During fruit ripening, various enzymes that degrade specific components of the cell walls are synthesized in the fruit. The events such as dissolution of the middle lamella, resulting in a reduction in intercellular adhesion, depolymerization, and solubilization of hemi cellulosic and pectic cell wall polysaccharides are accompanied by the increased expression of numerous cell wall degrading enzymes, including polysaccharide hydrolases, transglycosylases, lyases, and other wall loosening proteins, such as expansin (Rose *et al*., 2003; Brummell, 2006). Among the enzymes that accumulate in the fruits are cellulases, polygalacturonase (PG) and pectin methylesterase (PME), the last two are being involved in breakdown of the pectin cross linking molecules. These enzymes contribute to the softening of the fruit by reducing the rigidity of the cell wall structures. The expressions of the genes encoding these enzymes are regulated by ethylene.

2.5.3 Enzymes regulating fruit ripening

2.5.3.1 Polygalacturonase (PG)

Polygalacturonase or PG is an enzyme that degrades pectin in fruit cell walls and, along with other enzymes, provokes the softening of fruits during fruit ripening. antisense RNA techniques have been developed to generate novel mutant tomatoes in which the biochemical function of this enzyme and its involvement in fruit softening has been tested (Bird *et al*., 1988). The Calgene (Davis, CA) Flavr Savr TM tomato based on antisense PG gene was commercialized in the U.S. market in 1995, marketed as high flavoured fresh tomato. This tomato was modified by silencing the turning off PG synthesis using antisense technology (Redenbaugh *et al*., 1992). It was claimed that softening of the fruit was impeded in those fruit, allowing them to remain on the vine longer, with harvest later than the typical "mature green fruit" stage.

2.5.3.2 Pectin methyl esterase (PME)

PME is involved in the metabolism of pectins in the cell wall. Pectins are present in mature green fruit as long polymers, and PME is expressed during fruit ripening to break these large polymers into shorter molecules. It is likely that PME is one of the first

enzymes involved in the metabolism of pectins. Transgenic plants with low expression of pectin methyl esterase (PME) in the fruits were developed again using antisense RNA approach (Tieman *et al*., 1992; Hall, 1996). The pectins in these fruit with reduced PME activity remain large as the fruit ripen. They are not broken down into shorter pectins because there is no PME activity. However, transgenic plants with reduced PME levels ripen normally as it does not interfere with ethylene production, the central regulator of fruit ripening. Suppression in transgenic fruit resulted in reduced pectin depolymerization, however there was no effect on firmness during ripening (Tieman and Handa, 1994). The outcome of this modification is that using this transgenic tomato fruit has more viscous juice as the starting material. In turn, it requires less processing to produce tomato paste of the desired consistency.

2.5.3.3 Other enzymes

Softening following ripening proved to be significantly reduced in transgenic tomato fruit with suppressed β-galactosidase, an enzyme that is normally up regulated during the early stages of ripening and serves to remove pecticgalactan side chains (Smith *et al*., 2002). In addition, expansin proteins appear to play an integral role in fruit softening, probably by disrupting hydrogen bonding between cellulose micro fibrils and matrix polysaccharides, resulting in loosening of the cell wall structure (Brummell and Harpster, 2001).

2.5.3.4 Phospholipase D

Phospholipase hydrolyze phospholipids, which are the backbones of the biological membranes. The transgenic tomato fruits (*Solanum lycopersicon* cv. *Celebrity*) transformed with an antisense phospholipase D (PLD) cDNA construct, resulted in a 30-40 per cent reduction of PLD activity in ripe fruits. The transgenic fruits were firmer, possessed better red colour, and flavour. The dry matter and ash contents, as well as the precipitate weight ratio (PPT) of the transgenic fruit products were significantly higher when compared to the products from the control fruits. The vitamin C content of the transgenic fruits was also higher compared to the control fruits. The results suggested that a reduction in PLD activity may lead to increased membrane stability and preservation of membrane compartmentalization (Pinhero *et al*., 2003).

2.5.3.5 Deoxyhypusine synthase

Regulation of expression of programmed cell death, including senescence, in plants is achieved by integration of a gene or gene fragment encoding senescence-induced deoxyhypusine synthase, senescence-induced elF-5A or both into the plant genome in antisense orientation. DHS mediates the first of two sequential enzymatic reactions that activate eukaryotic translation initiation factor-5A (eIF 5A) by converting a conserved Lys to the unusual amino acid, deoxyhypusine. DHS levels were suppressed by anti-sense approach under constitutive promoter in tomato (Wang *et al*., 2005). The suppression of DHS had pleiotropic effects on growth and development of tomato. Fruit from the transgenic plants ripened normally, but exhibited delayed postharvest softening and senescence that correlated with suppression of DHS protein levels. Transgenic plants

in which *DHS* was more strongly suppressed were male sterile, did not produce fruit, and had larger, thicker leaves with enhanced levels of chlorophyll.

2.5.3.6 Delayed fruit ripening by manipulating ethylene metabolism

A number of approaches have been used to develop tomatoes with reduced synthesis of ethylene, to alter the ripening of tomatoes, besides improved quality as fresh market tomatoes. Expression of antisense versions of enzymes from the ethylene biosynthesis pathway can be used to regulate the genetic control of ethylene levels. The important transgenic strategies to lower the ethylene levels are as follow:

2.5.3.7 ACC synthase

ACC synthase is responsible for the conversion of SAM to 1-ACC. It is one of the rate limiting enzymes in ethylene biosynthesis and is the target for many strategies aimed at reducing ethylene. Oeller *et al*., (1991) to inhibit one tried tomato ACC synthase (ACS2) mRNA, for which the entire gene including the untranslated regions was inserted in opposite orientation under the constitutive CaMV 35S promoter into plant genome. These transgenic tomatoes showed a 99.5 per cent reduction in ethylene production and no ripening was observed. That effect can be reversed by exogenous ethylene treatment.

2.5.3.8 ACC oxidase

ACC oxidase is responsible for the conversion of 1-ACC to ethylene and it also encoded by a multigene family. Hamilton *et al*., (1999) over-expressed the antisense ACC oxidase gene in tomato. In other approach, RNAi-mediated gene silencing for the ACC oxidase gene was used for successful production of transgenic tomatoes with trace levels of ethylene and prolonged shelf life.

2.5.3.9 ACC deaminase

The enzyme ACC deaminase is shown to convert ACC to ammonia and β-ketobutyrate, both of them were further metabolized by microorganisms. This enzyme was first discovered in soil micro-organisms (Honma and Shimomura, 1978). These transgenic plants showed 90-97 per cent reduced ethylene production but they also had delayed pigment accumulation, altered ripening pattern and softening.

Vijaykumar *et al.* (2010) reported the prolonging of the shelf life of transgenic tomato resulting from the suppression of genes encoding cell wall–degrading proteins. N-glycans have been reported to play an important role during fruit ripening, although the role of any particular enzyme is yet unknown. They identified and targeted two ripening-specific N-glycoprotein modifying enzymes, α-mannosidase (α-Man) and β-D-N-acetylhexosaminidase (β-Hex). Analysis of transgenic tomatoes revealed ~ 2.5-fold and ~2-fold firmer fruits in the α-Man and β-Hex RNAi lines, respectively, and ~30 days of enhanced shelf life. Genetic manipulation of N-glycan processing can be of strategic importance to enhance fruit shelf life, without any negative effect on phenotype, including yield.

Handa and Mattoo, (2010) introduced the spermidine synthase gene from yeast into the transgenic tomato plants which led to increased production of spermidine in the tomatoes. Spermidine is a polyamine and is found in all living cells. Spermidine is important in reducing senescence. Spermidine synthase is an enzyme that catalyzes the transfer of the propylamine group from *S*-adenosyl methionine amine to putrescine in the biosynthesis of spermidine. It enhances nutritional and processing quality of tomato.

Tomato has been engineered to produce long shelf-life fruits, however but genetically modified crops are not yet well accepted by consumers. Considering the current scenario of India, approved for GM crop, except Bt cotton is still pending, where the next best option is to employ conventional breeding integrated with molecular marker assisted selection (MAS) for developing desired quality in this crop. Several spontaneous ripening mutants have been reported in tomato, were described in tomato, such as *rin* (*ripening inhibitor*), *nor* (*nonripening*) and *alc (alcobaca)* (Giovannoni, 2006) these gene can be well to improve the shelf life.

2.6 Breeding for high shelf life in tomato

Like all known species of the genus *Solanum*, tomato is a diploid; having 2n=24 chromosomes, with a genome size of 2.0 pg/2c = 9.5×105 Kb/1c (950 M bp), which is composed of 77 per cent heterochromatin and 23 per cent euchromatin (Paterson, 1996). The nine species have been grouped into two intracrossable and interincrossable groups (or complexes): the "*esculentum* complex," including *L. esculentum, L. esculentum* var. *cerasiforme, L. pimpinellifolium, L. cheesmanii* (including *L. cheesmanii* f. *minor*), *L. chmielewskii, L. parviflorum, L. hirsutum* (including f. *typicum*and f. *glabratum*) and *L. pennellii*, and the "*peruvianum* complex" including *L. peruvianum* and *L. chilense*.

All species within the *esculentum* complex can be hybridized with the cultivated tomato, and most of which (except *L. hirsutum* f. *typicum* and some *L. pennellii*) are self-compatible. The two species within the *peruvianum* complex are extremely diverse and represent a wealth of characteristics, which are potentially valuable for crop improvement. These species, which are mostly self-incompatible and produce green fruit, have been rather partial in their usefulness to cultivated forms due to various barriers present in sexual hybridization and gene transfer. However, they can be hybridized with members of the *esculentum* complex by the application of techniques such as embryo rescue or by the use of pollen mixture (with tomato pollen) when fertilizing tomato plants. There are documented examples of crosses with *peruvianum* complex which have been utilized in tomato breeding programme.

2.7 Use of molecular marker in evaluation of a population

Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as 'signs' or 'flags'. Genetic markers that are located in close proximity to genes (*i.e.* tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus') (Collard *et. al.*, 2005).

There are three major types of genetic markers:

(1) Morphological (also 'classical' or 'visible') markers, which themselves are phenotypic traits or characters;

(2) Biochemical markers, which include allelic variants of enzymes called isozymes; and

(3) DNA (or molecular) markers, which reveal sites of variation in DNA (Jones *et al.*, 1997; Winter and Kahl, 1995).

DNA markers may be broadly divided into three classes based on the method of their detection: (1) hybridization-based; (2) polymerase chain reaction (PCR)-based and (3) DNA sequence-based (Gupta *et al.*, 1999; Jones *et al.*, 1997; Joshi and Nguyen, 1993; Winter and Kahl, 1995). Essentially, DNA markers may reveal genetic differences that can be visualised by using a technique called gel electrophoresis and staining with chemicals (ethidium bromide or silver) or detection with radioactive or colorimetric probes. DNA markers are particularly useful if they reveal differences between individuals of the same or different species. These markers are called polymorphic markers, whereas markers that do not discriminate between genotypes are called monomorphic markers. Polymorphic markers may also be described as co-dominant or dominant. This description is based on whether markers can discriminate between homozygotes and heterozygotes. Co-dominant markers indicate differences in size whereas dominant markers are either present or absent. The different forms of a DNA marker (e.g. different sized bands on gels) are called marker 'alleles'. Co-dominant markers may have many different alleles whereas a dominant marker only has two alleles (Collard *et al.*, 2005).

Several different populations may be utilized for mapping within a given plant species, with each population type possessing advantages and disadvantages (Mc Couch and Doerge, 1995; Paterson, 1996). F_2 populations, derived from F_1 hybrids, and backcross (BC) populations, derived by crossing the F_1 hybrid to one of the parents, are the simplest types of mapping populations developed for self-pollinating species. Their main advantages are that they are easy to construct and require only a short time to produce.

It is critical that sufficient polymorphism exists between parents in order to construct a linkage map (Young, 1994). In general, cross pollinating species possess higher levels of DNA polymorphism compared to inbreeding species; mapping in inbreeding species generally requires the selection of parents that are distantly related. In many cases, parents that provide adequate polymorphism are selected on the basis of the level of genetic diversity between parents (Anderson *et al.*, 1993; Collard *et al.*, 2003; Joshi and Nguyen, 1993; Yu and Nguyen, 2000). The choice of DNA markers used for mapping may depend on the availability of characterized markers or the appropriateness of particular markers for a particular species.

Once polymorphic markers have been identified, they must be screened across the entire mapping population, including the parents (and F_1 hybrid, if possible). This is known as marker 'genotyping' of the population. Therefore, DNA must be extracted from

each individual of the mapping population when DNA markers are used. Significant deviations from expected ratios can be analyzed using Chi-Square tests. Generally, markers will segregate in a Mendelian fashion although distorted segregation ratios may be encountered (Sayed *et al.,* 2002; Xu *et al.,* 1997).

The cultivated tomato, *Lycopersicon esculentum*, is the second most consumed vegetable worldwide and a well-studied crop species in terms of genetics, genomics, and breeding. It is one of the earliest crop plants for which a genetic linkage map was constructed, and currently there are several molecular maps available, which have been constructed based on crosses between the cultivated and various wild species of tomato. The high-density molecular map, developed based on an *L. esculentum* × *L. pennellii* cross, includes more than 2200 markers with an average marker distance of less than 1 cM and an average of 750 K bp per cM. Different types of molecular markers such as RFLPs, AFLPs, SSRs, CAPS, RGAs, ESTs and COSs have been developed and mapped onto the 12 tomato chromosomes. Markers have been used extensively for identification and mapping of genes and QTLs for many biologically and agriculturally important traits and occasionally for germplasm screening, fingerprinting, and marker-assisted breeding. The utility of MAS in tomato breeding has been restricted largely due to limited marker polymorphism within the cultivated species and economic reasons. Also, when used, MAS has been employed mainly for improving simply-inherited traits and not much for improving complex traits. The latter has been due to unavailability of reliable PCR-based markers and problems with linkage drag. Efforts are being made to develop high-throughput markers with greater resolution, including SNPs. The expanding tomato EST database, which currently includes ~214 000 sequences, the new microarray DNA chips, and the ongoing sequencing project are expected to aid development of more practical markers. Several BAC libraries have been developed that facilitate map-based cloning of genes and QTLs. Sequencing of the euchromatic portions of the tomato genome is paving the way for comparative and functional analysis of important genes and QTLs (Fooland, 2007).

In order to use the wild germplasm in tomato breeding and genetic studies (Eshed and Zamir, 1994) developed a new kind of genetic resource which is composed of 50 *L. esculentum* lines each containing a single introgression from the green fruited species *L. pennellii* (LA 716). The lines contain on the average an introgression of 33 cM from a total genome size of 1200 cM. The size and identity of the introgressed segments was determined based on RFLP analysis of 350 markers.

An introgression line F_2 derived from *L. esculentum* (tomato) × *L. pennellii* and a backcross 1 (BC_1) population derived from *L. esculentum* × *L. pimpinellifolium* both place *fw 2.2* near TG-91 and TG-167 on chromosome 2 of the tomato high density linkage map. *fw 2.2* accounts for 30 per cent and 47 per cent of the total phenotypic variance in the *L. pimpinellifolium* and *L. pennellii* populations, respectively (Alpert *et al.,* 1995).

Five hundred DNA sequences of tomato were searched for SSRs and analyzed for the design of PCR primers. Of the 158 pairs of SSR primers screened against a set of 19

diverse tomato cultivars, 129 pairs produced the expected DNA fragments in their PCR products, and 65 of them were polymorphic with the polymorphism information content (PIC) ranging from 0.09 to 0.67. Among the polymorphic loci, 2-6 SSR alleles were detected for each locus with an average of 2.7 alleles per locus; 49.2 per cent of these loci had two alleles and 33.8 per cent had three alleles (He *et al.,* 2003).

Lycopersicon esculentum accessions bearing fasciated (multiloculed) fruit were characterized based on their flower organ and locule number phenotypes. The most significant of these, map to the bottoms of chromosomes 2 and 11 and correspond to the locule number and fasciated loci. All stocks tested were fixed for mutations at the fasciated locus, which maps to the 0.5 cM interval between the markers T302 and cLET24J2A and occurs in at least three allelic forms (wild type and two mutants). One of the fasciated mutant alleles is associated with nonfused carpels and repressed recombination. The other two loci controlling locule number correspond to the *lcn 1.1* and *lcn 2.2* loci located on chromosomes 1 and 2, respectively (Barrero and Tanksley, 2004)

Frary *et al*. (2004) used 150 molecular markers for genotyping 175 BC_2 plants for detection of QTLs for different yield and fruit quality traits in a population derived from the interspecific cross *Lycopersicon esculentum* (E6203) × *Lycopersicon pennellii* accession (LA1657).

In previous introgression studies, involving several different wild tomato species, have shown that the long arm of chromosome 4 contains QTLs for many horticulturally important traits including soluble solids content, fruit shape, lycopene content and biochemical composition. However, these earlier studies were unable to determine how many genes control these traits and whether genes affecting the same character from different wild species are allelic or not. In an effort to shed light on these issues, we have constructed a series of lines containing small, overlapping introgressions for portions of the long arm of chromosome 4 from *L. peruvianum* and *L. hirsutum* and tested these lines in replicated field trials. The results provide evidence for multiple, non-allelic loci controlling soluble solids and fruit weight. They also showed that the loci controlling some traits (e.g. fruit shape, fruit weight, epidermal reticulation) co-localize to the same portions of chromosome 4, a result that may be attributed to pleiotropy and gene dense areas with lower than average recombination (Yates *et al.,* 1965).

Tomato and potato expressed sequence tag (EST) sequences contained in the solanaceae genomics network (SGN) database were screened for simple sequence repeat (SSR) motifs. A set of 76 SSRs were placed on the *S. lycopersicum* (LA925) × *S. pennellii* (LA716) high-density map. A set of 76 selected cleaved amplified polymorphism (CAP) markers were also developed and mapped onto the same population. These 152 PCR-based anchor markers are uniformly distributed and encompass 95 per cent of the genome with an average spacing of 10.0 cM. These PCR based markers were further used to characterize *S. pennellii* introgression lines and should prove helpful in utilizing these stocks for high-resolution mapping experiments (Eshed and Zamir, 1994 and Frary *et al.,* 2005).

The comparative characterization was done by simple sequence repeat (SSR) and sequence characterized amplified region (SCAR) markers of two populations of F_6 lines derived from *Lycopersicon pimpinellifolium* (population, consisting of 142 lines) and *L. cheesmanii* (population, consisting of 115 lines) and sharing the female parent, *L. esculentum* cv. *cerasiforme.* A linkage map for each population was obtained, with the average distances between consecutive markers being 3.8 cM or 3.4 cM respectively, depending on the population (Villalta *et al.,* 2005).

Fruit shape features were measured in three inter-specific F_2 populations using the software application Tomato Analyzer. The largest effect QTL in the Rio Grande population were found to be *fs 8.1* on chromosome 8 was associated with TG-176 and *dblk 2.1* on chromosome 2 was associated with TG-645 (Gonzalo and Knap, 2008).

Ohyama *et al.,* 2009 developed nearly 700 non-redundant 2- or 3-base simple sequence repeat (SSR) markers from tomato using sequence data obtained from open genome databases. Among various types of core motifs, AT was most abundant in SSRs derived from cDNAs 53 per cent and bacterial artificial chromosome (BAC) ends 72 per cent. They found that there was a positive correlation between the rate of detection of polymorphic alleles (heterozygosity value; Hv) and the repeat number of the core motif in all markers showing polymorphism atleast among one pair of six cultivars or lines tested (r = 0.566**).

A total of 54 SSR primer pairs from 17 BAC clones on chromosome 6 were designed and validated. Polymorphism of these loci was evaluated in a panel of 16 genotypes comprising of *Solanum lycopersicum* and its wild relatives. Genetic diversity analysis based on these markers could distinguish genotypes at species level (Geethanjali *et al.,* 2010).

Using public genome sequence data in tomato, Shirasawa *et al.,* 2010 developed three types of DNA markers: expressed sequence tag (EST)-derived simple sequence repeat (SSR) markers (TES markers), genome-derived SSR markers (TGS markers) and EST-derived intronic polymorphism markers (TEI markers). A total of 2,047 TES, 3,510 TGS and 674 TEI markers were established and used in the polymorphic analysis of a cultivated tomato (*Solanum lycopersicum*) 'LA925' and its wild relative *Solanum pennellii* 'LA716', parents of the Tomato-EXPEN 2000 mapping population. A high-density genetic linkage map composed of 1,433 new and 683 existing marker loci was constructed on 12 chromosomes, covering 1,503.1 cM. In the present map, 48 per cent of the mapped TGS loci were located within heterochromatic regions, while 18 per cent and 21 per cent of TES and TEI loci, respectively were located in heterochromatin.

2.8 Molecular markers in tomato breeding

As there is need for breeding high quality tomato hybrid with both quality and quantity traits molecular markers allow the direction of such plant yield and fruit quality traits (Sacco *et al*., 2012). These molecular markers constitute an efficient tool for indirect selection in plant breeding. They have been widely used for following the introgression of monogenomic traits such as disease resistance (Singh *et al*., 2001). For

polygenic traits, several quantitative trait loci (QTLs) have been mapped and their individual effects estimated. MAS has been particularly efficient in investigation on trait with low heritability or traits that are expensive or difficult to assess and various MAS strategies have been proposed when all of the alleles come from a distant and inadequate line, the MABC (Bouchez *et al.,* 2002) have optimized the theoretical conditions required to the markers for introgressing several QTLs through MABC.

With all these researchers give importance to tomato molecular breeding, because both selfing and crossing in tomato. It is easy to do emasculation in cultivated tomato, as the flowers are large and anthers come off easily which is done about 2 days before opening of flower buds (Rick, 1991) pollen collection is also very easy in tomato since tomato flower itself acts as a natural pollen storage device. We can dig enough pollen out of the anther cones for pollinating few flowers. Drying anthers in the sun or under an incandescent lamp that is atleast 18'' away from the pollen work well. This pollen can be stored at -80 ^{0}C for extended period of time and thawed as needed (Sack and St. Clair, 1996).

2.9 Marker assisted selection (MAS) for shelf life in tomato

In tomato, most of the studies related to MAS are based on crosses carried out between cultivated tomato and related species. The level of genetic variation within the cultivated tomato, revealed by most of the common molecular markers, was very low, which is a burden for MAS with respect to a wide range of important agronomical traits. Most traits of agricultural importance, such as yield and quality, are complex and they have polygenic control and quantitative inheritance. There are many regions within the genomes which contain genes that are associated with a polygenic traits are called quantitative trait loci (QTLs). The identification of QTLs based only on conventional phenotypic evaluation is not possible. The efficiency of MAS backcrosses for the introgression of a quantitative trait locus (QTL) depends on the stability of QTL expression (Chaib *et al.,* 2006).

2.10 Suitable mapping population

It would be always advantageous using populations of early generations such as F_2, F_3, Backcross population etc. since the development of these populations require only 2 to 3 years compared to the late generation populations such as F_6, F_8, RIL, BC inbred lines etc. which requires about 7 to 8 years (Fig. 1). Development of these early generations are not a costly affair as in case the doubled haploid lines.

However predictions made involving early generations would be misleading because of the camouflaging effect of major genes on minor genes in early generations. Continuous inbreeding to evolve Recombinant Inbreed Lines (RILs) can eliminate this camouflaging effect. Thus, RILs can remain as the best choice of population for QTL analysis. As an alternative doubled haploid lines (DHLs) can also be used. The inherent homozygosity prevailing in the individuals of these two populations make the RILs and DHLs as immortals and help to have as many replications as required.

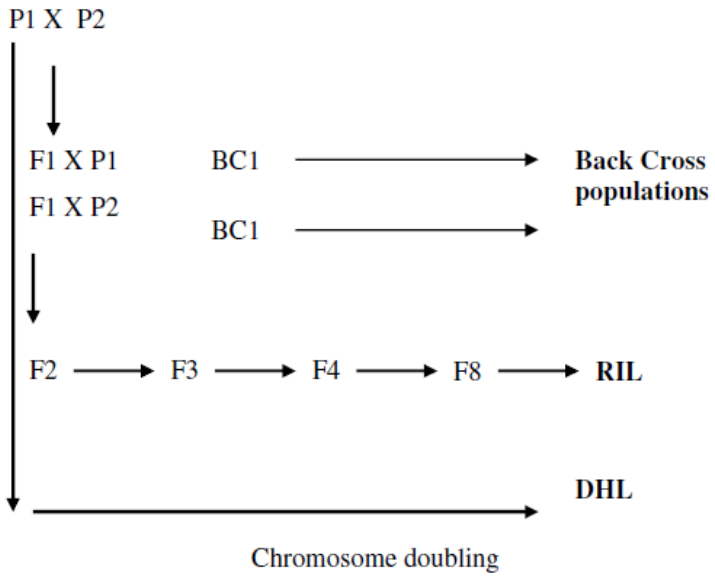


Fig. 1: Flow chart for development of mapping populations

2.11 Evaluation of Backcross population in tomato

Bernacchi and Tanksely, (1997) worked on producing improved processing tomato lines by molecular breeding strategy of advanced backcross QTL analysis. An evaluation of the agronomic performance of the NILs in five locations worldwide revealed that 22 out of the 25 (88%) quantitative factors showed the phenotypic improvement predicted by QTL analysis of the BC_3 populations, as NILs in at least one location.

The significant effect of the diallel mating scheme and simultaneous effect of the introgressed regions and of the genetic backgrounds were shown by using marker assisted introgression of five QTLs controlling fruit quality traits were reported by Lecomate *et al*. (2004).

Frary *et al*. (2004) used advanced back cross QTL mapping strategy to identify loci for yield, processing and fruit quality traits in a population derived from the interspecific cross between *L. esculantum* E6203 × *L. Pennelli* LA1657. A total of 84 different QTLs were identified, 45 per cent of which have been possibly identified in other wild-species-derived populations of tomato.

Yun *et al*. (2006) reported on validation of quantitative trait loci for multiple disease resistance in barley using advanced back cross lines developed with wild barley. They had conducted QTL analysis on population derived from diverse parental genotypes, providing the advantage of high levels of DNA polymorphisms for mapping and a high level of variation for trait of interest.

Pereira da costa *et al.* (2013) conducted research on QTL detection for fruit shelf life and quality trait across segregating population of tomato, this is done to detect QTL

associated with fruit shelf life and quality traits across different segregating population derived from an interspecific cross. Families derived from five BC_1 self-plant and BC_2 were used to validate the detected QTL in the BC_1 as well as to identify regions with wild type recessive alleles of QTLs controlling these traits. Thirty polymorphic markers (SSR) in parental genotypes and F_1 were used to analyze the segregating populations. The comparison among QTLs detected in the BC_1 and BC_2 generations and the families BC_1S_1 allowed assessing the consistency of six QTLs for length, shape, weight, pH, soluble solids content and fruit shelf life.

2.12 Single Markers analysis (SMA)

SMA is the method used in earliest studies on QTL mapping (Edwards *et al.*, 1987; Weller *et al.*, 1988). In this method one marker is involved at a time to find the QTL Marker association (Fig. 2). This single marker analysis can be implemented as a simple t test, ANNOVA, linear regression and likelihood ratio test and maximum likelihood estimation (Haley and Knott, 1992; Wang *et al.*, 1994). SMA can be performed using common statistical software.

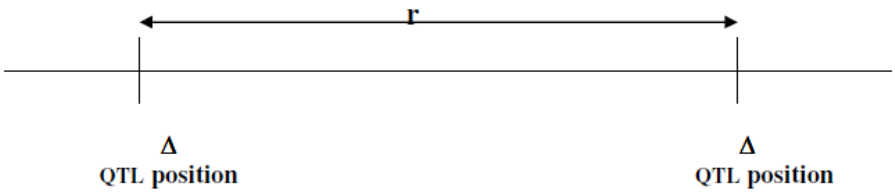


Fig. 2: Association of a marker with a putative QTL

III MATERIAL AND METHODS

The present investigation was carried out by conducting the field experiments during Kharif 2015 at the Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore, which is located at an altitude of 899 m above Mean Sea Level (MSL) and at 13° 00′ N latitude and 77° 35′ E longitude.

The details pertaining to the experimental location, materials used, layout of the field experiment, observations recorded, laboratory procedures followed for analysis of lycopene, total soluble solids, molecular characterization using SSR markers, validation of molecular markers linked to high shelf life and statistical procedures adopted for analysis of the data are briefly presented below.

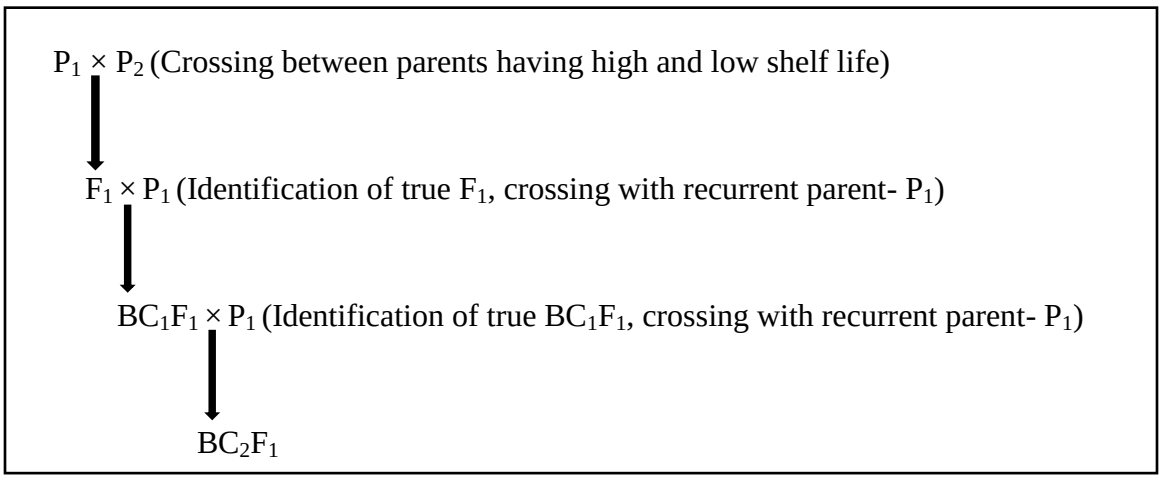
3.1 Experimental material

The Experimental material consists of two BC_2F_1 populations which were derived from crossing P_1 with P_2 and again back crossing with P_1 for twice. One population was developed by using Pusa Ruby as P_1 (plate: 2) and L121 as P_2 (plate: 3). Pusa Ruby was used as recurrent parent and it has semi-determinate growth habit. It is a commercially well accepted cultivar with good agronomic traits and has low shelf life of 10 days developed by IARI, New Delhi. L121 was used as donor parent and it has high shelf life of 40 days which is high compared to Pusa Ruby. L121 is a high shelf-life line because it contains *alc* gene. This gene is responsible for slow synthesis of ethylene hormone.

In the similar manner one more population of BC_2F_1 was derived by using Vaibhav as P_1 (plate: 4) and RIL126 as P_2 (plate: 5). Here Vaibhav is used as a recurrent parent. It is a commercially well accepted variety because of its high yielding capacity and it has low shelf life of 20 days released from University of Agricultural Sciences, GKVK, Bangalore. RIL126 developed by crossing L121 with Vaibhav, where L121 is used as female parent. This RIL 126 was used as donor parent and it has high shelf life of 50 days which was developed in our lab by Dr. P. H. Ramanjini Gowda.

3.2 Development of BC_2F_1 progenies

The seeds of P_1 and P_2 of both the cross were sown in Green house at Department of Plant Biotechnology, GKVK, Bangalore during Kharif 2014. F_1 hybrids were obtained by hand pollination using the cultivar P_1 as female parent and P_2 as male parent. BC_1F_1 progenies were developed by hand pollinating the true F_1 hybrids with that of recurrent parent during Rabi 2014 as a part of DBT project in our lab. Subsequently BC_2F_1 progenies were developed by hand pollinating the heterozygous BC_1F_1 progenies with that of recurrent parent (Fig. 3). The crossing plan that has been carried out to transfer genomic regions conferring high shelf life to low shelf life cultivar is as follows.



Development of BC_2F_1 populations.

The 70 BC_2F_1 progenies of the cross Pusa Ruby/L121//Pusa Ruby (plate: 6) and 92 BC_2F_1 progenies of the cross Vaibhav/RIL126//Vaibhav (plate: 7) were used for evaluation of shelf life and other characters.

3.3 Evaluation of backcross progenies and comparison with F_1 hybrids and parents

Total 162 BC_2F_1 progenies developed by backcrossing were evaluated in augmented design (windostat ver. 8.5) with their parents Pusa Ruby, L121, Vaibhav, RIL126 and F_1 hybrids of the cross Pusa Ruby/L121//Pusa Ruby and Vaibhav/ RIL126//Vaibhav during Kharif 2016. The experimental plot was ploughed and recommended FYM dose with fertilizers were incorporated into the soil before transplanting. 30 days old seedlings were transplanted into experimental plot with a spacing of 90 × 40 cm. Irrigation and other cultural practices were followed as per recommendations of the package of practices.

3.4 Recording of observations in backcross populations

The observations of plant morphological and yield characters in each plant were recorded. Three fruits per plant of parents (Pusa Ruby, L121, Vaibhav and RIL126) in each block, F_1 hybrids and every plant of two populations of BC_2F_1 at breaker stage defined by (Giovannoni, 2006) was harvested and was evaluated for fruit morphological, biochemical characters and shelf life. The average values were computed as treatment mean under each replication in parents F_1 hybrids. The characters studied and techniques adopted to record the observations given below.

3.4.1 Plant height (cm)

This was measured in cm from base of the plant to the tip after 65 days of transplanting.

3.4.2 Number of branches per plant

Number of branches in each plant was counted after 65 days of transplanting.

Plate 1: General view of the plot

Plate 2: Parental Line – Pusa Ruby (P_1)

Plate 3: Parental Line – L121 (P_2)

Plate 4: Parental Line – Vaibhav (P_1)

Plate 5: Parental Line – RIL126 (P_2)

Plate 6: BC_2F_1 (Pusa Ruby/L121)

Plate 7: BC_2F_1 (Vaibhav/RIL126)

3.4.3 Number of flowers per cluster

The number of flowers in each plant is counted after all the flowers in a cluster are initiated. Readings are taken from three clusters from each plant and averaged.

3.4.4 Number of fruits per cluster

Number of fruits per cluster is counted; average of three clusters per plant will be taken after all the flowers are set as fruits in that cluster.

3.4.5 Yield (g/plant)

Fresh weight of fruits per plant was recorded at each picking and total yield per plant was calculated and expressed in grams per plant.

3.4.6 Total number of fruits per plant

The total number of fruits per plant was counted in each plant at the time of picking.

3.4.7 Single fruit weight (g)

Three fruits of each plant will be weighed on a weighing balance and will be measured in g and averaged.

3.4.8 Fruit length (cm)

Length of the three fruits from each plant will be taken by using vernier calipus which will be measured in mm. Then these are converted to cm and are averaged to get mean value.

3.4.9 Fruit width (cm)

Width of the three fruits from each plant will be taken and measured by using vernier calipus in mm. Then these are converted to cm and are averaged to get mean value.

3.4.10 Number of locules per fruit

Three fruit from each plant will be cut in transverse section at fully ripened stage and number of locules will be counted.

3.4.11 Rind thickness (mm)

Three fruits from each plant were taken at red ripe stage and werecut into transverse section and rind thickness was measured by Vernier scale recorded in mm.

3.4.12 Shelf life (Days)

Three fruits at breaker (the stage at which green fruits turns to pink colour) stage from each plant will be kept for measuring shelf life at room temperature till shrinking and number of days will be counted from fruit breaker stage to shrinking stage.

3.4.13 Lycopene (mg/100g) (Ranganna, 1976)

Lycopene is responsible for red colour of tomato and it has various health benefits.

Principle:

The carotenoids in the sample were extracted in acetone and then taken up in petroleum ether. Lycopene has absorption maxima at 473 nm and 503 nm. One mole of lycopene when dissolved in one liter light petroleum (40-60 ºC) and measured in a spectrophotometer at 503 nm in 1 cm light path gives an absorbance of 17.2×10^4 Therefore, a concentration of 3.1206 µg lycopene/ml gives unit absorbance.

Materials:

Acetone (AR grade)

Petroleum ether 40-60 (AR grade)

Anhydrous Sodium sulphate

5 per cent Sodium sulphate.

Procedure:

1. Three to four tomato fruits were taken in a waring blender and pulp it well to a smooth consistency.

2. Five to ten g of this pulp was weighed.

3. The pulp was repeatedly extracted with acetone using pestle and mortar or a waring blender until the residue is colorless.

4. The acetone extracts were pooled and transferred to a separating funnel containing about 20 ml petroleum ether and mix it gently.

5. Twenty milliliters of five percent Sodium sulphate solution was added to it and the separating funnel was gently shaken. Volume of petroleum ether may be reduced during these processes because of its evaporation. Twenty ml of petroleum ether was added again to the separating funnel for obtaining clear separation of two layers. Most of the colour could be noticed in the upper petroleum ether layer.

6. The two phases were separated followed by re-extraction of the lower aqueous phase with additional 20 ml petroleum ether until the aqueous phase is colorless.

7. The petroleum ether extracts were pooled and washed once with a little distilled water.

8. The washed petroleum ether extract containing carotenoids was poured into a brown bottle containing about 10 g anhydrous sodium sulphate and kept undisturbed for 30 minutes or longer.

9. The petroleum ether extract was then decanted into a 100ml volumetric flask through a funnel containing cotton wool. Sodium sulphate slurry was washed with petroleum ether until turns colorless and transferred to the volumetric flask.

10. The volume was made up to 100 ml and the absorbance was measured in a spectrophotometer at 503 nm using petroleum ether as blank.

3.4.14 Calculation:

Absorbance (1 unit) = 3.1206 µg lycopene/ml.

$$\text{mg lycopene in 100g sample} = \frac{31.206 \times \text{Absorbance}}{\text{Wt. of sample (g)}}$$

3.4.15 Total soluble solids (TSS)

Total soluble solids were recorded from three randomly selected fruits of each plant by squeezing the juice on Erma hand refractometer ($0\text{-}32^0$ Brix) at room temperature and mean was recorded.

3.5 Statistical analysis

3.5.1 Analysis of variance for morphological, yield and fruit quality characters

The analysis of variance for different characters was used to partition the variance due to different sources following the method given by (Panse and Sukhatme, 1964).The significance was tested by comparing with the table values as given by (Yates, 1965). In this experiment analysis of variance was obtained by augment analysis for both the populations (Pusa Ruby/L121//Pusa Ruby) and (Vaibhav/RIL126//Vaibhav.

Analysis of variance for backcross progenies

Source of variation	**df**	**mss**
Blocks (b)	b-1	Mss (b)
Backcross progenies (r) + checks (c)	e-1	Mss (t)
Checks (c)	c-1	Mss (c)
Backcross progenies (v)	v-1	Mss (v)
Checks vs Backcross progenies (r)	(c-1) (v-1)	Mss (vc)
Error	(c-1) (b-1)	Emss

Where, v = number of progenies
c = number of check varieties
b = number of blocks
e = number of backcross progenies + checks
r = number of checks vs backcross progenies

The effect of each block (B_j) was computed as, $B_j = \bar{X}_j - \bar{X}\rightarrow$

Where,

$\bar{X}_j$ = Trait means of check entries in j^{th} block

$\bar{X}\rightarrow$ = Trait mean of all the checks in all the blocks.

B_j was used to adjust the trait means of the accessions relevant to the block. Thus, trait means of each accession evaluated in j_{th} block was adjusted by subtracting the block effect B_j of the j block from actual trait value of the accession. The significance was tested by referring to the table given by Fisher *et al*., (1932).

3.5.2 Phenotypic and Genotypic coefficient of variation (PCV and GCV)

The phenotypic and genotypic coefficient of variation was computed as per Burton and Dewane, (1953) for low moisture stress.

$$PCV = \frac{P}{\bar{X}} \times 100$$

$$GCV = \frac{G}{\bar{X}} \times 100$$

Where,

P = Phenotypic standard deviation

G = Genotypic standard deviation

$\bar{X}$ = Grand mean of character

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

PCV and GCV were classified according to Robinson *et al.,* (1949). (0-10 %) was considered as low, (10-20 %) as moderate and (20 %) and above as high.

3.5.3 Heritability (h^2)

Broad Sense Heritability was calculated using the formula.

$$h^2\,\% = \frac{Vg}{Vp} \times 100$$

Where,

h^2 % =Heritability percentage

Vg= Genotypic variance

Vp= Phenotypic variance

Heritability percentage was categorized as follows (Robinson *et al.,* 1949). (0-30 %) was considered as low; (30-60 %) was considered as moderate; (60 %) was considered as high.

3.5.4 Genetic advance (GA)

Genetic advance was calculated by using formula given by Johnson *et al.,* (1955).

$$GA = h^2 \times \sigma_p \times K$$

Where,

h^2 = Heritability (Broad sense)

σ_p =Phenotypic standard deviation

K = Selection differential which is 2.06 at 5 % intensity of selection (Lush, 1949).

3.5.5. Genetic advance as per cent mean

$$\text{GA as per cent mean} = \frac{GA}{\bar{X}} \times 100$$

Where,

GA = Genetic advance and

$\bar{X}$ =Treatment mean for the character.

The GA as per cent mean was classified (Johnson *et al.*, 1955) as given below. (0-10 %) was considered as low, (10-20 %) as moderate and (20 %) and above as high.

3.5.6. Correlation analysis

To estimate the degree of association between the traits studied, phenotypic correlation was computed by using the formula given by Webber and Moorthy, (1952).

$$r_p = \frac{COV\ (X, Y)}{[V(X).\ V\ (Y)]^{1/2}}$$

Where,

r_p = phenotypic correlation co-efficient.

COV (X, Y) = Phenotypic covariance.

V(X) and V(Y) = Phenotypic variances of the traits X and Y

3.5.7 Test of normality

3.5.7.1 Normal distribution

The normal distribution (the term first used by Galton, 1989) function is determined by the following formula:

$$f(x) = 1/[(2*\pi)\ 1/2*\sigma] *e**\{-1/2* (x-\mu)/ \sigma 2\}\ -\infty \text{ to } \infty$$

Where,

'μ' is the mean

'Σ' is the standard deviation

'e' is Euler's constant (2.71)

'π' is the constant Pi (3.14)

3.5.7.2 Skewness

Skewness is a measure of the extent to which the distribution of the respective variable is skewed to the left (negative value) or right (positive value), relative to the standard normal distribution (for which the skewness is 0). Genetic expectations of skewness reveal the nature of genetic control of the traits (Fisher *et al*., 1932). The adjusted mean values of each genotype for quantitative traits were used to estimate coefficient of skewness using software program. The skewness is calculated with the formula:

$$\text{Skewness} = n * M3 / [(n-1) * (n-2) * \sigma 3]$$

Where,

'M3' is equal to Σ (Xi – mean X) **3

'N'is the valid number of cases

'σ3' is the standard deviation (sigma) raise to the third power.

3.5.7.3 Kurtosis

Kurtosis is a measure of how wide flat or peaked the distribution is for the respective variable, relative to the standard normal distribution (for which the kurtosis is equal to 3). Kurtosis indicates the relative number of genes controlling the traits (Robson, 1956). The adjusted mean values of each genotype (line) for quantitative traits were used to estimate coefficient of kurtosis using software program. The kurtosis is calculated with the formula:

$$\text{Kurtosis} = [n* (n+1) * M4 - 3 * M2 * M2 * (n-1)] / [(n-1)* (n-2)* (n-3)* \sigma 4]$$

Where,

'Mj' is equal to Σ (Xj – mean X)**j

'N' is the valid number of cases

'σ4' is the standard deviation (sigma) raise to the fourth power.

3.6 Molecular characterization with SSR markers

3.6.1 Material

70 BC_2F_1 progenies of the cross from Pusa Ruby/L121//Pusa Ruby, BC_2F_1 progenies derived from Vaibhav/RIL126//Vaibhav and four parents were used for

molecular characterization studies. DNA was extracted from the young leaves using CTAB method as demonstrated explained by Saghi – Maroof *et al.* (1984).

3.6.2 Protocol for extraction of genomic DNA

a. One gram of fresh young leaf sample was taken in a mortar and powdered well using liquid nitrogen.

b. The ground tissue was transferred to a 50 ml sterile centrifuge tube by adding 15 ml of CTAB buffer (Extraction buffer- 2 per cent C-TAB, 1.4 NaCl, 20 mM EDTA (Disodium) & 10 mM Trisbase (pH 8). Then 50 µl of β- mercaptaethanol was added to each tube and the extract was mixed well by inverting the tubes several times. The tubes were incubated in a water bath maintained at 65 ^{0}C for an hour with constant stirring at an interval of 15 minutes.

c. After an hour, 15 ml of chloroform isoamyl alcohol (24:1) was added to the incubated sample and mixed well by inverting. The tubes were then centrifuged at 6000 rpm for 20 minutes.

d. The aqueous upper phase was carefully transferred using the 1 ml cut tips into fresh sterile centrifuge tubes. To this supernatant 0.7 volume (10.5 ml) of cold Isopropanol was added.

e. The tubes were carefully inverted and kept for 5 minutes in ice. DNA precipitation was seen in the form of strands. The tubes were then centrifuged at 6000 rpm for 20 minutes and sedimentation of DNA as a hard pellet was seen.

f. Further the supernatant was decanted gently and the tubes were inverted on a clean filter paper.

g. The pellet was washed twice by suspending in one ml of 70 per cent ethanol for 5 to 10 minutes and the DNA was centrifuged at 6000 rpm for two minutes.

h. Ethanol was drained of slowly and the pellet was vacuum dried in a desiccator for 5 to 10 minutes. The pellet was then dissolved in 500 µl of TE buffer by flicking the tubes. (TE buffer = 0.1 mM Tris + 0.05 mM EDTA).

i. To remove the RNA 5µl of RNase (10 mg/ml) was added into the DNA solution and is incubated at 37 ºC in a water bath for 1 hour.

j. Again the DNA solution was cleaned by washing with equal volume (500 µl) of Phenol: Choloroform: Isoamyl alcohol (25:24:1) by invert mixing several times and centrifuging at 6000 rpm for 15 minutes to separate the two phases.

k. The aqueous upper phase was transferred into a clean 1.5 ml eppendorf tube and twice the volume of 100 per cent ethanol was added to precipitate the DNA.

l. The DNA pellet was washed with 70 per cent ethanol twice and after removing ethanol the pellet was dried.

m. Finally, DNA pellet was dissolved in 500 µl of TE buffer and stored at 20 °C until use.

n. The extracted DNA was quantified using spectrophotometer.

3.6.3 Quantification of DNA

The genomic DNA was quantified spectrophotometrically both at 260 nm and 280 nm wavelength. The absorbance at 260 nm allows the calculation of DNA concentration in the sample. An OD of 1 at 260 nm corresponds to 50 µg of double stranded DNA. A pure sample of DNA shows the ratio of OD 260/280 as 1.8. Ratios less than 1.8 indicate contamination in the isolation either with phenol or with proteins. The values higher than this indicate the presence of RNA in the isolation

3.7 Molecular markers used in the study

In this study 30 SSR markers (Appendix I) were selected from database (He *et al.*, 2003; www.oardc.ohio-state.edu/tomato/SNPdata/SSRPrimerVMar05.xls; http://solgenomics.net/). And also from research paper (Pereira da Costa *et al*., 2013).

3.7.1 Simple sequence repeats (SSRs)

The microsatellites are also called as short tandem repeats (STR) or simple sequence repeats (SSR) or simple sequence length polymorphisms. Typically they may be dinucleotides (AC)n; trinucleotides (TCT)n; tetranucleotide (TATG)n and so on where n is the number of repeating units within the microsatellites locus. In addition to occurring at many different loci they can also be polyallelic. The (AT)n dinucleotides are the most abundant type of SSR in plants (Ma *et al*., 1996). The methodology used to isolate an SSR at particular locus starts with the construction of the small insert genomic library. PCR amplification is used to generate DNA banding patterns on a gel and to reveal the polymorphism based on different number of repeats at the two alleles of a locus. The marker thus has the advantage of being co-dominant; they are simple PCR based and extremely polymorphic. They are also highly informative due to number and frequency of alleles detected and ability to distinguish between closely related individuals. They find application as marker for mapping, cultivar identification, protecting germplasm, determination of hybridity, analysis of gene pool variation and as diagnostic marker for traits of economic value (Powell *et al*., 2012).

3.7.2 Standardization of annealing temperature for SSR primers

The annealing temperature was standardized by using gradient PCR technique. In this method different annealing temperatures (Tm ± 5 ^{0}C) were set to each block and amplification is carried as per below mentioned reaction conditions. After standardization of annealing temperature the parental lines were amplified using the same protocol.

3.7.3 PCR reaction mixture for standardization of annealing temperature

PCR for SSR marker was performed in a total volume of 20 µl. The components used for PCR reaction mixture are listed below.

Sl. No.	Components	Volume (µl)
1	10X PCR buffer	1.00
2	0.4mM dNTPs mix	0.40
3	1.0 mM of $MgCl_2$	1.00
4	1.5 M forward primer	1.50
5	1.5 M reverse primer	1.50
6	100 ng genomic DNA	2.00
7	0.3units of *Taq* polymerase	0.30
8	Nuclease free water	12.30
	Total	20.00

3.7.4 Amplification condition

Amplification was carried out on Master Cycler Gradient Techgene PCR.

The amplification profile was as follows:

a) Initial denaturation temperature	94 °C	5 min.	
b) Denaturation	94 °C	1 min	35 cycles
c) Primer annealing (primer specific)	50 °C- 52 °C	1 sec	
d) Primer extension	72 °C	1 min	
e) Complete primer extension	72 °C	5 min.	
f) Hold	4 °C	till removal	

3.7.5 Polyacrylamide gel electrophoresis (Wang *et al.*, 2003)

Polyacrylamide gel electrophoresis of 5 per cent concentration was prepared and PCR products were run using 1x TBE buffer for two and half hour at 150 V and photographed in trans-illuminator using digital camera. The bands are then scored for statistical analysis.

3.7.6 Scoring of generated bands

The bands generated by polymorphic primers were scored by giving code ‘1’ for Parent-1 (upper/ lower band), ‘3’ for Parent-2 (upper/ lower band) and ‘2’ for backcross

progenies having both the bands, ‘1’ for backcross progenies having recurrent parental type of band.

3.7.7 Single marker analysis

Single-marker analysis (also single-point analyses) is the simplest method for detecting QTLs associated with single markers. The statistical methods used for single marker analysis include t-tests, analysis of variance (ANOVA) and linear regression. We have done single marker analysis by ANOVA (Lander *et al.*, 1987).

IV EXPERIMENTAL RESULTS

The results obtained from the present investigation on "Evaluation and Molecular marker analysis in backcross progenies of (BC_2F_1) in tomato for shelf life" are presented below.

4.1 Mean performance of parents, their F_1 hybrids and backcross progenies in field conditions

The mean performance of parents used in development of backcross population BC_2F_1, their F_1 hybrids and backcross progenies (Table 1), with respect to morphological, yield and biochemical characters are briefly presented below.

4.1.1 Plant height

The mean plant height of the parent Pusa Ruby was 48.1 cm and parent L121 was 50.4 cm. Their F_1 hybrids and backcross progenies have recorded plant height of 60 cm and 54.8 cm, respectively. Among the progenies, the highest plant height of 80 cm and lowest plant height of 27 cm was observed.

The mean plant height of the parent Vaibhav was 82.2 cm and parent RIL126 was 64.3 cm while, their F_1 hybrids and backcross progenies have recorded plant height of 75 cm and 77.5 cm, respectively. Among the progenies the highest plant height of 99 cm and lowest plant height of 43 cm was observed.

4.1.2 Number of branches per plant

The mean number of branches per plant in the parent Pusa Ruby was 6and parent L121 was 5, while their F_1 hybrids and backcross progenies have 7 and 6 branches per plant respectively. Among the progenies the highest number of branches per plant was 9 and lowest was 3 branches per plant.

The mean number of branches per plant in the parent Vaibhav was 3 and in the parent RIL126 was 2 while, their F_1 hybrids and backcross progenies both have 3 branches per plant. Among the progenies the highest number of branches per plant was 9 and lowest was 2 branches per plant.

4.1.3 Number of flowers per cluster

The mean number of flowers per cluster in the parent Pusa Ruby was 6 and parent L121 was 5 while their F_1 hybrids and backcross progenies both have 6 flowers per cluster. Among the progenies the highest number of flowers per cluster was 9 and lowest was 3.

The mean number of flowers per cluster in both the parents Vaibhav and RIL126 was 5 while their F_1 hybrids and backcross progenies have 6 and 5 flowers per cluster respectively. Among the backcross progenies the highest number of flowers per cluster was 10 and lowest was 3.

Table 1: Mean performance of parents their F_1 hybrids, backcross progenies and commercial checks for morphological, yield and Biochemical characters

Sl. No.	Varieties	PHT (cm)	NBP	NFLC	NFRC	NFP	SFW (g)	FL(cm)	FW (cm)	NLF	RT (mm)	YLD (g/plant)	SL (Days)	TSS (%)	LYC (mg/100g)
1	**Pusa Ruby**	48.1	6	6	4	17	35.4	2.8	4.1	6	3.17	580.55	10	4.90	1.18
2	**L121**	50.4	5	5	3	12	85.4	3.9	6.0	7	4.15	963.80	40	4.60	0.81
3	**F_1**	60.0	7	6	4	28	60.0	4.5	5.5	6	4.50	1080.0	30	5.10	1.02
4	**BC_2F_1**	54.8	6	6	4	20	50.0	3.0	4.3	6	3.29	673.60	30	4.72	0.95
5	**Vaibhav**	82.2	3	5	5	16	39.3	5.2	4.1	2	5.21	560.21	20	4.20	3.69
6	**RIL126**	64.3	2	5	3	8	51.6	5.4	4.3	2	4.72	410.02	50	4.30	2.80
7	**F_1**	75.0	3	6	5	20	55.0	4.5	5.5	2	5.50	910.00	40	5.00	4.11
8	**BC_2F_1**	77.5	3	5	4	17	49.0	4.5	3.8	2	5.20	685.25	40	4.35	3.24

Legend

PHT = Plant height (cm)
NFLC = Number of flowers per cluster
SFW = Single fruit weight (g)
FL = Fruit length (cm)
NLF = Number of locules per fruit
YLD = Yield (g/plant)
TSS = Total Soluble Solids (%)

NBP = Number of branches per plant
NFRC = Number of fruits per cluster
NFP = Number of fruits per plant
FW = Fruit width (cm)
RT = Rind Thickness (mm)
SL = Shelf life (days)
LYC = Lycopene content (mg/100g)

4.1.4 Number of fruits per cluster

The mean number of fruits per cluster in the parent Pusa Ruby was 4 and parent L121 was 3 while their F_1 hybrids and backcross progenies both have 4 fruits per cluster. Among the progenies the highest number of fruits per cluster was 6 and lowest was 2.

The mean number of fruits per cluster in the parent Vaibhav was 5 and in RIL126 was 3 while their F_1 hybrids and backcross progenies have 5 and 4 fruits per cluster, respectively. Among the progenies the highest number of flowers per cluster was 8 and lowest was 2.

4.1.5 Number of fruits per plant

The mean number of fruits per plant in the parent Pusa Ruby was 17 and parent L121 was 12. Their F_1 hybrids and backcross progenies have 28 and 20 fruits per plant. Among the backcross progenies the highest number of fruits per plant was 43 and lowest was 3.

The mean number of fruits per plant in the parent Vaibhav was 16 and parent RIL126 was 8.Their F_1 hybrids and backcross progenies have 20 and 17 fruits per plant. Among the backcross progenies the highest number of fruits per plant was 47 and lowest was 5.

4.1.6 Single fruit weight (g per fruit)

The mean weight of single fruit in parents Pusa Ruby was 35.4 g and in L121 was 85.4 g. Their F_1 hybrids and backcross progenies have fruit weight of 60.0 and 50.0 g per fruit, respectively. Among the backcross progenies the maximum weight of single fruit was 96.0 g per fruit and minimum was 21.0 g per fruit.

The mean weight of single fruit in Vaibhav was 39.3 g and in RIL126 was 51.6 g, while their F_1 hybrids and backcross progenies have fruit weight of 55.0 g and 49.6 g per fruit, respectively. Among the progenies the maximum weight of single fruit was 86.0 g per fruit and minimum was 30.0 g per fruit.

4.1.7 Fruit length (cm)

The mean length of the fruit in Pusa Ruby was 2.8 cm and in L121 was 3.9 cm, while their F_1 hybrids and backcross progenies have fruit length of 4.5 cm and 3.0 cm, respectively. Among the backcross progenies the maximum length of the fruit was 4.5 cm and minimum was 2.4 cm.

The mean length of the fruit in Vaibhav was 5.2 cm and in RIL126 was 5.4 cm, while their F_1 hybrids and backcross progenies both have fruit length of 4.5 cm. Among the backcross progenies the maximum length of the fruit was 5.7 cm and minimum was 3.7 cm.

4.1.8 Fruit width (cm)

The mean width of the fruit in Pusa Ruby was 4.1 cm and in L121 was 6.0 cm, while their F_1 hybrids and backcross progenies have fruit width of 5.5 cm and 4.3 cm, respectively. Among the backcross progenies the maximum width of the fruit was 6.0 cm and minimum was 3.5 cm.

The mean width of the fruit in Vaibhav was 4.1 cm and in RIL126 was 4.3 cm, while their F_1 hybrids and backcross progenies have fruit width of 5.5 cm and 3.8 cm, respectively. Among the backcross progenies the maximum length of the fruit was 4.7 cm and minimum was 3.0 cm.

4.1.9 Number of locules per fruit

The mean number of locules per fruit in the parent Pusa Ruby was 6 and parent L121 was 7, while their F_1 hybrids and backcross progenies both have 6 locules per fruit. Among the progenies the maximum number of locules per fruit was 9 and minimum was 3.

The mean locules per fruit in the parent Vaibhav was 2 and in RIL126 was 2 while their F_1 hybrids and backcross progenies both have 2 locules per fruit, respectively. The maximum number of locules per fruit was 3 and minimum was 2.

4.1.10 Fruit Rind thickness (mm)

The mean rind thickness of the fruit in Pusa Ruby was 3.17 mm and in L121 was 4.15 mm, while their F_1 hybrids and backcross progenies have fruit rind thickness of 4.50 mm and 3.29 mm, respectively. Among the progenies the maximum rind thickness of the fruit was 5.5 mm and minimum was 2.2 mm.

The mean rind thickness of the fruit in Vaibhav was 5.21 mm and in RIL126 was 4.72 mm, while their F_1 hybrids and backcross progenies have fruit rind thickness of 5.50 mm and 5.20 mm, respectively. Among the backcross progenies the maximum rind thickness of the fruit was 7.6 mm and minimum was 2.8 mm.

4.1.11 Yield per plant (g/plant)

The mean yield per plant in the parent Pusa Ruby was 580.55 g/plant and parent L121 was 963.80 g/plant, while their F_1 hybrids and backcross progenies have 1080.00 g/plant and 673.60 g/plant, respectively. Among the progenies the maximum yield per plant was 3120.00 g / plant and minimum was 94 g/plant.

The mean yield per plant in the parent Vaibhav was 560.21 g/plant and in RIL126 was 410.02 g/plant, while their F_1 hybrids and backcross progenies have 910.00 g/plant and 685.25 g/plant respectively. Among the backcross progenies the maximum yield per plant was 1815.0 g/plant and minimum was 157.50 g/plant.

4.1.12 Total soluble solids (%)

The mean Total soluble solid in Pusa Ruby was 4.9 and in L121 were 4.6, while their F_1 hybrids and backcross progenies have TSS of 5.1 and 4.7, respectively. Among backcross the progenies the maximum TSS was 7.3 and minimum was 3.7.

The mean TSS in Vaibhav was 4.2 and in RIL126 was 4.3, while their F_1 hybrids and backcross progenies have TSS of 5.0 and 4.3 respectively. Among the backcross progenies the maximum TSS was 7.0 and minimum was 3.5.

4.1.13 Lycopene content (mg/100g)

The mean lycopene in Pusa Ruby was 1.18 mg/100g and in L121 was 0.81 mg/100g, while their F_1 hybrids and backcross progenies have lycopene of 1.02 mg/100g and 0.98 mg/100g, respectively. Among the progenies the maximum lycopene was 2.30 mg/100g and minimum was 0.2 mg/100g.

The mean lycopene in Vaibhav was 3.69 mg/100g and in RIL126 was 2.80 mg/100g, while their F_1 hybrids and backcross progenies have lycopene of 4.11 mg/100g and 2.23 mg/100g, respectively. Among the progenies the maximum lycopene was 4.40 mg/100g and minimum was 2.10 mg/100g.

4.1.14 Shelf life (Days)

The mean fruit keeping quality in Pusa Ruby was 10 and in L121 was 40, while their F_1 hybrids and backcross progenies have shelf life of and 30days, respectively. Among the progenies the maximum fruit keeping quality was 60 and minimum was 30 days.

The mean fruit keeping quality in Vaibhav was 20 and in RIL126 was 50, while their F_1 hybrids and backcross progenies both have shelf life of 40 days. Among the progenies the maximum fruit keeping quality was 60 and minimum was 30 days.

4.2.1 Analysis of variance for plant growth, yield and fruit quality parameters in field condition for the population 1 (Pusa Ruby/L121//Pusa Ruby)

The mean sum of squares due to various sources of variation for plant growth, yield and fruit quality parameters of the backcross progenies in population 1 was represented in (Table 2) and (Table 3). Significant differences were observed within the checks for all the characters except number of flowers per cluster and also there was significant difference for all the traits between the parents. There was significant difference between the checks and backcross progenies for all the characters except for number of flowers per cluster. The significant difference was also observed within the backcross progenies for all the traits except plant height, number of flowers per cluster, fruit length, yield (g/plant) and lycopene indicating variability for the yield associated traits and shelf life.

Table 2: Analysis of variance for growth and yield parameters in BC_2F_1 population of the cross Pusa Ruby/L121//Pusa Ruby

Source of variations	Mean sum of squares							
	df	Plant height (cm)	Number of branches/ plant	Number of flowers/ cluster	Number of fruits/ cluster	Yield (g/plant)	Number of fruits/ plant	Single fruit weight (g)
Blocks	03	512.41	0.09	0.81	0.14	157947.00	2.31	18.26
(BC_2F_1 progenies+checks)	74	620.37 *	2.41 **	1.23	1.52 **	230415.60 *	89.15 **	291.29 **
Checks	06	4172.87 **	11.36 **	1.32	5.47 **	590591.90 **	325.46 **	860.23 **
BC_2F_1 progenies	67	130.11	1.07 **	1.20	1.10 **	167930.30	65.13 **	236.38 **
Checks vs. BC_2F_1 progenies	01	12153.28 **	38.24 **	2.74	6.24 **	2255877.00 **	280.62 **	556.76 **
ERROR	18	231.08	0.17	0.67	0.14	88096.37	4.88	27.87

* Significant at 5 % ** Significant at 1 %

Table 3: Analysis of variance for fruit quality parameters in BC_2F_1 population of the cross Pusa Ruby/L121//Pusa Ruby

Source of variations	Mean sum of squares							
	df	Fruit length	Fruit width	Number of locules/ fruit	Rind thickness	TSS	Lycopene	Shelf life
Blocks	03	0.05	0.03	0.29	0.003	0.002	0.43	28.57
(BC_2F_1 progenies + checks)	74	0.92 **	0.49 **	4.59 **	1.04 **	0.40 **	0.90	227.01 **
Checks	06	3.63 **	3.48 **	18.90 **	2.71 **	0.45 ***	3.85 **	703.57 **
BC_2F_1 progenies	67	0.06	0.19 **	2.10 **	0.31 **	0.39 **	0.15	186.54 **
Checks vs. BC_2F_1 progenies	01	42.72 **	2.68 **	85.86 **	40.38 **	0.67 **	33.25 **	79.00
ERROR	18	0.04	0.06	0.64	0.06	0.001	0.47	25.79

* Significant at 5 % ** Significant at 1 %

4.2.2 Analysis of variance for plant growth, yield and fruit quality parameters in field condition for the population 2 (Vaibhav/RIL126//Vaibhav)

The mean sum of squares due to various sources of variation for plant growth, yield and fruit quality parameters of the backcross progenies in population 2 was represented in (Table 4) and (Table 5). There are no significant differences between the backcross progenies because these are considered as replications. Significant differences were observed within the checks for all the characters except number of flowers per cluster and yield per plant. There was significant difference between the checks and backcross progenies for all the characters except for plant height, number of flowers per cluster and lycopene. The significant differences were also observed within the backcross progenies for all the traits except plant height, number of flowers per cluster, yield (g/plant), fruit length, fruit width, number of locules per fruit and lycopene.

4.3 Test of normality for the population 1 (Pusa Ruby/L121//Pusa Ruby)

4.3.1 Skewness

Among all the backcross progenies all characters are positively skewed except for the traits like plant height, number of branches per plant, number of flowers per cluster, number of fruits per cluster, and shelf life which are negatively skewed (Figure 3, 4 and Table 6).

4.3.2 Kurtosis

The distribution curves for all the characters in the backcross progenies were found to be platykurtic with a kurtosis value less than 3, except fruit length, TSS and lycopene. Whereas, in plant height, number of locules per fruit, and shelf life are Leptokurtic with kurtosis was less than 3 (Figure 3, 4 and Table 6).

4.4 Test of normality for the population 2 (Vaibhav/RIL126//Vaibhav)

4.3.1 Skewness

Among all the backcross progenies all characters are positively skewed except for the traits like plant height, number of branches per plant, fruit length, fruit width, rind thickness, lycopene and shelf life which were negatively skewed. (Fig.5, 6 and Table 7).

4.3.2 Kurtosis

The distribution curves for all the characters in the backcross progenies were found to be platykurtic with a kurtosis value less than 3, except number of branches per plant, flowers per cluster and total soluble solids. The plant height was leptokurtic with kurtosis value less than 3 (Fig.5, 6 and Table 7).

4.4 Studies on genetic variability

The genetic variability parameters viz., minimum, maximum, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2) and genetic advance as per cent mean for plant growth, yield and fruit

Table 4: Analysis of variance for growth and yield parameters in BC_2F_1 population of the cross Vaibhav/RIL126//Vaibhav

Source of variations	Mean sum of squares							
	df	Plant height (cm)	Number of branches/ plant	Number of flowers/ cluster	Number of fruits/ cluster	Yield (g/plant)	Number of fruits/ plant	Single fruit weight(g)
Blocks	03	512.42	0.09	0.81	0.14	157949.10	2.32	18.26
(BC_2F_1 progenies + checks)	98	383.46	2.53 **	1.19	1.23 **	183880.40 *	105.05 **	132.29 **
Checks	06	4172.88 **	11.37 **	1.32	5.48 **	590591.90 **	325.47 **	860.24 **
BC_2F_1 progenies	91	136.42	1.52 **	1.13	0.89 **	146944.60	91.26 **	76.96 **
Checks vs. BC_2F_1 progenies	01	127.09	41.37 **	5.81 **	6.45 **	1104773.00 **	37.35 *	800.65 **
ERROR	18	231.08	0.18	0.67	0.14	88096.02	4.89	27.88

* Significant at 5 % ** Significant at 1 %

Table 5: Analysis of variance for fruit quality parameters in BC_2F_1 generation of the cross Vaibhav/RIL126//Vaibhav

Source of variations	Mean sum of squares							
	df	Fruit length (cm)	Fruit width (cm)	Number of locules/ fruit	Rind thickness (mm)	TSS (%)	Lycopene (g/100mg)	Shelf life (days)
Blocks	03	0.059	0.034	0.291	0.003	0.002	0.43	28.57
(BC_2F_1 progenies + checks)	98	0.352**	0.47 **	2.23 **	1.03 **	0.19 **	0.67	140.48 **
Checks	06	3.64**	3.49 **	18.91 **	2.71**	0.46**	3.86 **	703.57**
BC_2F_1 progenies	91	0.14 **	0.11	0.14	0.86**	0.17**	0.25	103.25 **
Checks vs. BC_2F_1 progenies	01	0.10	15.23**	92.86**	6.14**	1.08 **	19.79 **	149.59*
ERROR	18	0.04	0.06	0.64	0.07	0.001	0.47	25.79

* Significant at 5 % ** Significant at 1 %

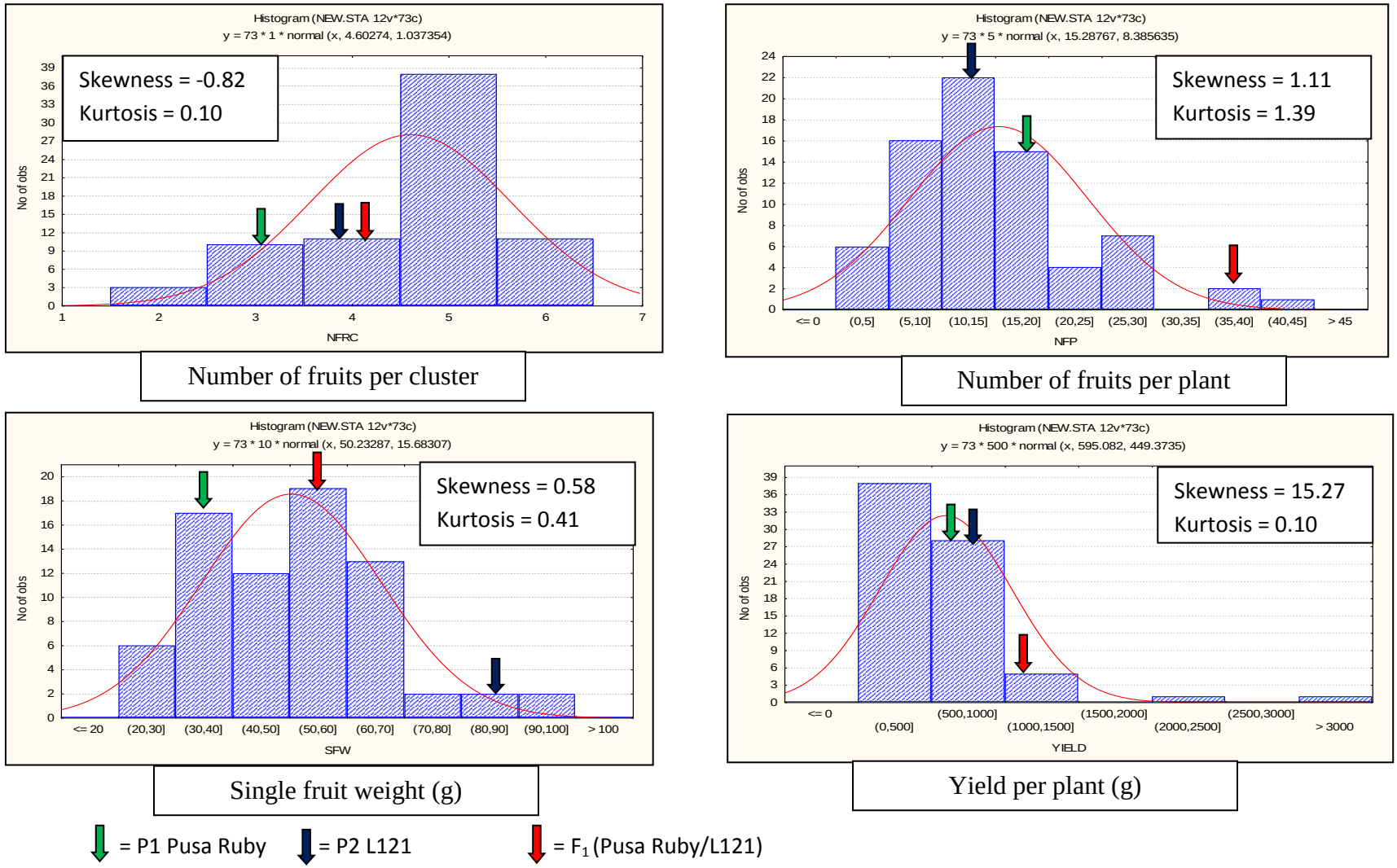


Fig. 3: Frequency distribution for yield and yield attributing traits in BC_2F_1 population of the cross Pusa Ruby/L121// Pusa Ruby

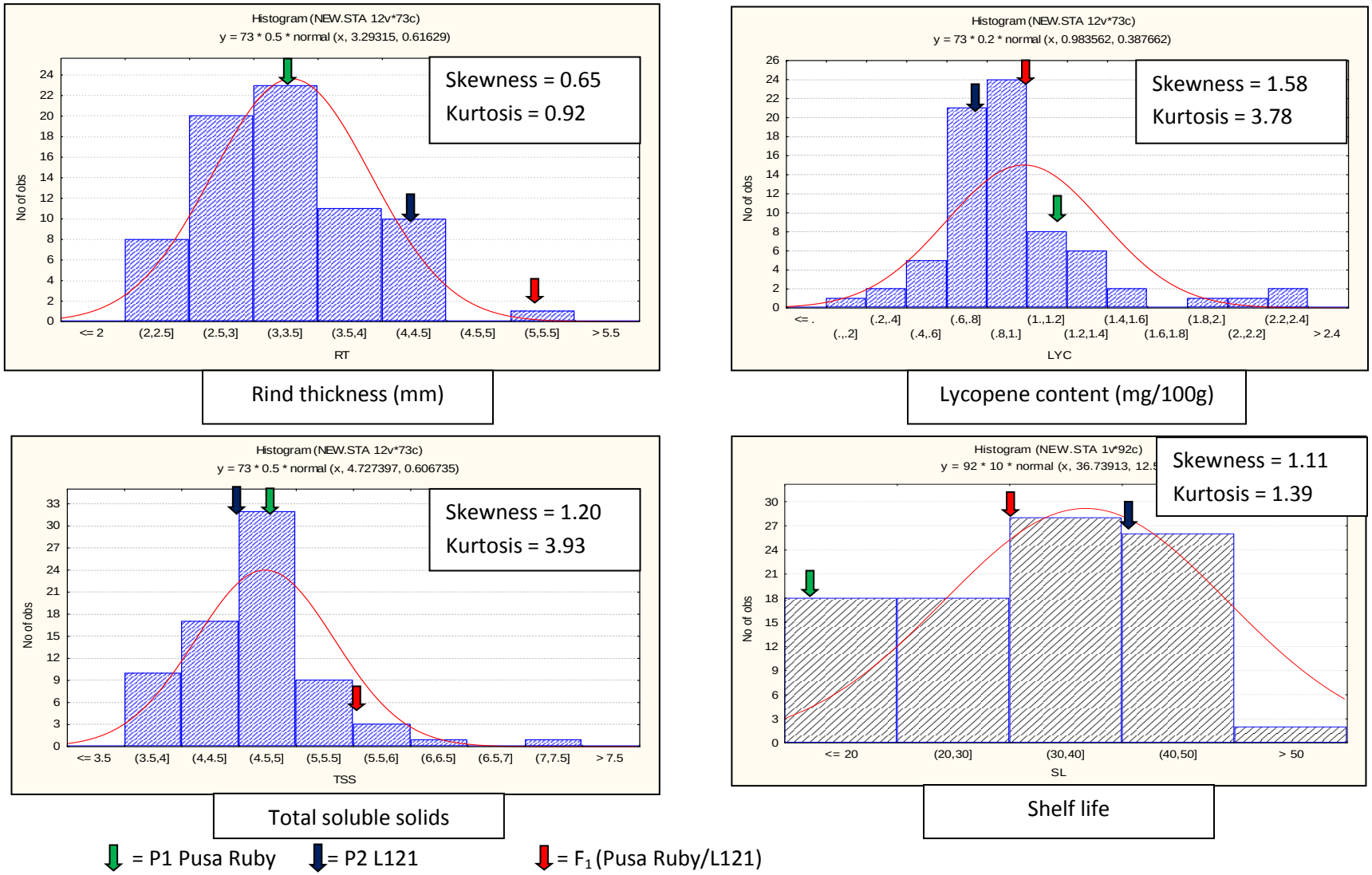
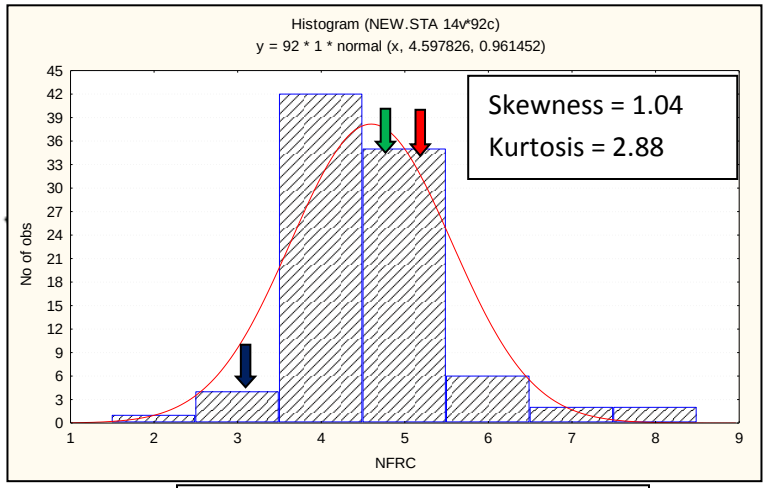
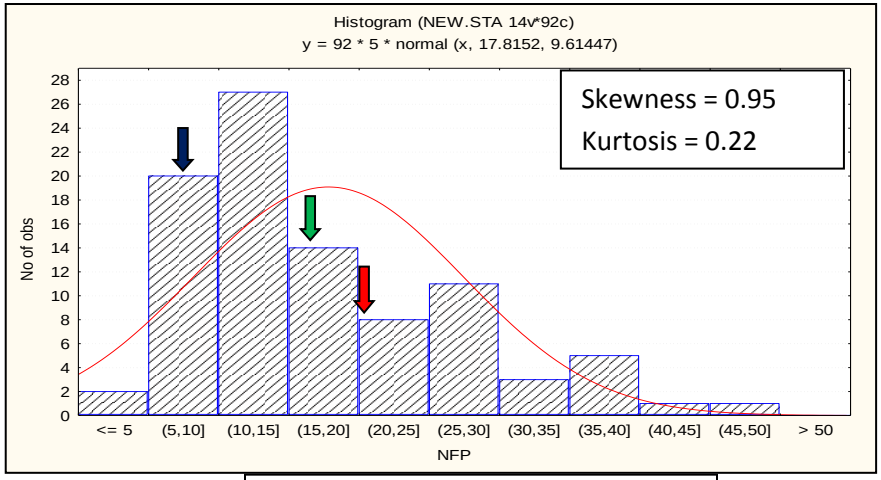


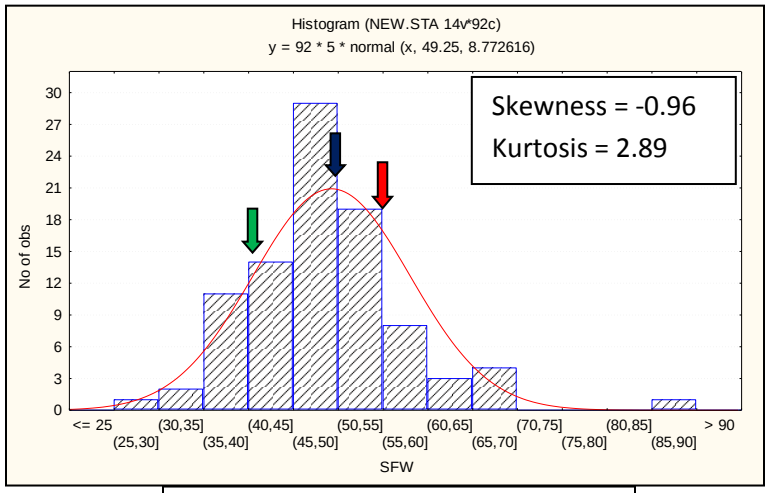
Fig. 4: Frequency distribution for fruit quality traits in BC_2F_1 population of the cross Pusa Ruby/ L121// Pusa Ruby



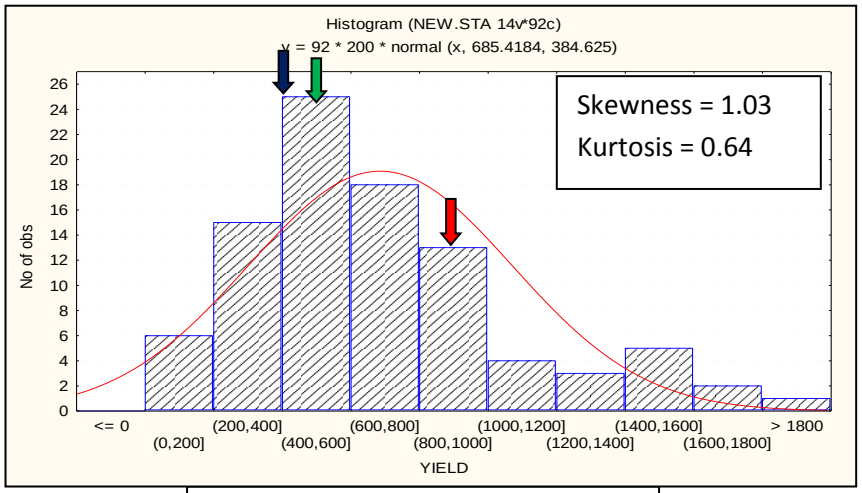
Number of fruits per cluster



Number of fruits per plant



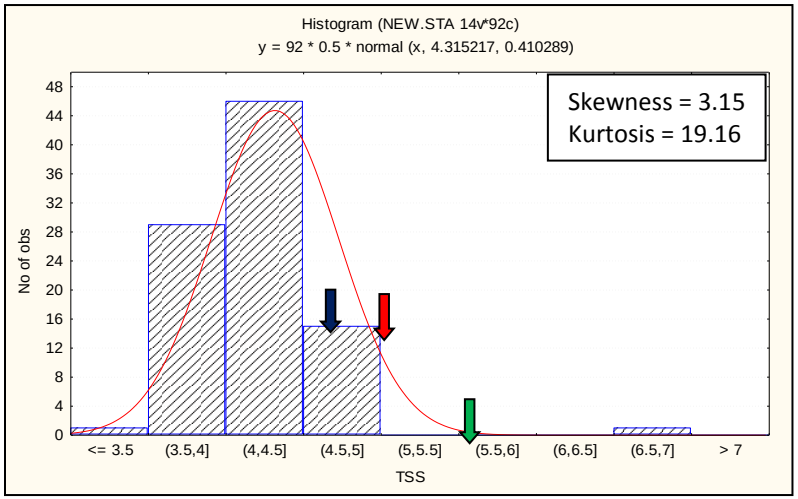
Single fruit weight (g)



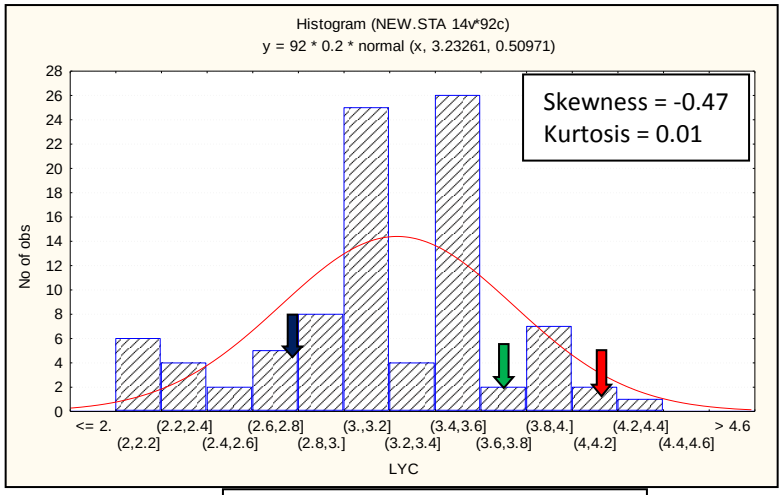
Yield per plant (g)

= P1 Vaibhav = P2 RIL126 = F_1 (Vaibhav/RIL126)

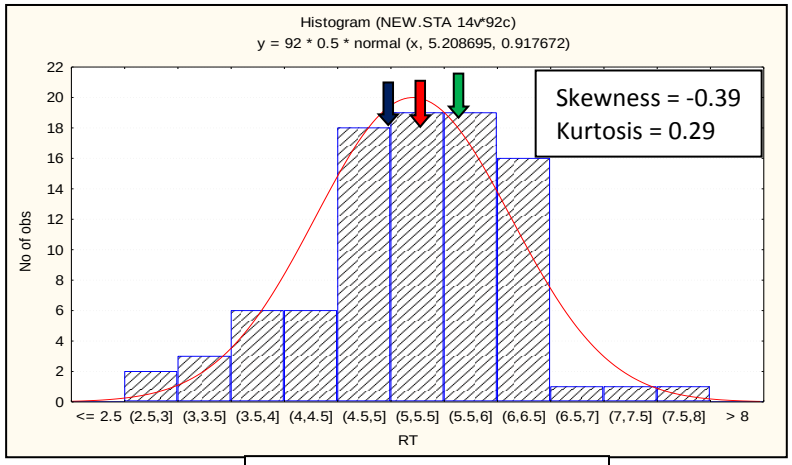
Fig. 5: Frequency distribution for yield and yield attributing traits in BC_2F_1 population of Vaibhav/RIL126// Vaibhav



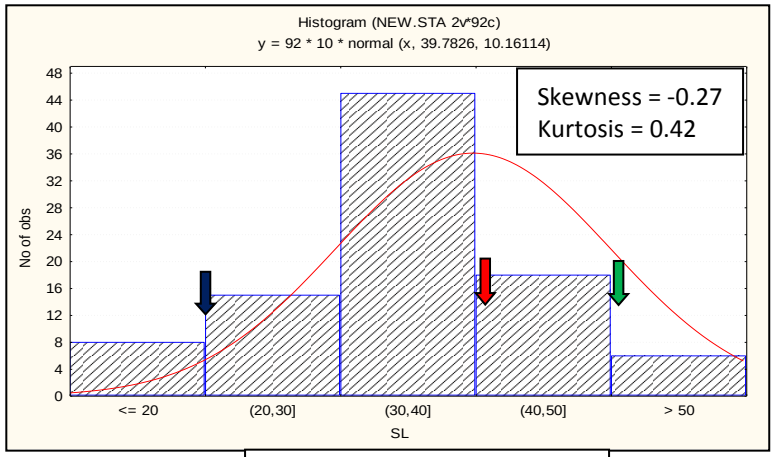
Total soluble solids



Lycopene content (mg/100g)



Rind thickness (mm)



Shelf life

= P1 Vaibhav = P2 RIL126 = F_1 (Vaibhav/RIL126)

Fig. 6: Frequency distribution for fruit quality traits in BC_2F_1 population of Vaibhav/RIL126// Vaibhav

Table 6: Estimates of mean, range and components of variability for the morphological, yield and shelf life characters in BC_2F_1 population of Pusa Ruby/L121//Pusa Ruby

Sl. No.	Characters	Mean ± SE	Minimum	Maximum	Std. Dev.	Skewness	Kurtosis
1	Plant height (cm)	54.83 ± 1.34	27.00	80.00	11.46	-0.26	-0.48
2	Number of branches per plant	6.0 ± 0.12	3.00	9.00	1.07	-0.58	1.26
3	Number of flowers per cluster	5.0 ± 0.13	3.00	9.00	1.12	-0.46	0.72
4	Number of fruits per cluster	4.0 ± 0.12	2.00	6.00	1.03	-0.81	0.10
5	Number of fruits per plant	15.0 ± 0.98	3.00	43.00	8.38	1.11	1.38
6	Single fruit weight (g)	50.23 ± 1.83	21.00	96.00	15.68	0.58	0.40
7	Fruit length (cm)	3.01 ± 0.03	2.40	4.50	0.33	1.58	4.95
8	Fruit width (cm)	4.37 ± 0.05	3.50	6.00	0.49	0.75	0.78
9	Number of locules per fruit	6.0 ± 0.16	3.00	9.00	1.40	0.03	-0.48
10	Rind thickness (mm)	3.29 ± 0.07	2.20	5.50	0.61	0.65	0.92
11	Yield (g / plant)	595.0 ± 52.59	94.00	3120.00	449.37	3.28	15.26
12	Total soluble solids	4.72 ± 0.07	3.70	7.30	0.60	1.20	3.93
13	Lycopene (mg / 100g)	0.98 ± 0.04	0.20	2.30	0.38	1.58	3.78
14	Shelf life (days)	35.0 ± 1.5	10.00	60.00	13.54	-0.26	-0.94

Table 7: Estimates of mean, range and components of variability for the morphological, yield and shelf life characters in BC_2F_1 population of Vaibhav/RIL126//Vaibhav

Sl. No.	Characters	Mean ± SE	Minimum	Maximum	Std.Dev.	Skewness	Kurtosis
1	Plant height (cm)	77.49 ± 1.19	43.00	99.00	11.42	-0.39	-0.04
2	Number of branches per plant	3.0 ± 0.13	2.00	9.00	1.25	1.21	3.10
3	Number of flowers per cluster	6.0 ± 0.11	3.00	10.00	1.06	0.92	3.30
4	Number of fruits per cluster	4.0 ± 0.10	2.00	8.00	0.96	1.04	2.88
5	Number of fruits per plant	17.81 ± 1.00	5.00	47.00	9.61	0.92	0.21
6	Single fruit weight (g)	49.25 ± 0.91	30.00	86.00	8.77	0.96	2.88
7	Fruit length (cm)	4.55 ± 0.04	3.70	5.70	0.39	-0.01	0.45
8	Fruit width (cm)	3.88 ± 0.03	3.00	4.70	0.33	-0.33	0.08
9	Number of locules per fruit	2.0 ± 0.03	2.00	3.00	0.38	1.74	1.08
10	Rind thickness (mm)	5.20 ± 0.09	2.80	7.60	0.91	-0.39	0.29
11	Yield (g / plant)	685.41 ± 40.09	157.50	1815.00	384.62	1.03	0.64
12	Total soluble solids	4.31 ± 0.04	3.50	7.00	0.41	3.15	19.16
13	Lycopene (mg / 100g)	3.23 ± 0.05	2.10	4.40	0.51	-0.47	0.01
14	Shelf life (days)	39.0 ± 1.05	10.00	60.00	10.16	-0.27	0.41

quality parameters of the backcross progenies of population 1 and population 2 are presented in the Table 8 and 9, respectively.

4.4.1 Studies on genetic variability for the population 1 (Pusa Ruby/L121//Pusa Ruby)

The GCV and PCV estimates are high for yield per plant (42.05 % and 66.69 %) followed by lycopene content (40.92 % and 50.94 %), number of fruits per plant (43.51 % and 45.87 %), shelf life (30.78 % and 34.00 %), single fruit weight (24.60 % and 26.71 %). The GCV and PCV estimates were less for the characters like plant height (7.75 % and 23.70 %), number of locules per fruit (16.30 % and 20.65 %), number of fruits per cluster (18.03 % and 19.79 %), number of flowers per cluster (10.40 % and 17.13 %), rind thickness (13.02 % and 15.19 %), number of branches per plant (13.26 % and 14.96 %), total soluble solids (11.29 % and 11.31 %). fruit width (7.18 % and 9.17 %), fruit length (3.52 % and 8.11 %) (Table 8).

Heritability in broad sense was highest for TSS (99.50 %) followed by number of fruits per plant (89.98 %), single fruit weight (84.24 %), number of fruits per cluster (83.02 %), shelf life (81.95 %), number of branches per plant (78.54 %), rind thickness (73.45 %), lycopene content (64.23 %), number of locules per fruit (62.35 %), fruit width (61.41 %) and it was moderate for yield (39.76 %), number of fruits per cluster (36.88 %) and it was lowest for fruit length (18.92 %), plant height (10.69 %) (Table 8).

Genetic advancement as percent of mean was high for lycopene content (86.81 %), number of fruits per plant (85.03 %), shelf life (73.56 %) and yield (69.99 %). It is moderate for single fruit weight (46.61 %), number of locules per fruit (33.99 %), number of fruits per cluster (33.9 %), number of branches per plant (31.05 %), TSS (29.73 %), rind thickness (29.45 %), and less for number of flowers per cluster (16.68 %), fruit width (14.87 %), plant height (6.68 %), fruit length (4.05 %) (Table 8).

4.4.2 Studies on genetic variability for the population 2 (Vaibhav/RIL126//Vaibhav)

The GCV and PCV estimates are high for number of locules per fruit (50.34 % and 54.25 %), yield per plant (31.42 % and 53.58 %) followed by lycopene (49.32 % and 51.11 %), number of fruits per plant (46.60 % and 48.25 %), number of branches per plant (30.82 % and 33.34 %), shelf life (19.59 % and 23.38 %), single fruit weight (12.60 % and 16.54 %). The GCV and PCV estimates are less for the characters like number of fruits per cluster (16.65 % and 18.55 %), plant height (11.18 % and 17.50 %), number of flowers per cluster (9.70 % and 16.45 %), rind thickness (15.12 % and 15.89 %), total soluble solids (8.30 % and 8.34 %), fruit width (4.99 % and 8.11 %), fruit length (5.89 % and 7.61 %) (Table 9).

Heritability in broad sense was highest for TSS (98.90 %) followed by number of fruits per plant (93.27 %), lycopene (93.13 %), rind thickness (90.55 %), number of locules per fruit (86.10 %), number of branches per plant (85.48 %), number of fruits per cluster (80.56 %), shelf life (70.20 %), fruit length (59.95 %) and it was moderate for single fruit weight (58.01 %), plant height (40.80 %), fruit width (37.89 %), yield (34.38 %) and number of flowers per cluster (34.38 %) (Table 9).

Table 8: Estimates of genetic components for different traits in for the morphological, yield and shelf life characters in backcross population (BC_2F_1) of Pusa Ruby/L121// Pusa Ruby

Sl. No.	Characters	GCV (%)	PCV (%)	Broad sense heritability h^2 (%)	Genetic Advancement as per cent mean
1	Plant height (cm)	7.75	23.70	10.69	6.68
2	Number of branches per plant	13.26	14.96	78.54	31.05
3	Number of flowers per cluster	10.40	17.13	36.88	16.68
4	Number of fruits per cluster	18.03	19.79	83.02	33.85
5	Number of fruits per plant	43.51	45.87	89.98	85.03
6	Single fruit weight (g)	24.60	26.71	84.24	46.61
7	Fruit length (cm)	3.52	8.11	18.92	4.05
8	Fruit width (cm)	7.18	9.17	61.41	14.87
9	Number of locules per fruit	16.30	20.65	62.35	33.99
10	Rind thickness (mm)	13.02	15.19	73.45	29.45
11	Yield (g/plant)	42.05	66.69	39.76	69.99
12	Total soluble solids (%)	11.29	11.31	99.50	29.73
13	Lycopene (mg/100g)	40.92	50.94	64.23	86.81
14	Shelf life (days)	30.78	34.00	81.95	73.56

Table 9: Estimates of genetic components for different traits in for the morphological, yield and shelf life characters in backcross population (BC_2F_1) of Vaibhav/RIL126// Vaibhav

Sl. No.	Character	GCV (%)	PCV (%)	Broad sense heritability h^2 (%)	Genetic advancement as per cent mean
1	Plant height (cm)	11.18	17.50	40.80	18.85
2	Number of branches per plant	30.82	33.34	85.48	75.24
3	Number of flowers per cluster	9.70	16.45	34.80	15.11
4	Number of fruits per cluster	16.65	18.55	80.56	39.47
5	Number of fruits per plant	46.60	48.25	93.27	118.81
6	Single fruit weight (g)	12.60	16.54	58.01	25.33
7	Fruit length (cm)	5.89	7.61	59.95	12.05
8	Fruit width (cm)	4.99	8.11	37.89	8.11
9	Number of locules per fruit	50.34	54.25	86.10	123.32
10	Rind thickness (mm)	15.12	15.89	90.55	37.99
11	Yield (g / plants)	31.42	53.58	34.38	48.64
12	Total soluble solids (%)	8.30	8.34	98.90	21.79
13	Lycopene (mg/100g)	49.32	51.11	93.13	125.66
14	Shelf life (days)	19.59	23.38	70.20	43.34

Genetic advancement as percent of mean was high for lycopene (125.66 %), number of locules per fruit (123.32 %), number of fruits per plant (118.81 %), number of branches per plant (75.24 %). It was moderate for yield (48.64 %), shelf life (43.34 %), number of fruits per cluster (39.47 %), rind thickness (37.99 %), single fruit weight (25.33 %), TSS (21.79 %) and less for plant height (18.85 %), number of flowers per cluster (15.11 %), fruit length (12.05 %) and fruit width (8.11 %) (Table 9).

4.6 Evaluation of shelf life

Three fruits from each plant were kept at room temperature for shelf life evaluation. The shelf life ranged from 10 days to 60 days. There was increase in shelf life in most of the progenies than the recurrent parent in both the populations (Plate 8 and 9).

4.7 Correlation studies

The results of the correlation analysis for population 1 and population 2 are presented in Table 10 and 11. The correlation studies in the population 1 shows that shelf life of tomato has significant effect with rind thickness and also lycopene content of the fruit and also number of fruits per cluster. Where as in population 2 there was no significant effect of rind thickness on shelf life but it shows the positive effect and traits like number of fruits per cluster and lycopene content have significant effect on shelf life.

In both the population number of fruits per plant has significant effect on yield and in population 2 fruit length and fruit width also shows the significant effect on yield. The fruit width has significant effect on number of locules per fruit and thus shows the indirect effect on yield of the plant.

4.8 Molecular marker analysis of backcross populations

Molecular marker analysis was done by using Simple Sequence Repeat (SSR) markers as they display co-dominant inheritance. The gradient PCR has been carried out for standardization of annealing temperature. Annealing temperatures was standardized for markers which showed locus specific amplification. After standardization, these primers were used to identify parental polymorphism and then for validation of SSR markers linked to high shelf life. The PCR amplification of the 30 selected SSR markers, followed by Polyacrylamide gel (5%) electrophoresis was done for the parental lines Pusa Ruby, L121, Vaibhav and RIL126 to identify the polymorphic markers. Out of them four SSR markers were polymorphic in nature (Plate 10 and 11).

4.8.1 Identification of true backcross progenies in BC_1F_1 generation.

Identification of true backcross progenies in BC_1F_1 was done for selecting the true backcross progenies to develop BC_2F_1 generation for both the population. Out of 30 SSR markers 4 markers were found to be polymorphic for the parents. Out of these 2 SSR markers TGS 293 and TGS 450 were showing the true backcross progenies (Plate 12, 13). In BC_1F_1 progenies of the cross (Pusa Ruby/L121//Pusa Ruby), P_4 and P_8 were identified as true backcross progenies. Thus seeds of these progenies in this cross (Pusa Ruby/L121//Pusa Ruby) were used to develop BC_2F_1 progenies. In BC_1F_1 progenies of the cross (Vaibhav/RIL126//Vaibhav), P_5 and P_9 were identified as true backcross

Table 10: Estimation of phenotypic correlation coefficient for shelf life and its contributing trait in BC_2F_1 generation of the cross Pusa Ruby/L121//Pusa Ruby

	NBP	NFLC	NFRC	NFP	SFW	FL	FW	LOC	RT	YLD	TSS	LYC	SL
PHT	0.5042**	0.4237**	0.1443	0.3182**	0.3119**	0.2414*	0.0922	-0.1949	-0.0734	-0.0164	-0.0914	-0.0123	-0.0732
NBP	**1.00**	0.8525**	0.4038**	0.0708	0.2538*	0.0326	0.0316	-0.1331	-0.1287	-0.1916	-0.1527	0.1474	0.0462
NFLC		**1.00**	0.5793**	0.0094	0.2004	0.0567	-0.0208	-0.0917	-0.1137	-0.2014	-0.1122	0.0568	0.0146
NFRC			**1.00**	- 0.1198	0.1000	0.1600	-0.0804	0.1486	-0.0038	-0.3066**	0.0257	0.0132	0.2261*
NFP				**1.00**	0.0789	0.1568	0.0898	-0.0596	0.2373*	0.6493**	-0.0812	-0.0166	-0.1860
SFW					**1.00**	0.2794*	0.269 *	0.0293	-0.0525	0.1641	- 0.1112	-0.2386*	0.0301
FL						**1.00**	0.515**	0.1292	0.2462*	0.1175	-0.0301	0.0590	-0.1199
FW							**1.00**	0.4615**	0.2992*	0.0048	- 0.2852*	0.1668	-0.0866
LOC								**1.00**	0.1749	-0.0769	- 0.2861*	0.1446	0.0781
RT									**1.00**	0.1831	0.1411	0.0024	0.4525**
YLD										**1.00**	-0.1292	0.0835	-0.0188
TSS											**1.00**	-0.1823	0.1183
LYC												**1.00**	0.3567**

Legend

PHT- Plant height (cm)
NBP-Number of branches per plant
NFLC- Number of flowers per cluster
NFRC-Number of fruits per cluster
YLD-Yield (g/plant)
SFW- Single fruit weight (g)
NFP- Number of fruits per plant
FL - Fruit length (cm)
FW - Fruit width (cm)
LOC- Number of locules per fruit
RT- Rind thickness (mm)
TSS– Total Soluble Solids (%)
LYC– Lycopene content (mg/100g)
SL - Shelf life (days)

Table 11: Estimation of phenotypic correlation coefficient for shelf life and its contributing trait in BC_2F_1 generation of the cross Vaibhav/RIL126//Vaibhav

	NBP	NFLC	NFRC	NFP	SFW	FL	FW	LOC	RT	YLD	TSS	LYC	SL
PHT	0.0411	0.2135*	0.1873	-0.0163	0.0383	0.0974	0.0295	-0.2181*	- 0.0390	0.0788	-0.1344	-0.1004	-0.1367
NBP	**1.00**	0.0765	0.2203*	0.3539**	0.0656	0.0371	0.0607	0.0746	0.0210	0.4606 **	0.0427	-0.0806	0.0233
NFLC		**1.00**	0.4994**	-0.2026	-0.0634	-0.1275	-0.1641	-0.0124	-0.0626	0.0083	0.0477	0.1055	0.1256
NFRC			**1.00**	-0.0775	-0.1325	-0.0391	-0.0529	-0.2278*	-0.0685	0.1624	-0.0733	0.0693	0.2100*
NFP				**1.00**	-0.0261	0.1314	0.2461*	- 0.0375	0.0699	0.5695**	0.0945	-0.0429	-0.0325
SFW					**1.00**	0.3703**	0.3063**	0.0435	0.0108	0.0971	0.0948	-0.1558	-0.0179
FL						**1.00**	0.0783	0.0514	0.1231	0.3824**	0.0251	-0.0158	-0.1881
FW							**1.00**	-0.0699	0.5093**	0.6604 **	0.0039	-0.0937	-0.1201
LOC								**1.00**	0.4678**	-0.0327	-0.0234	-0.2669*	0.0386
RT									**1.00**	0.1340	-0.0021	-0.1396	0.1532
YLD										**1.00**	0.0064	-0.0770	-0.0270
TSS											**1.00**	0.0982	0.0487
LYC												**1.00**	0.2100*

Legend

PHT- Plant height (cm)
NBP-Number of branches per plant
NFLC- Number of flowers per cluster
NFRC-Number of fruits per cluster
YLD-Yield (g/plant)
SFW- single fruit weight (g)
NFP- Number of fruits per plant
FL - Fruit length (cm)
FW - Fruit width (cm)
LOC- Number of locules per fruit
RT- rind thickness (mm)
TSS – Total Soluble Solids (%)
LYC– Lycopene content (mg/100g)
SL - shelf life (days)

Plate 8: Shelf life evaluation of 10 Tomato backcross progenies (BC_2F_1) of the cross Pusa Ruby/L121// Pusa Ruby along with their parents

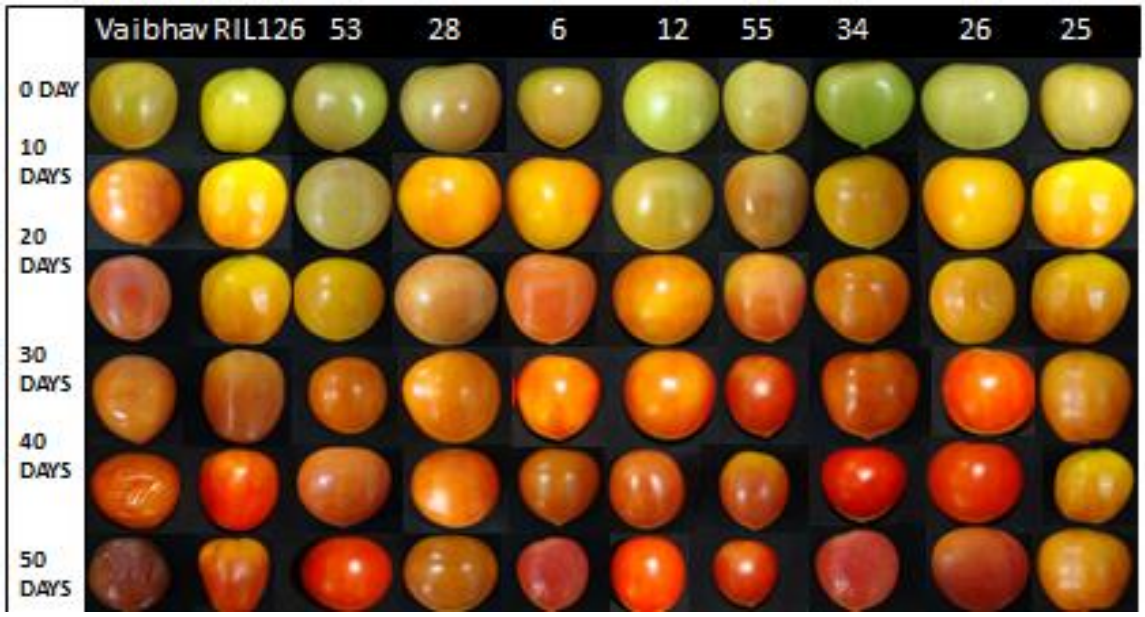


Plate 9: Shelf life evaluation of 10 Tomato backcross progenies (BC_2F_1) of the cross Vaibhav/RIL126// Vaibhav along with their parents

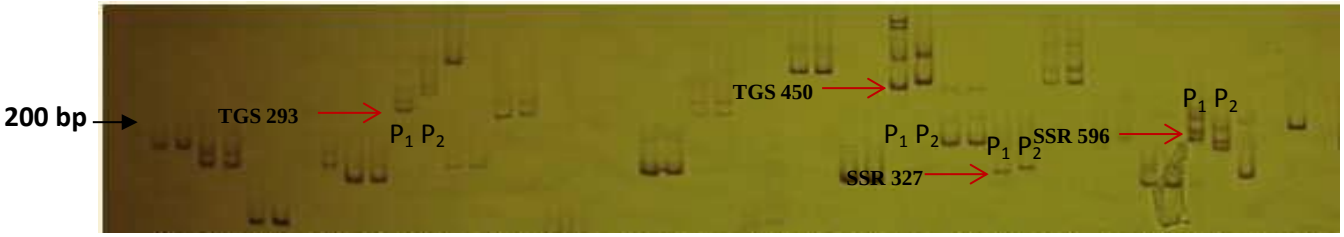


Plate 10: Identification of parental polymorphism between Pusa Ruby (P_1) and L121 (P_2) arrows indicate polymorphic markers

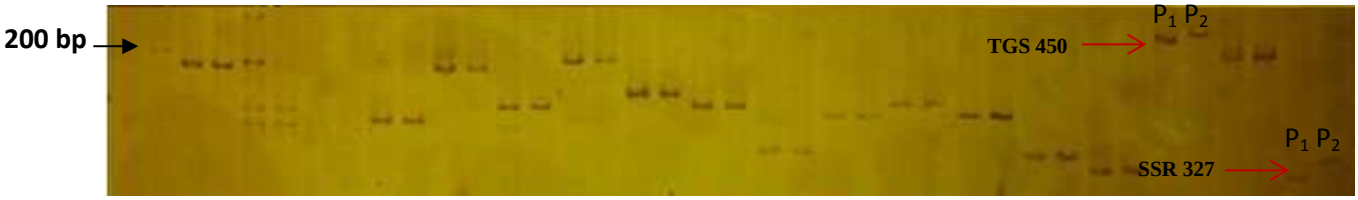


Plate 11: Identification of parental polymorphism between vaibhav (P_1) and RIL126 (P_2) arrows indicate polymorphic markers

Plate 12: Identification of true Backcross progeny in BC_1F_1 population using polymorphic primer TGS450

Single banding pattern indicate parental type and the double bands (arrow) indicate the true backcross progeny

Parents: 1. Vaibhav 2. Pusa Ruby 3. Arka Alok 4. L121 5. RIL-126

Plate 13: Identification of true Backcross progeny in BC_1F_1 population using polymorphic primer TGS293

Single banding pattern indicate parental type and the double bands (arrow) indicate the true backcross progeny

Parents: 1. Pusa Ruby 2. Arka Alok 3. L121 4. RIL-126

progenies. Thus seeds of these progenies were used to develop BC_2F_1 progenies of the cross (Vaibhav/RIL126//Vaibhav).

4.8.2 Validation of SSR markers linked to high shelf life in backcross population.

The primers which showed parental polymorphism were further used for screening 40 selected backcross progenies along with their parents (Plate 14). The single Marker analysis was done by single factor ANOVA performed between the marker loci and shelf life traits. The results of single marker analysis revealed that markers were associated with fruit shelf life. The SMA revealed that SSR markers TGS 293 was associated with fruit keeping quality (Plate 14).

Plate 14: TGS 293 Screened for 40 Progenies along with their parents

1-40: Backcross Progenies
Parent (P1): L121
Parent (P2): Pusa Ruby
Marker (M): 100bp Ladder (Genei)

V DISCUSSION

A significant impact of globalization on horticulture crops has been an increasing demand for quality improvement and the wider adoption of quality standards for fruit and vegetable. Tomato (*Solanum lycopersicum)* is a major vegetable crop with an estimated total area 45,82,438 thousand ha with production of 15,05,13,813 Mt and with productivity of 32.8 t ha^{-1}. (FAOSTAT, 2013). Tomato is very popular for genetic research. Because, it is a basic diploid with minimal DNA duplication and its 12 chromosomes are highly differentiated and distinguishable. The tomato is naturally self-pollinated, yet flowers are easily manipulated to yield large quantities of hybrid seed. The plants can be easily cultured in a wide range of environmental conditions; the tomato is amenable to sexual and asexual propagation. The genome is replete with conventional and molecular markers and has well developed linkage maps. The tomato offers the advantages of its edible crop status; much mutual benefit results from frequent exchanges between applied and basic research (Rick, 1991).

Tomato experiences great post-harvest losses because of its natural perishability, precarious transportation and storage conditions, and inadequate packaging. The post-harvest losses of fruits and vegetables in the developing countries account for almost 50 per cent of the produce. In India, losses up to 40 per cent of produce occur because of excessive fruit softening. The quality of tomato fruits depends entirely on their developmental stage. So, if harvested at immature stage, the colour, taste and flavour of fruits do not develop properly. Hence, on storage the fruits get shrivelled and post-harvest shelf life is reduced (Mutschler *et al*., 1992).

The enhancement of yield or economic product of any crop species is the ultimate aim of the plant breeder in the concerned crop improvement programme. The yield and its attributing characters are polygenic in nature and as such, are complex in their mode of inheritance. An understanding of the genetic nature and inheritance of such complex traits and extent of their association with yield is very much essential to help and guide the plant breeder in selection experiments. It also helps in systematic handling of breeding material to achieve maximum improvement by adopting highly sophisticated statistical techniques.

Integrating molecular approaches with traditional breeding for high shelf-life could significantly increase the storage life of tomato. Most traits of agricultural importance, such as yield and quality, are complex and they have polygenic control and quantitative inheritance. There are many regions within the genomes which contain genes that are associated with a polygenic trait and are called quantitative trait loci (QTLs). The identification of QTLs based only on conventional phenotypic evaluation is not possible. The efficiency of MAS backcrosses for the introgression of a quantitative trait locus (QTL) depends on the stability of QTL expression (Chaib *et al*., 2006). These marker assisted backcross breeding has become one of the essential tools in transferring novel genes to adapted varieties.

The main aim of the present research is to introgress the high shelf life genes from variety with high shelf life into the background of commercially well accepted variety using backcross breeding. Both phenotypic evaluation of these backcross progenies for shelf life and other related characters. This evaluation study shows the phenotypic variations between the parents and their progenies. The phenotypic variation measures the magnitude of variation arising out of difference in phenotypic values while, the genotypic variances cannot be used for comparing the magnitude of variation due to differences in genotypic values. The absolute values of the genotypic and phenotypic variances cannot be used for comparing the magnitude of variability for different characters since the mean and units of magnitude of variability for different characters since the mean and units of measurement of characters may be different. Hence, the co-efficient of variations expressed at phenotypic and genotypic levels have been used to compare the variability present in the characters. While, genotypic co-efficient of variation indicates the amount of heritable portion of variation indicates the amount of genetic variability present in the characters, the heritability estimates aid in determining the relative amount of heritable proportion of variation. However, heritability values themselves provide no indication of the amount of genetic progress that would result from selecting the best individuals. Ramanujam and Thirumalachar, (1967) while studying the genetic variability in red pepper discussed the limitation of estimating the heritability in a broad sense as it include both additive and non-additive genetic effects. According to them heritability estimates in broad sense would be reliable if accompanied by high genetic advance.

The results obtained in the current study are discussed under the following main headings.

5.1 Mean performance of parents, F_1 hybrids and backcross progenies of the cross Pusa Ruby/L21//Pusa Ruby (population-1)

5.2 Mean performance of parental lines, F_1 hybrids and backcross progenies of the cross Vaibhav/RIL126//Vaibhav (population-2)

5.3 Genetic variability studies

5.4 Test of normality.

5.5 Correlation studies.

5.6 Evaluation of shelf life in backcross progenies

5.7 Validation of SSR markers

5.1 Mean performance of parental lines, F_1 hybrids and backcross progenies of the cross Pusa Ruby/L21//Pusa Ruby (population-1)

In the present study the mean performance of the F_1 hybrids is superior to both the parents in plant height, number of fruits per plant, fruit length, rind thickness, yield and TSS which is due to the hybrid vigour. The characters like number of flowers per cluster, number of fruits per cluster, number of locules per fruit of F_1 hybrids are similar to Pusa Ruby whereas single fruit weight, lycopene and shelf life of F_1 hybrids lay in-between

both the parents. In BC_2F_1 progenies most of the characters were found to be similar to that of recurrent parent Pusa Ruby except for single fruit weight, yield, TSS, rind thickness and shelf life which are superior to Pusa Ruby.

The mean mass per commercial fruit was not affected by the *alcobaça* allele in heterozygosity (Freitas *et al*., 1996). Mutschler *et al*. (1992), Souza *et al*. (1999), and Faria *et al*. (2003) concluded that the mean fruit mass is not affected by the genetic makeup of the *alcobaca* locus, in agreement with the results of this research the gene related to high shelf life do not affect the total yield of the plant.

5.2 Mean performance of parental lines, F_1 hybrids and backcross progenies of the cross Vaibhav/RIL126//Vaibhav (population-2)

In this population the mean performance of the F_1 hybrids is superior to both the parents in plant height, number of flowers per cluster, number of fruits per plant, single fruit weight, fruit width, yield TSS and lycopene which is due to the heterosis. The characters like number of fruits per cluster, number of locules per fruit of F_1 hybrids are similar to vaibhav whereas yield and shelf life of F_1 hybrids lay between both the parents. In BC_2F_1 progenies most of the characters were found to be similar to that of recurrent parent Vaibhav except for single fruit weight, yield, TSS fruit length and shelf life which are superior to Vaibhav.

The mean performance of the population-2 in most of the characters is superior to population-1 except for number of locules per fruit, TSS, and it is early variety. The fruits with more number of locules contribute to the highest seed number which is not preferred by consumers but these are preferred due to its high TSS content (Rodriguez *et al.*, 2011). The population-2 is better for the table purpose and also has longer shelf life than population -1.

5.3 Genetic variability studies of backcross progenies

The analysis of variance revealed that highly significant differences among the genotypes for all the characters indicating sufficient variability existed in the present material selected for the study and indicating the scope for selection of suitable initial breeding material for crop improvement. However, the absolute variability in different characters does not permit identification of the characters showing the highest degree of variability. Therefore, PCV and GCV values were estimated. The coefficient of variation whether it is genotypic or phenotypic, both are useful in studying the extent of variability in different characters as it measures the range of variability.

The range in mean values does not reflect the total variance in the material studied. Hence, actual variance has to be estimated for the characters to know the extent of existing variability, so, the co-efficient of variation (GCV and PCV) which is calculated by considering the respective means have been used for the comparison. High values of these parameters indicate wider variability and vice versa. In the same context, narrow difference between the phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) implies lesser influence of environment on these traits. In both the population the phenotypic co-efficient of variation (PCV) were higher in

magnitude than the genotypic co-efficient of variation (GCV) indicating the involvement of environmental factors in manifestation of traits under study. Singh, (2005) reported the estimates of PCV and GCV were high for fruit yield per plant, yield per ha. Fruits weight, radial diameter, polar diameter and average fruit weight shows less influence of environment on these traits.

5.3.1 Genetic variability studies of backcross progenies of tomato in Pusa Ruby/ L21//Pusa Ruby (population-1)

In population-2 the difference between values of PCV and GCV were less for all traits except plant height, number of flowers per cluster, fruit length and yield. It means that these traits were less influenced by environment and hence, they could be improved by phenotypic selection for further studies. Shankar *et al*., (2013) observed highest PCV and GCV values particularly for lycopene and yield per plant due to very high variability available in these traits.

The PCV and GCV values were very high particularly for lycopene, number of fruits per plant, single fruit weight, yield per plant and shelf life due to very high variability available in these traits. Moderate PCV and GCV values for number of branches per plant, number of flowers per cluster, number of fruits per cluster, number of locules per fruit, pericarp thickness and TSS indicated the presence of moderate genetic variability for these characters in backcross progenies.

Whereas, low PCV and GCV was recorded for fruit length, fruit width and plant height suggesting less variability existed in these characters. This moderate to low variability indicates the need for improvement of base population through crossing with recurrent parent followed by selfing to increase the gene flow and to fix favourable alleles. Yogendra and Gowda, (2013) reported that the genetic variability studies in tomato RIL's shows the low genetic variability in fruit quality characters.

Perusal of results on heritability and genetic advance as per cent of mean (GAM) revealed that heritability estimates were high for most of the characters studied. This suggested the greater effectiveness of selection due to less influence of environment and improvement to be expected for these characters in future breeding programme. Johnson *et al*. (1955) suggested that high heritability coupled with high genetic advance as percentage of mean (GAM) were more useful than heritability alone in predicting the resultant effect during selection of best individual genotype. Genetic advance is the measure of genetic gain under selection and expression in percentage of mean. In the present research high heritability and genetic advance as per cent of mean (GAM) was recorded for number of branches per plant, number of fruits per cluster, number of fruits per plant, single fruit weight, number of locules per fruit, pericarp thickness, yield, shelf life, lycopene and TSS indicating predominance of additive gene action for these characters. Simple selection based on phenotypic performance of these characters would be more effective.

High heritability and moderate genetic advance as per cent of mean values were observed for the fruit width character. This indicates the influence of non-additive gene

action and considerable influence of environment in the expression of these traits. These traits could be exploited through manifestation of dominance and epistatic components through heterosis. Whereas the results of Shankar *et al*. (2013) showed, high heritability and moderate genetic advance as per cent of mean for number of flowers per cluster, TSS. This indicates the influence of non-additive gene action and considerable influence of environment in the expression of these traits.

5.3.2 Genetic variability studies of backcross progenies of tomato in Vaibhav/ RIL126//Vaibhav (population-2)

In population-2 the difference between values of PCV and GCV were less for all traits except plant height, number of flowers per cluster, fruit width and yield. It means that these traits were less influenced by environment and hence, they could be improved by phenotypic selection for further studies. The PCV and GCV values were very high particularly for lycopene, number of fruits per plant, number of locules per fruit, yield per plant and shelf life due to very high variability available in these traits.

Moderate PCV and GCV values for plant height, number of branches per plant, number of flowers per cluster, number of fruits per cluster, single fruit weight and pericarp thickness indicated the presence of moderate genetic variability for these characters in backcross progenies.

Whereas, low PCV and GCV was recorded for fruit length, fruit width and TSS suggesting less variability existed in these characters. Shankar *et al*. (2013) observed higher estimates of PCV and GCV are obtained for plant height, number of fruits per cluster, Average fruit weight, yield per plant, titrable acidity, ascorbic acid content and lycopene indicated a good deal of variability in those characters signifying the effectiveness of selection of desirable types for improvement. The moderate to low variability indicates the need for improvement of base population through crossing with recurrent parent followed by selfing to increase the gene flow and to fix favorable alleles.

In the population-2 high heritability and genetic advance as per cent of mean (GAM) was recorded for number of branches per plant, number of fruits per cluster, number of fruits per plant, single fruit weight, fruit weight, number of locules per fruit, pericarp thickness, yield, shelf life, lycopene and TSS indicating predominance of additive gene action for these characters. Simple selection based on phenotypic performance of these characters would be more effective. This suggested the greater effectiveness of selection due to less influence of environment and improvement to be expected for these characters in future breeding programme.

High heritability and moderate genetic advance as per cent of mean values were observed for the fruit length character. This indicates the influence of non-additive gene action and considerable influence of environment in the expression of these traits. These traits could be exploited through manifestation of dominance and epistasis components through heterosis. These results are in agreement with Shankar *et al*. (2013). Hence, the breeder should adopt suitable breeding methodology to utilize both additive and non-

additive gene effects simultaneously, since varietal and hybrid development will go a long way in the breeding programme especially in case of tomato.

Both the population show similar type of variability for the characters studied. In Population-1 there is high heritability for shelf life than population-2 indicating there is more additive gene action in population-1 than population-2.

5.4 Test of normality

From the results of Shapiro-Wilk's W test (Shapiro *et al*., 1968) it was found that the BC_2F_1 populations of the cross Pusa Ruby/L121//Pusa Ruby has been shown normal distribution for most of the characters. The study of distribution properties such as coefficients of skewness and kurtosis provides insight about the nature of gene action (Fisher *et al*., 1932) and number of genes controlling the traits (Robson, 1956), respectively. All the reported genetic analysis of quantitative traits based on first degree (gene effects through generation mean analysis) and second degree (components of genetic variance analysis) statistics.

Skewness and kurtosis are more powerful than variances which reveal interactions and genetic effects (Choo and Reinbergs, 1982). The skewed distribution of a trait in general suggests that the trait is under the control of non-additive gene action, especially epistasis and influenced by environmental variables (Pooni *et al.*, 1977; Kimbeng and Bingham, 1998; Roy, 2000). While positive skewness is associated with complementary gene interactions, negative skewness is associated with duplicate (additive × additive) gene interactions. The genes controlling the trait with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait. The traits with leptokurtic and platykurtic distribution are controlled by fewer and large number of genes, respectively. Kurtosis is negative or close to zero in the absence of gene interaction and is positive in the presence of gene interactions (Pooni *et al*., 1977; Choo and Reinbergs, 1982; Kotch *et al*., 1992).

5.4.1 Test of normality in backcross progenies of the cross Pusa Ruby/L21//Pusa Ruby

Negatively skewed and platykurtic distribution is an evidence for involvement of large number of dominant genes with majority of them having increasing effects and duplicate type of epistasis in the inheritance of number of branches per plant, number of flowers per cluster, number of fruits per cluster.

Positively skewed and platykurtic distribution is an evidence for involvement of moderate number of genes among which equal frequency of genes had increasing and decreasing effects with complementary type of epistasis on the expression of number of fruits per plant, single fruit weight, fruit length, fruit width, rind thickness, yield and TSS. Maximizing the genetic gain in respect of the traits with positively skewed distribution requires intense selection from the existing variability (Roy, 2000).

Leptokurtic and positively skewed distribution can be seen in rind thickness, suggested the involvement of relatively fewer number of segregating genes with majority of them having decreasing effects and dominance based complementary type of interaction in the inheritance of fruit yield per plant.

5.4.2 Test of normality in backcross progenies of the cross Vaibhav/RIL126// Vaibhav

Negatively skewed and platykurtic distribution is an evidence for involvement of large number of dominant genes with majority of them having increasing effects and duplicate type of epistasis in the inheritance of fruit length, fruit width, rind thickness, lycopene and shelf life.

Positively skewed and platykurtic distribution is an evidence for involvement of moderate number of genes among which equal frequency of genes had increasing and decreasing effects with complementary type of epistasis on the expression of number of branches per plant, number of flowers per cluster, number of fruits per cluster, number of fruits per plant, single fruit weight, number of locules per fruit, yield and TSS. Maximizing the genetic gain in respect of the traits with positively skewed distribution requires intense selection from the existing variability (Roy, 2000). Leptokurtic and negatively skewed distribution can be seen in plant height suggested the involvement of relatively fewer number of segregating genes with additive gene effects.

Some of the traits in the both the populations exhibiting deviation from normal distribution due to:

1. Natural selection and meiotic distortion.
2. Due to the phenomenon of linkage drag and linkage disequilibrium.

5.5 Correlation studies

As the present investigation is related to shelf life the correlation analysis was carried out to understand the relationship between different traits in backcross population. As fruit shelf life is a complex polygenic trait, direct selection based on these trait would not yield fruitful results without giving due importance to their genetic background. Besides total plant yield, other most important character has to be understood by this correlation studies. The relative association of these traits and their component traits reflects the nature and degree of relation between them. The correlation analysis helps in examining the possibility of improving shelf life and yield through indirect selection of their component traits which are highly correlated.

5.5.1 Correlation studies of backcross progenies of tomato in backcross progenies of the cross Pusa Ruby/L21//Pusa Ruby

In this population the correlation analysis was carried out in 68 BC_2F_1 progenies. Highly significant and positive correlations were observed for fruit shelf life with number of fruits per cluster, rind thickness and lycopene. Rest of the characters did not have

significant association with fruit shelf life. Shelf life has negative correlation with number of fruits per plant and single fruit weight.

Yield per plant which is most important trait has significant and positive correlation with number of fruits per plant. Reports of Aruna, (1992), Premalakshmi, (2001), Ghosh *et al.*, 2010 and Indurani *et al*., (2010) are in conformity with the above results. The traits like plant height and number of branches per plant were negatively correlated to yield which are on par with the results of Mohanty, 2003 and Ghosh *et al.*, 2010. The TSS has negative association with yield. Results were conformity with results of and Indurani *et al*. (2010).

The studies in the population-1 suggest that rind thickness and lycopene should be considered together as primary shelf life determining traits in tomato. Besides favorable association of characters with shelf life, an inter relation of characters would simplify selection schemes.

5.5.2 Correlation studiesof backcross progenies of tomato in backcross progenies of the cross Vaibhav/RIL126//Vaibhav (population-2)

In the population-2 the correlation analysis was carried out in 92 BC_2F_1 progenies. Highly significant and positive correlations were observed for fruit shelf life with number of fruits per cluster and lycopene. Yield per plant has significant positive association with number of branches per plant, number of fruits per plant, single fruit weight and fruit width.

5.6 Evaluation of shelf life in backcross progenies

The mean values of shelf life in Pusa Ruby were significantly lower compared to L121 which carries *alc* gene responsible for high shelf life. The Vaibhav also shows significant variation with respect to shelf life in comparison to RIL126. The F_1 hybrids and backcross progenies showed high shelf life compared to their respective recurrent parents.(Pratta *et al*., 2011) showed the evidence for prolonged shelf life is due to genes carried by both parents (the low and high shelf life genotypes),we have also observed the transgressive segregation in our studies for shelf life.

About 25 per cent of the backcross progenies have low shelf life rest 75 per cent of the progenies shows improved shelf life. This shows the additive effect of the genes governing shelf life of these progenies. The effects of the alc allele on several plant and fruit traits were studied by (Mutschler *et al*., 1992). However, the possible effects of the genetic background were not prioritized in these studies, even though studies previously conducted have shown that losses in fruit mass, coloration and firmness could be result from the genetic background utilized.

5.7 Validation of SSR markers

SSR markers which are co-dominant in nature have specific positions on chromosome regions. In the present study 30 SSR markers were used but out of these 4 markers were found to be polymorphic. In backcross population the shelf life is linked by

SSR marker, with the help of single marker analysis. Genetic polymorphism was scored among the backcross population of cross Pusa Ruby/L121//Pusa Ruby using polymorphic SSR marker TGS 293.The association of markers to fruit quality traits was detected using SMA. The association of a single marker to a number of traits is important because it reveals the nature of the particular marker (Yogendra and Gowda, 2013). The SMA revealed that the SSR marker TGS 293 is associated with fruit shelf life.

Pratta *et al*. (2011) also reported the presence of favourable alleles for shelf life in the cultivated genotypes of tomato. He also observed that heterozygous individuals of backcross population have longer shelf life than homozygous individuals which indicate the heterotic effect.

VI SUMMARY

Tomato is the second most important vegetable crop next to potato and has been the subject of genetic study for more than a century. The demand for tomato is increasing day by day mostly because of the increased per capita fresh fruit consumption. The postharvest losses of fruits and vegetables in the developing countries account for almost 50 percent of the produce. In India, post-harvest losses up to 40 percent of produce occur because of excessive fruit softening. Different approaches are used to improve the shelf life of tomato, these include Post harvest treatments, breeding methods and genetic engineering approaches.

An investigation was carried out in tomato (*Solanum lycopersicum*) at Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore, during Kharif, 2015 to evaluate parents, F_1 hybrids and backcross populations of the cross 'Pusa Ruby/L121//Pusa Ruby' and 'Vaibhav/RIL126//Vaibhav' in field conditions for shelf life using SSR markers.

The salient features of the present investigation are summarized below

An important trait in commercial fresh market tomatoes is the fruit shelf life. Biotechnology has made considerable progress in modifying metabolic pathways that delay the tomato ripening process and discovering complex networks of transcription factors that regulate ripening, making it plausible that these features could be added through bioengineering. However, some consumers do not support genetic engineering of crops due to unanswered questions of food and environmental safety. Hence the transgenic technologies through plant breeding should be applied backcrossing is the most convenient method of plant breeding to introgress the favourable genes into the background of commercial variety. This present investigation was carried out to transfer the high shelf life character to commercially accepted varieties (Pusa Ruby and Vaibhav) with low shelf life.

All the checks and progenies shown considerable amount of variations in their mean performance with respect to all the characters studied, this indicates genotypes under study were genetically variable. The evaluation of *per se* performance of parents, F_1 hybrids and backcross progenies of both the crosses indicated that, F_1 hybrids were superior in respect of most of the traits studied. This is due to hybrid vigour whereas backcross progenies did not show higher performances than the F_1 hybrids may be due to inbreeding depression. However, among these most of the backcross progenies have shown the increased shelf life than their recurrent parental line this shows the transfer of desirable trait from donor parent to the recurrent parent. In some of the progenies we could see the decreased shelf life and also yield attributing characters, this may be due transfer of some undesirable genes from donor parent to the recurrent parent as linked genes.

Most of the traits showed less difference between GCV and PCV which indicate the less environmental effects. The heritability rates for fruits per cluster, fruits per plant, TSS, single fruit weight and shelf life were high which indicate the genetic stability for

these traits. This suggested the greater effectiveness of selection due to less influence of environment and improvement to be expected for these characters in future breeding programme.

Highly significant and positive correlations were observed for fruit shelf life with number of fruits per cluster, rind thickness and lycopene. Rest of the characters did not have significant association with fruit shelf life. Shelf life has negative correlation with number of fruits per plant and single fruit weight. Yield per plant which is most important trait has significant and positive correlation with number of fruits per plant. The traits like plant height and number of branches per plant were negatively correlated to yield

Negatively skewed and platykurtic distribution is an evidence for involvement of large number of dominant genes with majority of them having increasing effects and duplicate type of epistasis in the inheritance of number of branches per plant, number of flowers per cluster, number of fruits per cluster.

Positively skewed and platykurtic distribution is an evidence for involvement of moderate number of genes among which equal frequency of genes had increasing and decreasing effects with complementary type of epistasis on the expression of number of fruits per plant, single fruit weight, fruit length, fruit width, rind thickness, yield and TSS. Maximizing the genetic gain in respect of the traits with positively skewed distribution requires intense selection from the existing variability.

Leptokurtic and positively skewed distribution can be seen in rind thickness, suggested the involvement of relatively fewer number of segregating genes with majority of them having decreasing effects and dominance based complementary type of interaction in the inheritance of fruit yield per plant.

The polymorphic markers TGS 293 and TGS 450 were used to identify the heterozygous plants which were used in further crossing. This study indicates, the shelf life trait can be introduced by backcross breeding using molecular markers. The screening of 40 backcross progenies using TGS293 marker showed the improved shelf life in heterozygous progenies and this indicate the marker is linked to the trait of interest.

The use of *alcobaca* genes and their effects in heterozygosity are independent from the utilized background, and fallowed the same tendencies regardless of fruit position in the plant. The genotypic background can be manipulated in addition to using the *alc* ripening mutant, to promote the shelf life fruits.

Among both the populations studied the population-2 of the cross Vaibhav/RIL126//Vaibhav has high genetic stability with favourable traits. So this population can be further used for various selection and genetic improvement studies.

Future line of work:

1. The backcross progenies (BC_2F_2 and BC_3F_1) developed by using these BC_2F_1 can be used for further stabilization of yield and shelf life characters.
2. Further validation of polymorphic SSR markers linked to shelf life can be used for screening of stabilized populations.
3. Development of NILs and BILs for commercialization.

VII REFERENCES

ADAMS, D. AND ADAMS, S., 2000, Tomato lycopene and its role in human health and chronic diseases. *J. Sci. Food Agric.,* **163**: 739-744.

AGAR, I. T., ABAK, K. AND YARSI, G., 1994, Effect of different maturity stages on the keeping quality of *nor* (non-ripening), *rin* (ripening inhibitor) and normal type tomatoes. *Acta Hort.,* **368**: 742-753.

ALBA, R., PAYTON, P. AND FEI, Z., 2005, Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. *Plant cell,* **17**(11): 2954-2965.

ALMEIDA, J. L. F., 1961, A new aspect of improvement of tomato. *Agriculture*, **10**: 43-44.

ALPERT, K. B., GRANDILLO, S. AND TANKSLEY, S. D., 1995, fw 2.2: a major QTL controlling fruit weight is common to both red- and green-fruited tomato species. *Theor. Appl. Genet.,* **91**: 994-1000.

ALI, M. S., NAKANO, K. AND MAEZAWA, S., 2004, Combined effect of heat treatment and modified atmosphere packaging on the color development of cherry tomato. *Postharvest Biol. Tech*., **34**: 113-116.

ANDERSON, J., CHURCHILL, G., AUTRIQUE, J., TANKSLEY, S. AND SORRELLS, M., 1993, Optimizing parental selection for genetic linkage maps. *Genome,* **36**: 181-186.

ANONYMOUS, 2015, http://dictionary.sensagent.com/postharvest/en-en/

ARUNA, S., 1992, Genetic variability in certain exotic collection of tomato (*Lycopersicon esculentum* Mill.). *M.Sc.* (*Agri*) *Thesis,* Tamil Nadu Agricultural University, Coimbatore.

BADSHAH, N., MUHAMMAD, S., QAIM, M. AND AYAZ, S., 1997, Shelf life study in tomato storage with different packaging materials. *J. Agril*., **13**(4): 347-350.

BARONE, A., ERCOLANO, M. R., LANGELLA, R., MONTI, L. AND FRUSCIANTE, L., 2005, Molecular marker assisted selection for pyramiding resistance genes in tomato. *Adv. Hort. Sci.,* **19**: 147-152.

BARRERO, L. S. AND TANKSLEY, S. D., 2004, Evaluating the genetic basis of multiple locule fruit in a broad cross section of tomato cultivars. *Theor. Appl. Genet.,* **109**: 669-679.

BARRY, C. S., BLUME, B., BOUZAYEN, M., COOPER, W., HAMILTON, A. J. AND GRIERSON, D., 1996, Differential expression of the 1-aminocyclopropane 1-carboxylate oxidase gene family of tomato. *Plant J*., **9**: 525-535.

BARRY, C. S. AND GIOVANNONI, J. J., 2008, Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. *Proc. Natl. Acad. Sci*., USA, **103**: 7923-7928.

BERNACCHI, D. AND TANKSLEY, S. D., 1997, An interspecific backcross of *L. esculentum* × *L. hirsutum*: linkage analysis and a QTL study of sexual compatibility factors and floral traits. *Genetics*, **147**: 861-877.

BHATTARAI, D. R. AND GAUTAM, D. M., 2006, Effect of harvesting method and calcium on postharvest physiology of tomato. *Plant Sci*., **7**: 37-41.

BIRD, C. R., SMITH, C. J. S., RAY, J. A., MOUREAU, P., BEVAN, M. J., BIRDS, A. S., HUGHES, S., MORRIS, P. C., GRIERSON, D. AND SCHUCH, W.,1988, The tomato polygalacturonase gene and ripening specific expression in transgenic plants. *Plant Mol. Biol*., **l**: 651-662.

BRUMMELL, D. A., 2006, Cell wall disassembly in ripening fruit. *Funct. Plant Biol*., **33**: 103-119.

BRUMMELL, D. A. AND HARPSTER, M. H., 2001, Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Mol. Biol*., **47**: 311-340.

BOUCHEZ, A., HOSPITAL, F., CAUSSE, M., GALLAIS, A. AND CHARCOSSET, A., 2002, Marker assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. *Genet*., **162**: 1945-1959.

CARA, B. AND GIOVANNONI, J. J. Molecular biology of ethylene during tomato fruit development and maturation. 2008, *Plant Sci*., **175**: 106-113.

CAUSSE, M., DUFFEÉ, P., GOMEZ, M.C., BURET, M., DAMIDAUX, R., ZAMIR, D., GUR, A., CHEVALIER, C., LEMAIRE-CHAMLEY, M. AND ROTHAN, C., 2004, A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. *J. Exp. Bot*., **55**: 1673-1685.

CAUSSE, M., FRIGUET, C., COIRET, C., LE'PICIER, M., NAVEZ, B., LEE, M., HOLTHUYSEN, N., SINESIO, F., MONETA, E. AND GRANDILLO, S., 2010, Consumer preferences for fresh tomato at the European scale: a common segmentation on taste and firmness. *J. Food Sci*., **75**(9): 53-541.

CAUSSE, M., SALIBA-COLOMBANI, V., LECOMTE, L., DUFFÉ, P., ROUSSELLE, P. AND BURET, M., 2002, QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. *J. Exp. Bot*., **53**: 2089-2098.

CHAIB, J., LECOMTE, L., BURET, M. AND CAUSSE, M., 2006, Stability over genetic backgrounds, generations and years of quantitative trait locus (QTLs) for organoleptic quality in tomato. *Theor. Appl. Genet.,* **112**: 934-944.

CHOO, T. M., AND REINBERGS, E., 1982, Analysis of skewness and kurtosis for detecting gene interaction in a double haploid population, *Crop. Sci.,* **22**: 231-235.

COLLARD, B. C. Y., JAHUFER, M. Z. Z., BROUWER, J. B. AND PANG, E.C.K., 2005, An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, **142**: 169-196.

COLLARD, B. C. Y., PANG, E. C. K., AND TAYLOR, P. W. J., 2003, Selection of wild *Cicer* accessions for the generation of mapping populations segregating for resistance to ascochyta blight. *Euphytica*, **130**: 1-9.

COX, S., 2000, I Say Tomayto, You Say Tomahto. http://lamar.colostate.edu/ ~samcox/Tomato.html.

DARWIN, S.C., KNAPP, S. AND PERALTA, I. E., 2003, Taxonomy of tomatoes in the Galapagos Islands: native and introduced species of *Solanum lycopersicum* (Solanaceae). *Syst. Bio. Diver*., **1**: 29-53.

DIAS, T. J. M., MALUF, W.R., FARIA, M. V., DE FREITAS, J. A., AUGUSTO GOMES, L. A., RESENDE, J. T. V. AND DE AZEVEDO, S. M., 2003, *Alcobaça* allele and genotypic backgrounds affect yield and fruit shelf life of tomato hybrids. *Sci. agric.*, **60**: 269-275.

DHATT, A. S., 2001, Evaluation of F_1 hybrids incorporating *nor, rin* and *alc* alleles for yield, quality and shelf life of tomato (*Lycopersicon esculentum* Mill.). Ph. D. Thesis, Punjab Agricultural University, Ludhiana, India.

EDWARDS, M. D., STUBBER, C. W. AND WENDEL, J. F., 1987, Molecular marker facilitated investigations of quantitative trait loci in Maize, genomic distributions and type of gene action. *Genetics*, **116**: 113-125.

ESHED, Y. AND ZAMIR, D., 1994, An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield associated QTLs. *Genetics*, **141**: 1147-1162.

FAOSTAT., 2013, Statistical data bases. Food and Agricultural Organization (FAO) of United Nations, Rome.

FARIA, M.V., MALUF W.R., AZEVEDO S. M., ANDRADE, V.C., GOMES, L.A.A., MORETTO, P., LICURSI, V., 2003, Yield and postharvest quality of tomato hybrids heterozygous at the loci alcobaca, old gold-crimson or high pigment. *Genet. Mol. Res*., **2**: 317-327.

FISHER, F. A., IMMER, F. R. AND TEDIN, O., 1932, The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. *Genetics*, **17**: 107-124.

FOOLAND, M. R., 2007, Genome Mapping and Molecular Breeding of Tomato. *Int. J. Plant Genomics.* **125**: 52-92.

FRARY, A., FULTON, T. M., ZAMIR, D. AND TANKSLEY, S. D., 2004, Advance backcross QTL analysis of a *Lycopersicon esculentum* × *L. pennellii* cross and identification of possible orthologs in the Solanaceae. *Theor. Appl. Genet*., **108**: 485-496.

FRARY, A., XU, Y., LIU, J., MITCHELL, S., TEDESCHI, E. AND TANKSLEY, S., 2005, Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. *Theor. Appl. Genet*., **111**: 291-312.

FREITAS, J. A., MALUF, W. R., GOMES, L. A. A., AZEVEDO, S. M., 1996, Effects of the alleles *alc*, *ogc* and *hp* on ripening and postharvest shelf life characteristics of tomato fruits. *Cienciai Agrotecnologia*, **23**: 569-577.

FULTON, T.M., BUCHELI, P., VOIROL, E., LÓPEZ, J., PÉTIARD, V. AND TANKSLEY, S.D., 2002,Quatitative trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly contributing to flavour, identified in four advanced backcross populations of tomato. *Euphytica*, **127**: 163-177.

GABRIEL, O. S., GUSTAVO, D. T. AND ADELA, A. F., 1999, Controlled atmosphere storage of tomato fruit; low oxygen or elevated carbon dioxide levels alter galactosidase activity and inhibit exogenous ethylene action. *J. Sci. Food Agric*., **79**: 1065-1070.

GALTON, F., 1989, Storage temperature and fruit calcium alter the sequence of ripening events of pears. *Hort. Science*, **34**: 316-318.

GEESON, J. D., BROWNE, K. M., MADDISON, K., SHEPHERD, J. AND GUARALDI, F., 1985, Modified atmosphere packaging to extend the shelf life of tomatoes. *J. Food Technol.,* **20**: 339-349.

GEETHANJALI, S., CHEN, K. Y., PASTRANA, D. V. AND WANG, J. F., 2010, Development and characterization of tomato SSR markers from genomic sequences of anchored BAC clones on chromosome 6, *Euphytica*, **173**: 85-97.

GHOSH, K. P., ISLAM, A. K. M. A., MIAN, M. A. K. AND HOSSAIN, M M., 2010, Variability and character association in F_2 segregating population of different commercial hybrids of Tomato (*Solanum lycopersicum* L.). *J. Appl. Sci. Environ. Manage.,* **14**(2):91-95.

GIOVANNONI, J. J., TANKSLEY, S. D., VREBALOV, J. AND NOENSIE, E., 2008, *NOR* gene for use in manipulation of fruit quality and ethylene response. *US Patent No.* **5:** 234-834.

GIOVANNONI, J. J. 2006, Genetic regulation of fruit development and ripening. *Society*. **16**: 170-180.

GONZALO, M. J. AND KNAP, E. V., 2008, A comparative analysis into the genetic bases of morphology in tomato varieties exhibiting elongated fruit shape. *Theor. Appl. Genet.,* **116**: 647-656.

GRAY, J., PICTON, S., GIOVANNONI, J. J. AND GRIERSON, D., 1994, The use of transgenic and naturally occurring mutants to understand and manipulate tomato fruit ripening. *Plant Cell Environ.*, **17**: 557-57.

GUPTA, P., VARSHNEY, R., SHARMA, P. AND RAMESH, B., 1999, Molecular markers and their applications in wheat breeding. *Plant Breed.,* **118**: 369-390.

HALEY, C. S. AND KNOTT, S. A., 1992, A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity,* **69**: 315-324.

HALL, A., 1996, Liarozole amplifies retinoid-induced apoptosis in human prostate cancer cells. *J. Natl. Cancer Inst.*, **7**: 12-20.

HANDA, A.K. AND MATTOO, A. K., 2010, Differential and functional interactions emphasize the multiple roles of polyamines in plants. *Plant Physiol.Biochem.*, **48**: 540-546.

HAMILTON, C. M., FRARY, A., XU, Y., TANKSLEY, S. D. AND ZHANG, H. B., 1999, Construction of tomato genomic DNA libraries in a binary BAC (BIBAC) vector. *Plant J*., **18**(2): 223-229.

HE, C., POYSA, V. AND YU, K., 2003, Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars, *Theor. Appl. Genet*., **106**: 363-373.

HEISER, C. AND ANDERSON, G., 1999, New Solanums. IN: JANICK, J. (ED) Perspectives on New Crops and New Uses. *ASHS Press*, Alexandria, Virginia, 379-384.

HONG, J. H. AND GROSS, K. C., 2001, Maintaining quality of fresh cut tomato slices through modified atmosphere packaging and low temperature storage. *J. Food Sci.*, **66**(7): 960-965.

HONMA, M. AND SHIMOMURA, T., 1978, Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric. Biol. Chem.*, **43**: 1825-1831.

IGNATOVA, S. I., GARANKO, I. B. AND BOTYAEVA, G. V., 1999, The genotype environment interaction with using genes *nor, rin* and *alc*. *Acta Hort.*, **487**: 367-372.

INDURANI, C., MUTHUVEL, I. AND VEERARAGAVATHAM, D., 2010, Correlation and Path coefficient for yield components and quality traits in tomato (*Lycopersicon esculentum* Mill.), *Agric. Sci. Digest.*, **30**(1): 11-14.

JOHNSON, H.W., ROBINSON, H.I. AND COMSTOCK, R.E., 1955, Estimation of genetic and environmental variability in soybean. *Agron. J.*, **47**:314-318.

JONES, N., OUGHAM, H. AND THOMAS, H., 1997, Markers and mapping: We are all geneticists now, *New Phytol.*, **137**: 165-177.

JOSHI, C. AND NGUYEN, H., 1993, RAPD (random amplified polymorphic DNA) analysis based inter-varietal genetic relationships among hexaploid wheat. *Plant Sci*., **93**: 95-103.

KADER, A. A., 2005, Increasing Food Availability by Reducing Postharvest Losses of Fresh Produce. *Acta. Hort.*, **682**: 2169-2175.

KIMBENG, C. A. AND BINGHAM, E. T., 1998, Population improvement in Lucerne (*Medicago sativa* L.): components of inbreeding depression are different in original and improved populations. *Aus. J. Exp. Agril.*, **38:** 831-836.

KINZER, S. M., SCHWAGER, S. J. AND MUTSCHLER, M. A., 1990, Mapping of ripening-related or specific cDNA clones of tomato (*Lycopersicon esculentum*). *Theor. Appl. Genet.*, **79**(4): 489-496.

KITAGAWA, M., ITO, H., SHINA, T., NAKAMURA, N., INAKUMA, T., KASUMI, T., ISHIGRO, Y., YABE, K. AND ITO, Y., 2005, Characterization of tomato fruit ripening and analysis of gene expression in F_1 hybrids of the ripening inhibitor (*rin*) mutant, *Plant physiol.*, **123**: 331-338.

KNAPP, S. AND DARWIN, S. C., 2007, Proposal to conserve the name *Solanum cheesmaniae* (*L. Riley*) Fosberg against *S. cheesmanii* Geras. (Solanaceae).*Taxon.*, **55**: 806-807.

KNAPP, S., 2002, Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. *J. Experimental Botany*, **53**: 2001-2022.

KOPELIOVITCH, E., MIZRAHI, Y., RABINOWITCH, H. D. AND KEDAR, N., 1980, Physiology of the tomato mutant *alcobaca*. *Physiol. Plant.,* **48**: 307-311.

KOTCH, G. P., ORTIZ, R., AND PELOQUIM, S. J., 1992, Genetic analysis by use of potato haploid populations. *Genome,* **35**: 103-108.

LANAHAN, M. B., YEN, H. C., GIOVANNONI, J. J., KLEE, H. J., 1994, The Never ripe mutation blocks ethylene perception in tomato. *Plant Cell,* **6**: 521-530.

LANDER, E. S., GREEN, P., ABRAHAMSON, J., BARLOW, A., DALY, M. J., LINCOLN, S. E. AND NEWBURG, L., 1987, MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics,* **1**:174-181.

LEAL, N. R., 1973, Heranca da conservacao natural poscolheita de frutos do tomateiro (*Lycopersicon esculentum* MILL). Conservacao de frutos e anatomia do pericarpo de hibridos entre aintroducaoalcobaca or algunscultivares. *M. Sc.Thesis, Vicosa University, Minas Gerais,* Brazil.

LEAL, N. R. AND TABIM, M. E., 1974, Testes de conservacao natural poscolheitaalem dos 300 dias de frutos de algunscultivares de tomateiro *(Lycopersicon esculentum* MILL). E. hibridosdestes corn *alcobaca*. *Rev. Ceres*., **21**: 310-328.

LECOMTE, L., SALIBA-COLOMBANI, V., GAUTIER, A., GOMEZ-JIMENEZ, M. C., DUFFÉ, P., BURET, M. AND CAUSSE, M., 2004, Fine mapping of QTLs of chromosome 2 affecting the fruit architecture and composition of tomato. *Mol. Breed.,* **1**: 1-14.

LINNAEUS, C., 1753, Species Plantarum, 1st ed. Stockholm: *L. salvius*.

LUSH, J. L., 1949, Intrasire correlation and regression of offspring on dams as a method of estimating heritability of characters. *Proc. Am. Soc. Anim. Prod.,* **33**: 293-301.

MA, Z. Q., RODER, M. AND SORRELLES, M. S., 1996, Frequency and sequence characteristics of di, tri and tetra-nucleotide microsatellites in wheat. *Genome*, **39**: 123-130.

MATAS, A. J., GAPPER, N. E., CHUNG, MI-Y., GIOVANNONI, J. J. AND ROSE, J. K. C., 2009, Biology and genetic engineering of fruit maturation for enhanced quality and shelf-life. *Curr. Opin. Biotechnol*., **20**: 197-203.

Mc BRIDE, J.F., 1962, Solanaceae, in Flora of Peru. *Field Mus. Nat. Hist. Ser*., **13**: 3-267.

Mc COUCH, S. R., AND DOERGE, R. W., 1995, QTL mapping in rice. *Trends Genet*., **11**: 482-487.

MILLER AND RICE-EVANS, 1997, Antioxidant properties of phenolic compounds. *Trends* plant sci., **2**(4): 152-159.

MOHANTY, B.K., 2003, Genetic Variability, Correlation and Path Coefficient Studies in Tomato. *Indian J. Agric. Res.,* **37**(1): 68-71.

MOORE, S., VREBALOV, J., PAYTON, P. AND GIOVANNONI, J., 2005, Use of genomics tools to isolate key ripening genes and analyse fruit maturation in tomato. *J. Exp. Bot.,* **53**: 2023-2030.

MUKESHKUMAR, SINGH, P., SINGH, N., SINGH, L. AND PRASAD, R. N., 2007, Studies on Quality Traits of Open Pollinated Varieties and Hybrids of Tomato Responsible for Their Shelf Life at Ambient Conditions. *Ind. J. Agric. Biochem.,* **20**(1): 17-22.

MULLER, C. H., 1940, A Revision of the Genus *Lycopersicum*. USDA Miscellaneous Publication N0.382, USDA, Washington, DC.

MUTSCHLER, M. A., WOLFE, D. W., COBB, E.D. AND YOURSTONE, K.S., 1992, Tomato fruit quality and shelf life in hybrids heterozygous for the *alc* ripening mutant. *Hort. Sci.,* **27**: 352-355.

NAGETEY, F. K., MAO, L. C. AND MAO, L. C., 1999, Hot water immersion alleviates chilling injury in tomato fruit. *Acta. Horti.*, **11**(2): 67-72.

NAIK, D. M., MULEKAR, V. G., CHANDEL, C. G. AND KAPSE, B. M., 1993, Effect of prepackaging on physico-chemical changes in tomato (*Lycopersicon esculentum* Mill.) during storage. Indian Food Packer, July-August, **pp**. 9-13.

NASRIN, T. A. A., MOLLA, M. M., ALAMGIR, M., HOSSAEN, ALAM, M. S. AND YASMIN, L., 2008, Effect of Postharvest Treatments on Shelf Life and Quality of Tomato. *Bangladesh, J. Agril. Res.,* **33**(3): 579-585.

NAVEEN, G., DEVINDER, S. C. AND AJMER, S. D., 2008, Genetics of yield, quality and shelf life characteristics in tomato under normal and late planting conditions. *Euphytica*, **159**: 275-288.

NESBITT, T. C., TANKSLEY, S. D., 2002, Comparative sequencing in the genus *Lycopersicon*: implication for the evolution of fruit size in the domestication of cultivated tomatoes. *Genetics,* **162**: 365-379.

OELLER, P. W., WONG, L. M., TAYLOR, L. P., PIKE, D. A. AND THEOLOGIS, A., 1991, Reversible inhibition of tomato fruit senescence by antisense RNA. *Science*, **254**: 437-439.

OHYAMA, A., ASAMIZU, E., NEGORO, S., MIYATAKE, K., YAMAGUCHI, H., TABATA, S. AND FUKUOKA, H., 2009, Characterization of tomato SSR markers developed using BAC-end and cDNA sequences from genome databases. *Mol. Breeding*, **23**: 685-691.

ONWUZULU, O. C., PRABHA, T. N. AND RANGANNA, S., 1995, Modified atmosphere storage of ripening tomatoes: effect on quality and metabolism of 14C-glucose and 14C acetate. *Tropical Science*, **35**: 251-258.

PANSE, V. G. AND SUKATHME, P. V., 1964, Statistical methods for agricultural workers. *ICAR*, NewDelhi, 145 pp.

PATERSON, A. H., 1996, Making genetic maps. Genome Mapping in Plants, R. G. Landes Company, San Diego, California; *Academic Press*, Austin, Texas, pp. 23-39.

PERALTA, I. E. and SPOONER, D. M., 2005, Morphological characterization and relationships of wild tomatoes (*Solanum*L. Section *Lycopersicon*). In: Keating RC, Hollowell VC, Croat T (eds) Festschrift for William G. Darcy: the legacy of a taxonomist (Monograph in Systematic Botany 104). *MBG Press*, Missouri, USA, pp. 227-257.

PERALTA, I. E. AND SPOONER, D. M., 2007, History, origin and cultivation of tomato. IN: RAZDAN MK, MATTOO AK (EDS) Genetic improvement of Solanaceous crops. *Sci. Publ. Enfield.,* **2**: 1-24.

PEREIRA DA COSTA, B., GUSTAVO RUBÉN RODRÍGUEZ, GUILLERMO RAÚL, PRATTA, LILIANA AMELIA PICARDI, AND ROXANA ZORZOLI, 2013, QTL detection for fruit shelf life and quality traits across segregating populations of tomato. *Scientia Horti.*, **156:** 47-53.

PEREIRA DA COSTA, J. H., MARTÍNEZ, V. A., RODRÍGUEZ, G. R., PRATTA, G. R. AND ZORZOLI, R., (2009), Influence of exotic genes on shelf life and weight of Tomato Fruit. *Agri. Scientia*, **16**(1): 7-13.

PINHERO, R. G., ALMQUIST, K. C., NOVOTNA, Z., AND PALIYATH, G., 2003, Developmental regulation of phospholipase D in tomato fruits. *Plant Physiol. Biochem.*, **41**: 223-240.

POONI, H. S., JINKS, J. L., AND CORNISH, M. A., 1977, The causes and consequences of non-normality in pretending the properties of recombinant inbred lines. *Heredity,* **38**: 329-338.

POWELL, A. L., NGUYEN, C. V., HILL, T., CHENG, K. L., FIGUEROA-BALDERAS, R., AKTAS, H., ASHRAFI, H., PONS, C., FERNÁNDEZ-MUNOZ, R., VICENTE, A., LOPEZ-BALTAZAR, J., BARRY, C. S., LIU, Y., CHETELAT, R., GRANELL, A., VAN DEYNZE, A., GIOVANNONI, J. J. AND BENNETT, A. B., 2012, Uniform ripening encodes a golden 2-like transcription factor regulating tomato fruit chloroplast development. Science, **336**: 1711-1715.

PRATTA, G. R., RODRIGUEZ, R. G., ZORZOLI, R., VALLE, M. E., AND PICARDI, A. L., 2011, Phenotypic and molecular characterization of selected tomato recombinant inbred lines derived from the cross *Solanum lycopersicum* × *S. pimpinellifolium.* **90**(2): 232-237.

PREMALAKSHMI, V., 2001, Breeding for yield and postharvest qualities in tomato (*Lycopersicon esculentum* Mill.). *Ph.D. Thesis*, Tamil Nadu Agricultural University, Coimbatore.

RAMANUJAM, S. AND TIRUMALACHAR, D. K., 1967, Genetic variability of certain characters in red pepper (*C. annum* L.). *Mysore J. Agric. Sci.,* **1**: 32-36.

RANGANNA, S., 1976, *In: manual of analysis of fruits and vegetable products*, McGraw hill, New Delhi, pp. 77.

REDENBAUGH, K., HIATT, W., MARTINEAU, B., KRAMER, M. AND SHEEHY, R., 1992, Safety Assessment of Genetically Engineered Fruits and Vegetables: A Case Study of the FLAVR SAVR Tomato. Boca Raton, FL: CRC Press, pp. 267.

RICK, C. M., 1976, Tomato *Lycopersicon esculentum* (*Solanaceae*). IN: SIMMONDS NW (ED.) Evolution of crop plants. *Longman*, London: 268-273.

RICK, C. M., 1991, Tomato Paste: A Concentrated Review of Genetic Highlights from the Beginnings to the Advent of Molecular Genetics. *Genetics*, **128**: 1-5.

ROBSON, D. S., 1956, Application of K4 statistics to genetic variance component analysis. *Biometrics*, **12**: 433-444.

ROBINSON, H. F., COMSTOCK, R. E. AND HARVEY, V. H., 1949, Estimates of heritability and degree of dominance in corn. *Agron. J.*, **41**: 353 - 359.

RODRÍGUEZ, G. R., PRATTA, G. R., LIBERATTI, D. R., ZORZOLI, R. AND PICARDI, L. A., 2011, Inheritance of shelf life and other quality traits of tomato fruit estimated from F_1's, F_2's and backcross generations derived from standard cultivar, nor homozygote and wild cherry tomato. *Euphytica*, **176**(1): 137-147.

ROSE, J. K. C., CATALA, C., GONZALEZ-CARRANZA, C. Z. H. AND ROBERTS, J. A., 2003, Plant cell wall disassembly in rose *The Plant Cell Wall*, **8**: 264-324.

ROY, D., 2000, Plant breeding: The Analysis and exploitation of variability. PB: Narosa Publishing House. New Delhi. India, pp. 198.

SACKS, E. J. AND St. CLAIR, D. A., 1996, Cryogenic storage of tomato pollen: Effect on fecundity. *Hort. Sci.,* **31:** 447-448.

SACCO, A., BJØRNSTAD, A. AND NDJIONDJOP, M. N., 2012, An overview of molecular marker methods for plants. *Afr. J. Biotechnol.,* **25**: 2540-2568.

SAGHI-MAROOF, M. A., SOLIMAN, R. A., JORGENSEN, S. AND ALLARD, R. W., 1984, Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci..,* **81**: 8014-8018.

SAYED, H., KAYYAL, H., RAMSEY, L., CECCARELLI, S. AND BAUM, M., 2002, Segregation distortion in doubled haploid lines of barley (*Hordeum vulgare* L.) detected by simple sequence repeat markers. *Euphytica,* **225**: 265-272.

SHANKAR, A., REDDY, R., SUJATHA, M. AND PRATAP, M., 2013, Genetic variability studies in F_1 generation of tomato (*Solanum lycopersicon* L.). *Int. J. Curr. Microbiol. App. Sci.,* **4**(5): 31-35.

SHAPIRO, S. S., WILK, M. B. AND CHEN, H. J., 1968, A comparative study of various tests for normality. *J. Am. Statistical Assoc*., **63**: 1343-1372.

SHIRASAWA, K., ASAMIZU, E., FUKUOKA, H., OHYAMA, A., SATO, S., NAKAMURA, Y., TABATA, S., SASAMOTO, S., WADA, T., KISHIDA, Y., TSURUOKA, H., FUJISHIRO, T., YAMADA, M. AND ISOBE, S., 2010, An interspecific linkage map of SSR and intronic polymorphism markers in tomato. *Theor. Appl. Genet.,* **121**: 731-739.

SIMS, W. L., 1980, History of tomato production for industry around the world, *Acta Hort*., **100**: 25.

SINGH, A. K., 2005, Genetic variability, correlation and path coefficient studies in tomato under cold arid region of Ladkh. *Prog. Hortic.,* **37**: 437-443.

SINGH, S., SIDHU, J. S., HUANG, N., VIKAL, Y., LI, Z., BRAR, D. S., DHALIWAL, H. S. AND KHUSH, G. S., 2001, Pyramiding three bacterial blight resistance genes (xa 5, xa 13 and Xa 21) using marker-assisted selection into indica rice cultivar PR106. *Theor. Appl. Genet.,* **102**: 1011-1015.

SMITH AND ANDREW, F., 1994, The tomato in America: early history, culture, and cookery. Columbia, USA: University of South Carolina Press.

SMITH, D. L., ABBOTT, J. A., AND GROSS, K. C., 2002, Down-regulation of tomato beta-galactosidase 4 results in decreased fruit softening. *Plant Physiol.,* **129**: 1755-1762.

SNEDECOR, G. 1964, Statistical Methods, fifth ed. Editorial companion, Mexico D.F. 631.

SOUZA, A., 1999, Segregation distortion in doubled haploid lines of barley (*Hordeum vulgare* L.) detected by simple sequence repeat markers. *Euphytica.,* **225**: 265-272.

STEARNS J. C. AND GLICK B. R., 2003, Transgenic plants with altered ethylene biosynthesis or perception. *Biotechnol. Adv.,* **21**: 193-210.

STEVENS, M. A. AND RICK, C. M., 1986, Genetics and breeding. In: Atherton, J.G., Rudich, J.C. (Eds.), The Tomato Crop. Chapman and Hall, New York, pp. 35-100.

TANKSLEY, S. D., 2004, Enzyme-coding genes in tomato (*Lycopersicon esculentum*). *Isozyme Bulletin,* **18**: 43- 45.

TAYLOR, I. B., 1986, Biosystematics of the tomato.IN: ANTHERTON, J. AND RUDICH, G., The Tomato Crop. A Scientific Basis for Improvement. Chapman & Hall, Newyork, 1-34.

THAKUR, A. K., SINGH, A. AND PANDEY, M., 2000, Inhibition of respiration, ethylene synthesis and cell wall softening enzyme activity in tomato fruit during ripening by ethanol. *Adv. Hortic. Sci.*, **14**:176-181.

TIEMAN, D. M. AND HANDA, A. K., 1994, Reduction in pectin methylesterase activity modifies tissue integrity and cation levels in ripening tomato (*Lycopersicon esculentum* Mill.) fruits. *Plant Physiol*., **106**: 429-436.

TIEMAN, D. M., HARRIMAN, R. W., RAMAMOHAN, G., AND HANDA, A. K., 1992, An antisense pectinmethylesterase gene alters pectin chemistry and soluble solids in tomato fruit. *Plant Cell,* **4**: 667-679.

VIJAYKUMAR, S. M., GHOSH, S., PRABHA, T. N., CHAKRABORTY, N., CHAKRABORTY, S., AND DATTA, A., 2010, Enhancement of fruit shelf life by suppressing N-glycan processing enzymes, *Proc. Natl. Acad. Sci.*, **107**(6): 2413-2418.

VILLALTA, I., REINA-SA NCHEZ, A., CUARTERO, J., CARBONELL, E. A., and ASINS, M. J., 2005, Comparative microsatellite linkage analysis and genetic structure of two populations of F_6 lines derived from *Lycopersicon pimpinellifolium* and *L. cheesmanii*, *Theor. Appl. Genet.*, **110**: 881-894.

VREBALOV, J., RUEZINSKY, D., PADMANABHAN, V., WHITE, R., MEDRANO, D., DRAKE, R., SCHUCH, W., AND GIOVANNONI, J., 2002, A MADS-box gene necessary for fruit ripening at the tomato ripening inhibitor (*rin*) locus. *Science*, **296:** 343-346.

WANG, T. W., ZHANG, C. G., WU, W., NOWACK, C. M., MADEY, E. AND THOMPSON, J. E., 2005, Antisense Suppression of Deoxyhypusine Synthase in Tomato Delays Fruit Softening and Alters Growth and Development. *Plant Physiol*., **138**(3): 1372-138.

WANG, Z., WEBER, J. L., ZHONG, Z. AND TANKSLEY, S. D., 1994, Survey of plant short tandem DNA repeats. *Theor. Appl. Genet.*, **88**: 1-12.

WANG, S. Y. AND LIN, H. S., 2003, Compost as a soil supplement increases the level of antioxidant compounds and oxygen radical absorbance capacity in strawberries. *J. Agric. Food Chem.*, **51**: 6844-6850.

WEBER, C. R. AND MOORTHY, B. R., 1952, Heritability and non-heritability relationships and variability of oil content and agronomic characters in the F_2 generation of soybean crosses. *Agron. J*., **44**: 202-209.

WELLER, J. I., SOLLER, M. AND BRODY, T., 1988, Linkage analysis of quantitative trait in an intraspecific cross of tomato (*Lycopersicon esculentum* × *Lycopersicon pimpinellifolium*) by means of genetic markers. *Genetics*, **118**: 329-339.

WILKINSON, J. Q., LANAHAN, M. B., YEN, H. C., GIOVANNONI, J. J., AND KLEE, H. J., 1995, An ethylene inducible component of signal transduction encoded by Never-ripe. *Science,* **270:** 1807-1809.

WINTER, P. AND KAHL, G., 1995, Molecular marker technologies for plant improvement, *World. J. Microb. Biotechnol.*, **11**: 438-448.

www. Indiastat.Com. 2015.

XU, Y., ZHU, L., XIAO, J., HUANG, N. AND Mc COUCH, S. R., 1997, Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). *Mol. Gen. Genet.*, **253**: 535-545.

YAHIA, E. M., SOTO, G., BRECHT, J., GARDEA, A. AND STETA, M., 2003, The effect of hot air treatments in air or in low oxygen atmosphere on the quality and antioxidants of tomato fruit. *Acta Hort.,* **604**(1): 285-291.

YATES, H. E., FRARY, A., DOGANLAR, S., FRAMPTON, A., NANCY, T., EANNETTA, N. T., UHLIG, J. AND TANKSLEY, S. D., 1965, Comparative fine mapping of fruit quality QTLs on chromosome 4 introgressions derived from two wild tomato species. *Euphytica*, **135**: 283-296.

YOGENDRA, K. N. AND GOWDA, P. H., 2013, Phenotypic and molecular characterization of a tomato (*Solanum lycopersicum* L.) F_2 population segregation for improving shelf life. *Genet. Mol. Res*., **12(**1): 506-518.

YOUNG, N. D., 1994, Constructing a plant genetic linkage map with DNA markers, In: I. K.V. Ronald & L. Phillips (Eds.), DNA-based markers in plants. Kluwer, Dordrecht/Boston/London. **Pp**. 39-57.

YU, L. X. AND NGUYEN, H., 2000, Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.), *Theor. Appl. Genet*., **87**: 668-672.

YUN, S., GYENIS, Y. L., HAYES, P. M., MATUS, I., SMITH, K. P. AND MUEHLBAUER, B. J., 2006, Quantitative loci for multiple disease resistance in wild Barley. *Crop Sci*., **45**: 2563-2572.

ZHONG, A. S., YAO, Q. H., PENG, R. H. AND LI, X., 2013, Different effects on ACC oxidase gene silencing triggered by RNA interference in transgenic tomato. *Plant Cell Rep.,* **23**: 639-646.

ZARZOLI, R., PRATTA, G. AND PICARDI, L. A., 2000, Variabili dadpara la vidapost cosecha y el peso de los frutos en tomate parafamilias F_3 de unhíbrido interespecífico. *Pesq. Agrop. Bras*, **35**: 2423-2427.

ZURIAGA, E., BLANCA, J. M., CORDERO, L., SIFRES, A., BLAS CERDAN, W. G. AND MORALES, R., 2009, Genetic and bioclimatic variation in *Solanum pimpinellifolium*. *Genet. Res. Crop Evol.* **56**: 39-51.

APPENDIX-I

The simple sequence repeat markers, their flanking primer sequences, chromosome number and expected fragment sizes of the PCR products

Primers	Sequence (5'-3')	Chromosome number	Expected band size (bp)
SSR20	F:GAGGACGACAACAACAACGA	12	130
	R:GACATGCCACTTAGATCCACAA		
SSR22	F:GATCGGCAGTAGGTGCTCTC	3	210
	R:CAAGAAACACCCATATCCGC		
SSR26	F:CGCCTATCGATACCACCACT	2	190
	R:ATTGATCCGTTTGGTTCTGC		
SSR27	F:CCCAAATCAAGGTTTGTGGT	3	210
	R:TCAGATGCCACCACTCTCAG		
SSR32	F:TGGAAAGAAGCAGTAGCATTG	2	180
	R:CAACGAACATCCTCCGTTCT		
SSR34	F:TTCGGATAAAGCAATCCACC	10	190
	R:TCGATTGTGTACCAACGTCC		
SSR327	F:GTTTCTATAGCTGAAACTCAACCTG	8	130
	R:GGGTTCATCAAATCTACCATCA		
TGS293	F:TGCAGGTATGTCTCACACCA	10	192
	R:TTGCAAGAACACCTCCCTTT		
TGS450	F:CTCCAAATTGGGCAATAACA	10	230
	R:TTAGGAAGTTGCATTAGGCCA		
SSR45	F:TGTATCCTGGTGGACCAATG	7	260
	R:TCCAAGTATCAGGCACACCA		
SSR47	F:TCCTCAAGAAATGAAGCTCTGA	6	205
	R:CCTTGGAGATAACAACCACAA		
SSR51	F:CTACCCTGGTCTTGGTGGAA	1	100
	R:AAAGGATGCTCTAGCTTCTCCA		
SSR52	F:TGATGGCAGCATCGTAGAAG	7	200
	R:GGTGCGAAGGGATTTACAGA		
SSR596	F:CCACAAACAATTCCATCTCA	10	210
	R:GCTTCCGCCATACTGATACG		
SSR288	F:GGCAGGAGATTGGTTGCTTA	1	215
	R:TTCCTCCTGTTTCATGCATTC		
SSR66	F:TGCAACAACTGGATAGGTCG	2	190
	R:TGGATGAAACGGATGTTGAA		
SSR67	F:GCACGAGACCAAGCAGATTA	11	300
	R:GGGCCTTTCCTCCAGTAGAC		
SSR69	F:TTGGCTGGATTATTCCTGTTG	9	250
	R:GCATTTGATAGAAGGCCAGC		
SSR70	F:TTTAGGGTGTCTGTGGGTCC	9	100
	R:GGAGTGCGCAGAGGATAGAG		
SSR71	F:AAATGGCATGGAGAATGGAA	-	210
	R:CATCCACTGAGAGCCCAAAG		
SSR80	F:ATCCGTTAGCTATTGTGCCG	11	195
	R:TTGCCATGCACTTATCTTCG		
SSR96	F:GGGTTATCAATGATGCAATGG	2	210
	R:CCTTTATGTCAGCCGGTGTT		

APPENDIX-II

1. Cetyl trimethyl ammonium bromide (CTAB) extraction buffer (100 ml)
   CTAB 2 per cent (w/v)
   Tris HCl (pH 8.0) 100 mM
   Sodium Chloride 4 M
   EDTA 20 mM
   0.2 per cent β – Mercaptoethanol
   2 per cent Polyvinyl Poly Pyrrolidone (PVP)

2. TE Buffer – for dissolving DNA pellet.
   Tris EDTA (TE) buffer
   Tris HCl (pH 8.0) 10 mM
   EDTA (pH 8.0) 1 mM
   (This was dissolved and made up to 100 ml, autoclaved and stored at 4 °C).

3. 3X loading dye
   5 M NaOH 200 µl
   95 per cent formamide (v/v) 95 ml
   50 per cent bromophenol blue (w/v) 50 g
   0.5 per cent xylene cyanol (w/v) 50 mg
   (Dissolved in sterile double distilled water and made up the volume to 100 ml)

4. 10X Tris Borate EDTA buffer (TBE) – for PAGE
   Tris base 121 g
   Boric acid 51.30 g
   EDTA ($Na_2.2H_2O$) 3.70 g
   (Dissolved in 800 ml of sterile double distilled water and made up to 1000 ml)

5. PAGE gel 5 per cent
   Water 86.45 ml
   10X TBE 13 ml
   40 per cent acrylamide: bisacrylamide 16.25 ml
   TEMED 195 µl
   10 per cent APS 975 µl

6. Buffers for silver nitrate staining of PAGE gel
   Developer- 30 g Sodium sulphate, 3 ml (40 %) Formaldehyde – 1 litre
   Staining – 2.5 g silver nitrate, 3 ml (40 %) Formaldehyde - 1 litre
   Fixing buffer- 100 ml of commercial grade acetic acid – 1 litre