

**EVALUATION OF γ -RAY IRRADIATED SEED DERIVED PROGENIES AND
GERMPLASM FOR PLANT GROWTH, FRUIT QUALITY TRAITS,
BACTERIAL BLIGHT RESISTANCE AND ANALYSIS OF MARKER TRAIT
ASSOCIATION IN POMEGRANATE (*Punica granatum* L.)**

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JULY, 2017

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Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

**DOCTOR OF PHILOSOPHY
IN
MOLECULAR BIOLOGY AND BIOTECHNOLOGY**

By
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CERTIFICATE

This is to certify that the thesis entitled “**EVALUATION OF g-RAY IRRADIATED SEED DERIVED PROGENIES AND GERMPLASM FOR PLANT GROWTH, FRUIT QUALITY TRAITS, BACTERIAL BLIGHT RESISTANCE AND ANALYSIS OF MARKER TRAIT ASSOCIATION IN POMEGRANATE (*Punica granatum* L.)**” submitted by **Mr. DADEPEER A. PEERAJADE** for the degree of **DOCTOR OF PHILOSOPHY** in **MOLECULAR BIOLOGY AND BIOTECHNOLOGY** of College of Agriculture, University of Agricultural Sciences, Dharwad, is a record of research work carried out by him during the period of his 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.

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ACKNOWLEDGEMENT

First of all, my thanks to almighty god, the Al mighty, who helped me from the beginning to the end of this study.

When, I write this I am afraid if these words only make mode and no substance. I feel great at this juncture to recapitulate all my teachers, friends, near and dear ones. Hard indeed it is to reasonably evaluate the help, assistance and guidance received. But academic decency demands that I mention at least a few persons who stand out as near and dear.

*Indeed the words at my command are not adequate to convey the depth of my feelings and gratitude to my honorable major advisor **Dr. NARAYAN MOGER**, Professor, Department of Biotechnology and Chairman of my Advisory Committee, for his valuable guidance, sustained encouragement, critical observations and constructive suggestions. It was a privilege and luck for me to be associated with him.*

*My sincere thanks and heartfelt respect to **Dr. H. B. Patil**, Dean, College of Horticulture, UHS, Bagalkot and the Co- Chairman of my Advisory Committee for the stead-fast support, persistent encouragement, unceasing interest shown at every stage of my investigation and preparation of thesis. His efforts for providing me with the atmosphere and facilities that I needed for completing this work successfully, are better appreciated that can be expressed here.*

*I also owe my heartfelt thanks to the members of my advisory committee **Dr. H. L. NADAF**, Principal Scientist and Head, AICRP on Groundnut, UAS, Dharwad, **Dr. RAMESH BHAT**, Professor, Department of Biotechnology, UAS Dharwad, **Dr. M. M. JAMADAR**, Professor and Head, Department of Plant Pathology, AC, Bijapur, UAS, Dharwad and **Dr. PRASHANTHI S. K.**, Professor, Department of Biotechnology, UAS, Dharwad.*

I am profoundly thankful to Dr. I. S. Katageri, Professor and Head, Department of Biotechnology, UAS, Dharwad, Dr. P. U. Krishnaraj, Professor and Head, Department of Agricultural Microbiology, College of Agriculture, Vijayapura, UAS, Dharwad, and all my teachers, Dr. Sumangala Bhat, Dr. Spurthi N Nayak and Mr. K. M. Sumesh and all the teachers from other departments for valuable suggestion and inspiration during my course of study at UAS, Dharwad

*I also owe my gratitude to **Dr. Rajeeve Varshney**, Director, Center of Excellence in Genomics, ICRISAT, Hyderabad for his valuable suggestions and kind support during research.*

I am very much thankful to Horticulture Research and Extension Station, Vijayapur (Tidagundi), UHS, Bagalkot staff Dr. R. S. Jawadagi, Dr. A. M. Nadaf, Smt. Sunita M Patil, Dr. Raghvendra G., Dr. S. K. Pattanashetti, Dr. Jagadeesh R. C. and

Dr. D. R. Patil, for their constant encouragement, love and affection throughout my study period. I also thankful to lab and field assistants, Basavaraj, Shafeeq, Raju, Sunil, Mahantesh, Somu, Pradeep Krishna, Patil G. D., Patted A. M., for their timely help during laboratory and field work.

I would like to thank my Classmates Anil, Shrinvas, Meera and, Madhuri for their cooperation during academic tenure. I also thankful to seniors and juniors, Rajakumar B. K., Chidanand, Pasha, Mallikarjun and Sukruth, for their kind co-operation and timely help throughout the study.

This thesis would be incomplete if I do not reckon the sacrifices, love, affection and support of my family members father Shri. Ajeejapeer Peerajade, mother Mrs. Khairunbi Peerajade, father-in-law Janab Alhaj M. S. Mudhol, mother-in-law Alhaj Mrs. Haseena Begum Mudhol, My beloved wife Fouziya Zareen, daughters Aafiya Afsheen and Sayyeda Amna, brother Murtuja, Ismail, Rehman, Ameer, sister Sufiyan, brother-in-law Hashimpeer Makandar, Dr. Javeed Mulla & family, Mr. Aasif Sayyed and Family, Mr. Khalid and family and Dr. Sajid and Family, whose love and affection have no equivalent and are an everlasting source of inspiration to me.

With at most pleasure and sincerity, I thankful to acknowledge whole heartedly for the financial support given by University of Horticultural Sciences, Bagalokot, which lessened the burden on my parents.

*Last but not the least, I convey my thanks to **Mr. Arjun and Kalmesh (Arjun Computers, Dharwad)** for their patience, precise delivery and timely printing of this thesis.*

Finally, I take pleasure in thanking Mr. M. I. Kumbar for timely co-operation in preparing this thesis.

.....any omission of this brief acknowledgement does not indicate lack of gratitude.

DHARWAD

JULY, 2017

(DADEPEER A. PEERAJADE)

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LIST OF ABBREVIATIONS

%	Per cent
μl	Micro liter
μg	Microgram
@	At the rate of
°C	Degree Celsius
Anon.	Anonymous
bp	Base pair
CD	Critical difference
cm	Centimeter
cM	Centimorgan
CV	Coefficient of variation
DNA	Deoxyribo Nucleic Acid
e.g.	For example
<i>etc.</i>	Etcetera
Fig.	Figure
g	Gram
GAM	Genetic Advance as percent of mean
GCV	Genotypic coefficient of variation
GCV	Genotypic coefficient of variation
GLM	General Linear Model
h^2	Heritability (broad sense)
ha	Hectare
hr	Hour
<i>i.e.</i>	That is
kbp	Kilo base pair
LD	Linkage Disequilibrium
mm	Millimeter
MAF	Minor Allele Frequency
MAS	Marker Assisted Selection
Max	Maximum
Mbp	Mega base pair
mg	Milligram
min	Minutes
ml	Milliliter

Contd.....

MLM	Mixed Linear model
mM	Milimolar
MTA	Marker-Trait Association
ng	Nanogram
PCV	Phenotypic coefficient of variation
PDI	Per cent disease index
pM	Picomoles:
ppm	Parts per million
Q	Population Structure
Q-Q	Quantile-Quantile plot
QTL	Quantitative Trait Loci
r^2	Correlation coefficient
R^2	Linkage disequilibrium between two markers or Phenotypic variation explained by marker
RNA	Ribonucleic acid
rpm	Revolutions per minute
S.E.m.	Standard Error of mean
SMA	Single marker analysis
SNP	Single Nucleotide Polymorphism
SSR	Simple sequence repeats
TASSEL	Trait Association analysis by Evolution and Linkage
TBE	Tris Borate EDTA
U	Unit
UAS	University of Agricultural Sciences
viz.,	Namely
Vol.	Volume
<i>Xap</i>	<i>Xanthomonas axonopodis</i> pv. <i>punicae</i>

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1. INTRODUCTION

Pomegranate (*Punica granatum* L.), a favourite table fruit in tropical and sub-tropical regions of the world, belongs to family Punicaceae, having chromosome number $2n = 16$ and is an ancient fruit with rich history and believed to have originated from Iran, but extensively cultivated in Mediterranean region especially in Spain, Morocco, Egypt and Afghanistan. It is also grown in drier parts of Southeast Asia, Burma, China, Japan, USA, West Indies, Russia, Bulgaria, Tropical America, Southern Italy and India (Priya *et al.*, 2016). In India it is cultivated over an area of 131.00 thousand hectare with a production of 1,346.00 thousand MT (Anon., 2015). Amongst different states growing pomegranate, Maharashtra is the largest producer with a total area of 90 thousand hectare and production of 945.00 thousand MT followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. In Karnataka the area and production of pomegranate is 16.6 thousand hectare and 134.20 thousand MT, respectively (Anon., 2015). Area under pomegranate is increasing worldwide because of its hardy nature, wider adaptability, drought tolerance, higher yields, excellent keeping quality and remunerative prices in domestic as well as export markets.

India is the leading pomegranate producer, which contributes nearly 50 per cent of the world's production (Wayde *et al.*, 2015). It thrives well in dry tropics and subtropics and performs well even in soils of low fertility status and saline conditions. The fruit has a wide consumer preference for its attractive, juicy, sweet, acidic and refreshing arils. Pomegranate fruits are good source of carbohydrates and minerals such as calcium, iron and sulphur that possess pharmaceutical and therapeutic properties. It is rich in vitamin C and citric acid is the predominant organic acid in pomegranate (Malhotra *et al.*, 1983a). Glucose (5.46 %) and fructose (6.14 %) are the main sugars with no sucrose in fruits. Sweet varieties are mildly laxative, whereas sour types are good against inflammation of stomach and heartache. Flower buds are very useful in ayurveda for managing bronchitis. The stem bark and fruit rind is used to treat diarrhoea and indigestion (Anon., 1969) which contains number of alkaloids belonging to pyridine group. The bark is also used in tanning industry (Patil and Karle, 1990). Inherent constraints of pomegranate are non-availability of suitable varieties, prone to environmental vagaries, nutritional deficiencies, physiological disorders, glut in market,

post-harvest losses, improper storage, lack of marketing facilities and price fluctuations. Biotic constraints are pest and disease problems. Although, 25 to 30 per cent of total cost of production is spent on plant protection, yet the biotic constraints could not be managed effectively. Among the diseases infecting pomegranate, a bacterial disease popularly known as ‘bacterial blight’ caused by *Xanthomonas axonopodis* pv. *punicae*, which was once a minor disease has emerged as a major production constraint resulting in severe yield loss both in terms of quality and quantity in the recent years in India (Hingorani and Mehta, 1952; Chand and Kishun, 1993; Vauterin *et al.*, 1995; Benagi and Kumar, 2009 and Sharma *et al.*, 2010). Chand and Kishun (1991) reported the epidemics of bacterial blight of pomegranate causing 60 to 80 per cent losses at Indian Institute of Horticultural Research (IIHR) experimental plots. They isolated the causal organism and identified it as *Xanthomonas campestris* pv. *punicae* based on its pathological, cultural, biochemical and physiological features. Later on during 1995, Vauterin *et al.*, re-named the causal organism as *Xanthomonas axonopodis* pv. *punicae* depending on the presence or absence of metabolic activity on different carbon substrates. The disease continued to damage the crop for subsequent years and farmers have adopted all possible and available protection measures yet, the disease could not be managed effectively due to rapid build up of inoculum and eventually the boon of this commercial fruit crop turned as a big bane to the farmers. The disease accounted up to 70 – 100 per cent loss during 2006 in Karnataka and Maharashtra resulting in wipe out of pomegranate. During the year 2007, the total production of pomegranate in India was down by 60 per cent (Raghavan, 2007). Many growers finding no options to mitigate the disease effectively uprooted their crop due to unbearable losses. The disease has been observed as serious threat to the cultivation of recommended varieties of pomegranate. Of the several disease management strategies, varietal resistance is considered as the best alternative.

Genetic improvement of fruits is essential for increasing the productivity. The major problems with fruit breeding work are long life cycle and the large juvenile period has hampered fruit breeding work. Preliminary screening of available pomegranate germplasm revealed that all the accessions were susceptible to bacterial blight (Ashish and Arora, 2014; Katwal 2015; Priya *et al.*, 2016 and Jyoti, 2017) and not much variability is existing. Exploiting natural or induced genetic diversity is a

substantiated strategy in the improvement of all major food crops and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability (Parry *et al.*, 2009). Breeding strategies in pomegranate include seedling selection, hybridization and mutation breeding. In pomegranate, seed propagation is relatively straightforward, but seed-derived progenies represent a heterogeneous population (Jalilop, 2010). It is known that irradiation of seeds increases mutation frequency and in turn promotes gene recombination and widens the mutation spectrum (Larik *et al.*, 2009) and introduces considerable variability among many useful traits including plant size, blooming time and fruit ripening, fruit colour, self-thinning and resistance to pathogens (Jain, 2010).

Molecular markers have been used to estimate genetic diversity and examine genetic relationships that exist between cultivars in a range of horticultural crops. There are some reports using RAPD markers (Sarkhosh *et al.*, 2006 and Sarkhosh *et al.*, 2009), SSR markers (Curro *et al.*, 2010; Soriano *et al.*, 2011; Raina, 2013 and Singh *et al.*, 2015), RAPD and morphological markers (Zamani *et al.*, 2007) as well as AFLP markers (Yuan *et al.*, 2007 and Jbir *et al.*, 2008) to analyze the genotypic characteristics and genetic relationships of pomegranate cultivars. However, molecular markers allow a direct comparison of the effects of genotypes at the DNA level. The explorations of DNA markers like SSRs undertaken to identify the DNA polymorphism and proven to be very useful for genetic diversity studies in a wide range of plants due to their high polymorphism, abundance, their distribution in the whole genome and co-dominance (Gupta *et al.*, 2005). Furthermore, because of their high reproducibility and reliability these are the markers of choice for efficient and effective selection of desirable genotypes.

Marker-trait association (MTA) and association mapping plays an important role in plant marker assisted selection/quantitative trait loci (MAS/QTL) breeding programs, especially in plants that no other genetic information such as linkage map and quantitative trait loci are available (Ruan, 2010). These strategies utilise phenotypic and genotypic information to increase gain from selection and reduce selection cycle time and facilitate selection for low heritability traits. Pomegranate being an out crossing, molecular based selection along with the phenotype provides more efficient selection strategies. In this regard, the present study is more relevant in pomegranate to fulfil the

present and basic requirement of pomegranate improvement. Hence, an attempt has been made to characterize the pomegranate genotypes for both morphological and molecular studies with respect to plant growth, fruit quality traits, bacterial blight resistance and marker trait association analysis. With this background, the current study was taken up with the following objectives:

1. To isolate and assess the variability of the pathogen causing bacterial blight of pomegranate.
2. To evaluate γ - ray irradiated seed derived progenies and germplasm of pomegranate for plant growth, fruit quality traits and bacterial blight disease resistance.
3. Genotyping of γ - ray irradiated seed derived progenies and germplasm of pomegranate with SSR markers.
4. To ascertain association of markers with plant growth, fruit quality traits and bacterial blight disease resistance.

2. REVIEW OF LITERATURE

Pomegranate is a tropical fruit crop being grown in all parts of India and its consumption is driven by both fresh market and processing industry. It is crucial to acknowledge all fruit characteristics to not only classify varieties from a botanical point of view, but also to meet current market demand for quality fruits. However, the production of pomegranate decreased due to the biotic and abiotic stress factors. Among the biotic factors, bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae*, is a serious threat for pomegranate cultivation in terms of both quality and quantity.

In any successful varietal improvement programme the availability of adequate variability in the basic genetic stocks and their proper use through breeding for building up improved strains are imperative. The induction of variability for fruit quality traits, biotic and abiotic stresses is a prerequisite to isolate desirable types, as the natural variability is relatively low in pomegranate races. In this direction, mutation breeding has become a major tool in plant breeding to supplement existing germplasm variability and to add useful characteristics to the delicate system of genetic balance. Improved plant type with desirable morphological and quality traits, besides adaptation to diverse growing conditions has been the basis of the present day crop improvement programmes.

Since spontaneous mutations occur at very low frequency, induced mutations are rewarding to develop improved varieties at a swifter rate (Mirosław, 1990). First observation about artificial induction of genetic changes dates back to the beginning of the 20th century but proper proof of Mendelian inheritance of such induced changes came only by Muller (1927) and Stadler (1928) using x-rays. The use of induced mutations over the past 50 years played a major role in the development of superior crop varieties translating into a tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and millions of cultivated hectares.

Various biometrical and molecular techniques have been developed for studying the genetics of quantitative and qualitative traits. The information can be used for

developing more sophisticated and efficient approach to select the parents. The review of literature related to this particular study is presented under the following headings.

- 2.1 Importance of pomegranate as fruit crop
- 2.2 Origin and taxonomy of pomegranate
- 2.3 Bacterial blight disease and its resistance in pomegranate
- 2.4 Genetic variability and diversity for plant growth, fruit quality traits and bacterial blight disease resistance
- 2.5 Molecular characterization and diversity in pomegranate
- 2.6 Marker trait association (MTA) and association mapping for plant growth, fruit quality traits and bacterial blight disease resistance in pomegranate

2.1 Importance of pomegranate as fruit crop

Pomegranate (*Punica granatum* L., Punicaceae) is a small shrub with hard wood and stems. It belongs to family Punicaceae. Recent advancement of science has shown that, entire tree of pomegranate (fruit, flowers, bark and leaves) is rich with bioactive phytochemicals. The fruit has a wide consumer preference for its attractive, juicy, sweet, acidic and refreshing arils. Pomegranate fruits are good source of carbohydrates and minerals such as calcium, iron and sulphur. It possesses pharmaceutical and therapeutic properties also. It is rich in vitamin C. Citric acid is the predominant organic acid in pomegranate (Malhotra *et al.*, 1983a). Glucose (5.46 %) and fructose (6.14 %) are the main sugars with no sucrose in fruits. Sweet varieties are mildly laxative, whereas sour types are good against inflammation of stomach and heart ache. Flower buds are very useful in Ayurveda for managing bronchitis. The stem bark and fruit rind is used to treat diarrhoea and indigestion (Anon., 1969) which contains number of alkaloids belonging to pyridine group (Table 1). The bark is also used in tanning industry (Patil and Karle, 1990).

Table 1: Pomegranate fruit parts and their compounds

S. N.	Plant component	Chemical composition
1.	Pomegranate juice	Anthocyanins; glucose, ascorbic acid; ellagic acid, gallic acid, caffeic acid; catechin, EGCG (Epigallocatechin gallate); quercetin, rutin; numerous minerals, particularly iron and amino acids
2.	Pomegranate seed oil	95-per cent punicalic acid; other constituents, including ellagic acid; other fatty acids; sterols
3.	Pomegranate pericarp (peel, rind)	Phenolic punicalagins; gallic acid and other fatty acids; catechin, EGCG (Epigallocatechin gallate); quercetin, rutin, and other flavonols; flavones, flavonones anthocyanidins
4.	Pomegranate leaves	Tannins (punicalin and punicafolin); and flavones glycosides, including luteolin and apigenin
5.	Pomegranate flower	Gallic acid, ursolic acid; triterpenoids, including maslinic and asiatic acid; other unidentified constituents
6.	Pomegranate roots and bark	Ellagitannins, including punicalin and punicalagin; numerous piperidine alkaloids

Therefore, its demand in the market is increasing but the capability to fulfil the requirement is reducing due to depleting cultivating land area and frequent occurrence of diseases, which belongs to wide class of microbes such as bacteria and fungus. Among these, bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae* is widely spread and devastating pathogen in the crop, which reduces the crop yield by 60-80 per cent (Wayde *et al.*, 2015). The information available on this disease, pathogen and resistant variety is very meagre. Apart from this, a sound knowledge of genetic variability for different traits and their association with fruit quality traits helps the breeder to identify high yielding and resistant genotypes.

2.2 Origin distribution taxonomy and botany of pomegranate

Wild pomegranates are grown today in central Asia from Iran and Turkmenistan to India. Goor and Liberman (1956) defined southwest Asia as the pomegranate origin. Culture of pomegranate began in prehistoric times. It is estimated that pomegranate domestication began somewhere in the Neolithic era (Levin, 2006; Still, 2006). Pomegranates are thought to have been domesticated initially in the Transcaucasian-Caspian region and northern Turkey (Zohary and Spiegel-Roy, 1975 and Harlan, 1992). Evidence for consumption of pomegranates in the Middle East is dates back to 5,000 years ago. Pomegranate artefacts and relics dating to 3000 BCE were found in Egypt, Israel Armenia and Mesopotamia (Goor and Liberman, 1956; Still, 2006 and Stepanyan, 2007). Carbonized fragments of pomegranate rinds dating from early Bronze Age were found in Jericho and Arad, Israel (Still, 2006), in Nimrod, Lebanon (Still, 2006), in Egypt (Still, 2006) and in Armenia (Stepanyan, 2007). Pomegranates were introduced throughout the Mediterranean region to the rest of Asia, North Africa and Europe. They travelled to the Indian peninsula from Iran about the first century and started cultivation in India. In the 17th century the Greeks and the successor empires distributed the pomegranate all over the Europe and Spanish. Later, Spanish Jesuit missionaries introduced pomegranates into Mexico and California in the 1700s (Goor and Liberman, 1956 and Morton, 1987). The ability of pomegranate trees to adjust to variable climatic conditions is reflected in the wide distribution of the wild form throughout Eurasia to the Himalayas (Levin, 2006).

Commercial orchards of pomegranate trees are now grown in the Mediterranean basin (North Africa, Egypt, Israel, Syria, Lebanon, Turkey, Greece, Cyprus, Italy, France, Spain, Portugal) and in Asia (Iran, Iraq, India, China, Afghanistan, Bangladesh, Myanmar, Vietnam, Thailand; and in the former Soviet republics: Kazakhstan, Turkmenistan, Tajikistan, Kirgizstan, Armenia and Georgia). In the New World, pomegranates are grown in the United States and Chile. New orchards are now established in South Africa, Australia, Argentine and Brazil.

Punicaceae contains only two species, *Punica protopunica* and *Punica granatum* L. Balf. f. 1882. *P. protopunica* is endemic to the Socotra Island (Yemen) and is the only congeneric relative of *P. granatum* (Zukovski, 1950; Levin and Sokolova, 1979; Guarino *et al.*, 1990; Mars, 2000 and Levin, 2006). Based on xylem anatomy, *P. protopunica* has been suggested as the ancestral of the genus (Shilkina, 1973). The haploid chromosome number is 8 (Yasui, 1936; Darlington and Ammal, 1945; Raman *et al.*, 1971; Sheidai and Noormohammadi, 2005) or 9 (Darlington and Ammal, 1945).

The pomegranate is self-pollinated as well as cross-pollinated crop with the help of insects mainly by bees (Morton, 1987). Emasculation and bagging studies on Indian, Turkmen, Israeli and Tunisian pomegranate cultivars indicate that pomegranate flowers can self-pollinate and produce normal fruit (Nalawadi *et al.*, 1973; Mars 2000; Levin 2006 and Holland *et al.*, 2007). The degree of fruit set by self-pollination varies among different pomegranate cultivars (Levin, 2006 and Kumar and Rana, 2014). In hermaphrodite flowers, six per cent to 20 per cent of pollen may be infertile; in male flowers, 14 to 28 per cent are infertile. The size and fertility of the pollen vary with the cultivar and season (Morton, 1987).

Pomegranate tree produces two types of flowers viz., hermaphrodite flowers (vase shape) and male flowers (bell shape). Both types have several hundred stamens. The bell-shape flower has poorly developed or no pistil and atrophied ovaries containing few infertile ovules. Therefore, the bell shape flower is referred as a male flower and will drop without fruit set. The vase-shape flower is fertile with a normal ovary capable of developing into a fruit. The stigma of the hermaphrodite flower is at the anthers height or emerging above them. This position allows for self-pollination as well as pollination by insects. The factor that determines the fruit set capacity is the

number of vase-shape flowers. Therefore, cultivars with higher vase-shape to bell-shape ratio will have a higher fruit yield potential.

2.3 Bacterial blight disease and its resistance in pomegranate

For the first time in India, Hingorani and Mehta (1952) reported the occurrence of leaf spot of pomegranate and observed the association of bacterium. Rangaswamy (1962) observed bacterial leaf spot of pomegranate at Annamalainagar of Tamil Nadu. The disease was reported by Sohi *et al.* (1964) in Solan area of Himachal Pradesh generally occurring on leaves and fruits. In Karnataka for first time the severe disease incidence (60-80 %) was observed in the experimental plots of IIHR Bangalore by Chand and Kishun (1991). However, the outbreak of the disease was noticed in major pomegranate growing areas of Bellary and Vijayapur districts causing severe loss both in terms of yield and quality (Anon, 2002 and Ravikumar *et al.*, 2004). The disease has been found epidemic in Vijayapur and Bagalkot districts (Jalaraddi, 2006). Later it spread to other districts viz., Raichur (22 % yield loss annually); villages of Chitradurga followed by Ananthpur, Koppal and Bellary. Among the varieties, Bhagwa was found more susceptible (Yenjerappa, 2009) and disease was more severe during *mrigbahar* cropping season. The pathogen surveillance and infestation capability varies with respect to environment conditions. Raju *et al.* (2011) found that the disease was more severe in *mrigbahar* season (June-July) compared to *Hasta bahar* (October - September) and *Ambe bahar* (January-March) seasons.

2.3.1 Symptomatology

The infestation occurred on stem, leaves and fruits. Initially, symptoms appeared on leaves as irregular spots varying from 2 mm to 5 mm in diameter with light brown in colour surrounded by water soaked margin (Hingorani and Mehta, 1952). Later, number of adjacent spots coalesced with each other and forms elongated and irregular lesions. In many cases, spots were restricted on leaves by veins resulted into linear stripes (Kanwar, 1976; Kishun, 1993; Manjula and Khan, 2002; Katwal, 2015 and Gadhe *et al.*, 2016). Adjacent spots coalesced and covered larger areas. The spots were initially light brown in colour, surrounded by water soaked margin, later turned to dark brown as the disease progressed and turned into necrotic spots surrounded by chlorotic halos with

translucent water-soaked appearance (Rangaswamy, 1962). Under very severe infestation leaves will shed off. On fruits the symptoms appeared as water soaked spots in the earlier stages, later become dark brown, slightly raised from the surface with oily appearance (Kanwar, 1976, Kishun, 1993 and Manjula and Khan, 2002). The infestation occurs on petals of flowers, the symptoms appears as small, brown, water-soaked spots, which later became black and bigger in size and in advanced stages of infection, flower buds drop off (Yenjerappa, 2009). Symptoms were also noticed on immature fruits as small, pin head like, circular with dull green colour, which later turned deep brown or black with yellowish zone surrounding the spots. The necrotic lesion on the fruit increased as the fruit size increased with age leading to L, Y or star shaped cracks within the spots. Severely infected fruits split opened, partially exposing the arils (Kirankumar, 2007). Under heavy infection condition, fruits remained undeveloped and deformed.

Yenjerappa (2009) noticed bacterial infection on stem in the form of long narrow, elongated brown to black coloured lesions on the main stem and branches. The lesions later on became rough and cankerous, leading to stem girdling and breaking at the point of infection. It resulted into complete drying of the branches. As the disease advanced, spots on fruits coalesced together and formed irregular lesions; as a result, skin of the fruit became rough.

2.3.2 Isolation and pathogenicity

The bacterial blight infestation occurs on stem, leaf and fruit. However, the isolation of pathogen from leaf is a general practice. The leaf that shows typical symptoms viz., water soaked spot with brownish central area were major source of pathogen and further the infected tissue were surface sterilized with either sodium hypochlorite or mercuric chloride with subsequent water washing. Several studies reported that, the tissue was homogenized and serial diluted up to 10^{-6} and spread on *Xanthomonas* specific medium i.e., Starr's medium or nutrient agar (NA) medium. As per the Bergey's manual the *Xanthomonas* colonies appears light yellow colour after incubation at 30 °C for 72 hr on medium. Apart, from morphological identification it is essential to re-confirm the pathogen by Koch's postulates. Therefore, in several studies (Hingorani and Mehta, 1952; Kanwar, 1976; Chand and Kishun, 1991 and Manjula,

2002) after the pathogen isolation have shown reproved the pathogenicity or Koch's postulate on desired plants.

The traditional method to prove the pathogenicity is spraying the inoculum over the plants is most common practices and followed by many scientists. Hingorani and Mehta (1952) sprayed the pathogen inoculum directly on healthy pomegranate plant. After 710 days incubation they observed infection on the tender leaves of artificially inoculated young potted plants. Isolation from the infected plants yielded an organism similar to one used in the inoculation experiments. However, efficiency of pathogen to enter the healthy plant is always under the question. Hence, few reports concentrated on mechanical damage to the plant and infecting. Kanwar (1976) sprayed the bacterial suspension of 48 hr old culture onto the injured and uninjured leaves, flowers and fruits of healthy plants. Infection occurred more rapidly on injured parts than uninjured ones. Meantime, the inoculation method and pathogen concentration are very important to check the pathogenicity.

Chand and Kishun (1991) standardized the inoculation method by inoculating the bacterial suspension (25×10^8 cfu/ml) on 40 days old leaves by pinprick, rubber block pressure, leaf cut and atomization methods. They observed that, leaf cut was found superior, where they recorded 100 per cent infection covering 70 to 90 per cent leaf area within 21 days. The atomization of bacterial suspension was also induced the infection within 17 to 40 days. Yenjerappa (2009) sprayed the 5×10^5 cfu/ml pathogen concentration and observed the disease infestation within 15 days of inoculation. Similar observations were made by Kirankumar (2007) and Raju (2011).

2.3.3 Variability and identification of the pathogen

2.3.3.1 Morphological characters

The bacterium was short, rod shaped with rounded ends, single or in pairs, sometimes in chains, no involution formed, measuring 1 to 2.5×0.5 μm in size, motile with a single polar flagellum, gram negative, no endospores and was not acid fast. It readily stains with common dyes like gentian violet and carbolfuchsin (Hingorani and Singh, 1959). Further, with respect to the presence and absence of capsule there is a ambiguity in the literature. As per the Hingorani and Singh (1959) and Jalaraddi (2006)

the bacteria has capsule around its cell wall, in contrast few studies reported absence of capsule around cell wall of bacterium (Kanwar, 1976; Manjula, 2002; Kirankumar, 2007; Yenjerappa, 2009 and Wayde, 2015).

2.3.3.2 Molecular identification

The highly conserved genetic characteristics, 16s-23s intergenic spacer (IGS) of the rDNA operon has also been used to infer the phylogenetic relationship and identification of bacteria at different taxonomy levels (Gurtler and Stanisich, 1996). Hirokaku and Hisatoshi (1999) identified 49 *Xap* isolates through 16s and 23s rRNA gene specific primers. They observed unique amplicon with the size of 1600bp and 550bp for 16s and 23s rRNA, respectively. Similarly, Mondal *et al.* (2012) identified *Xap* isolates through 16s rRNA gene based universal primers (forward- 5' AGAGTTTGATCCTGGCTAG3' and reverse 5'AGGAGGTGATCCAGCCGCA3'). They also attempted to understand the genetic variability and phylogenetic relationship among the collected *Xap* isolates from diverse geographical origins viz., Delhi, Maharashtra and Andhra Pradesh with two housekeeping genes viz., 16s rRNA and *gyrB*. Results revealed that all 15 strains collected from different regions formed one cluster and sharing cent per cent identity among them, indicating that geographical distance does not necessarily contribute to the variation in *Xap* strains with respect to the 16s rRNA and *gyrB*.

2.3.3.3 Cultural variability

Cultural characters of the pathogenic bacterium have been studied on various media by different workers. Hingorani and Singh (1959) opined that nutrient agar, yeast, glucose, chalk agar and potato cylinders were the best media for the cultivation of bacterium, because of luxuriant growth obtained on them. The pathogen was a facultative anaerobe. They also described that colonies on nutrient agar were filiform, slightly raised, glistening, pale yellow and odourless. Similar characters were also found on yeast glucose chalk agar with an exception that colour of the colony was bright yellow in the beginning and it gradually changed to quite dark brown with age. In 1991, Chand and Kishun had recovered pathogen from leaf, fruit and node. All the infected parts of pomegranate viz., leaf, fruit and node yielded mucoid, circular, convex, yellow, round, glistening and raised colonies on nutrient agar medium. Based on pathological,

cultural, biochemical and physiological characters, the authors identified the organism as *Xanthomonas campestris* pv. *punicae* (Hingorani and Singh, 1959). Joseph (1997) recorded the highest recovery of the colonies of *Xanthomonas campestris* pv. *dieffenbachiae* of anthurium on tween media followed by cellobiose starch medium.

2.3.3.4 Molecular variability

The RAPD technique is widely applicable as it has provided a relatively simple tool of genetic variability in biological system. RAPD is the best assay when the nucleotide sequence is not known. Unlike other polymerase chain reaction (PCR) protocols, which utilize two or more primers of defined sequence, RAPD detects nucleotide polymorphism using only one primer of an arbitrary nucleotide sequence.

DNA amplification approaches using arbitrary primers have been employed to a large number of genetic studies including the estimation of genetic relationship (Wilde *et al.*, 1992). Kim *et al.* (1993) reported that PCR had been widely and successfully employed for the diversified analysis of important plant pathogenic bacteria and fungi. Manulis *et al.* (1994) distinguished the strains of *Xanthomonas campestris* pv. *pelargonii* from 21 other *Xanthomonas* species and/or pathogens using random amplified polymorphic DNA method. Among the 42 arbitrarily chosen primers evaluated, 3 were found to reveal diagnostic polymorphism. The distinctive *X. campestris* pv. *pelargonii* patterns were clearly different from those obtained with any of 46 other *Xanthomonas* strains tested.

Pooler *et al.* (1996) studied genetic relationships among 25 isolates of *Xanthomonas fragariae* from diverse geographic regions by using RAPD PCR, repetitive extragenic palindromic (REP) PCR and enterobacterial repetitive intergenic consensus (ERIC) PCR. The results indicated that pathogenic strains were very closely related to each other and there was no correlation between DNA amplification product patterns and geographic sites of isolates suggesting that this bacterium has spread largely through exchange of infected plant germplasm.

Siraree *et al.* (2004) generated the RAPD profiles using single decamer primers in polymerase chain reaction to study the diversity in *Xanthomonas campestris* pv. *campestris* population prevalent in the region. They standardized the amplification

conditions so as to get polymorphism specific for individual isolate. Amplification products ranged from 6 to 12 with fragment size ranging from 100 bp to 5.0 kb.

Giri *et al.* (2011) worked on RAPD profiles and showed a high level of genetic variability among the different isolates of *Xanthomonas axonopodis* pv. *punicae*. He had collected 16 isolates from different parts of Karnataka to know the existence of molecular diversity. The cluster analysis based on similarity co-efficients separated the sixteen strains into two major clusters.

Arshiya *et al.* (2014) isolated 56 strains of *Xanthomonas axonopodis* pv. *citri* (*Xac*) from different citrus growing areas of Marathwada region of Maharashtra state and twenty representative strains of *Xac* were selected for genetic diversity. Repetitive DNA polymerase chain reaction-based fingerprinting (rep-PCR) using repetitive extragenic palindromic (rep) and enterobacterial repetitive intergenic consensus (ERIC) and BOX primers were used to assess the genetic diversity. Cluster analysis done by combining the banding patterns of REP-PCR, ERIC-PCR and BOX-PCR clearly separated two diverse groups within species of *Xanthomonas axonopodis* pv *citri* based on the geographic region of isolation.

Wayde *et al.* (2015) collected seven strains of *X. axonopodis* and differentiated into seven major region representing Malegaon, Nampur, Satana, Khakurdi, Ramnagar, Rahuri and Nashik region. However, the genetic similarity within seven strains of *X. axonopodis* was ranged between 72.72 and 100 per cent. Similarly the genetic relationship of strains *X. axonopodis* was also established and showed 72.72, 72.72, 72.72, 92.30, 94.11 and 100 per cent similarity with region of Malegaon, Nampur, Satana, Khakurdi, Ramnagar, Rahuri and Nashik region, respectively. Average polymorphism in strains of *X. axonopodis* based on RAPD analysis was 84.95 per cent. The combined data analysis by using UPGMA method also revealed the two distinct groups representing seven strains of *X. axonopodis* and in first group M1 and second group R4 and subgroup RH6, N2, NS7, S3, K5.

Gadhe *et al.* (2016) studied the variability among the five isolates of *Xanthomonas axonopodis* pv. *punicae* using 10 RAPD primers, out of which seven primers produced a total of 26 scorable bands with an average of 3.7 bands per primer.

Out of 26 bands, 21 bands were found to be polymorphic and level of polymorphism was 80.76 per cent. The Jaccard's similarity coefficient showed that the isolate Loni (*Xap1*) was found to have higher value of similarity coefficient 0.88 with Astagaon (*Xap2*), whereas Talegaon (*Xap3*) was found to have lower value of similarity coefficient 0.20 with Rahuri (*Xap4*). The cluster analysis based on similarity coefficients divides the five isolates into two major and two sub-clusters.

2.3.3.5 Pathogenic variability

Joshi *et al.* (2014) tested the six isolates of *Colletotrichum gloeosporioides* with 11 pomegranate varieties and assessed virulence of the isolates and reaction of the particular variety was expressed in diseased reaction index (DRI). There was a considerable variation in virulence and susceptibility with these isolates and varieties. Isolate Cg86 was found virulent (DRI 7.2), Cg 81 as less virulent (DRI 2.87) and other isolates were moderately virulent irrespective of varieties. Fruit varieties Aarakta and Bhagwa were found highly susceptible (DRI 10.38 and DRI 8.56), respectively. Resistant varieties Yarcaud local and Bedana thin skin showed uniform resistant reaction against studied six isolates of pathogen. Ismail *et al.* (2014) conducted study on five different isolates of the bacterium (*Xanthomonas campestris*) including *Xac1*, *Xac2*, *Xac3*, *Xac4* and *Xac5* isolated from grapefruit (*Citrus paradisi*), lime (*Citrus limon*), rough lemon (*Citrus jambhiri*), red blood malta (*Citrus sinensis*) and kinnow (*Citrus reticulata*), respectively. These isolates were tested to find the pathogenic variability on three test hosts including grapefruit, lime and rough lemon. The results of these isolates on pathogenicity, symptoms development and host susceptibility indicated that the isolate *Xac3* showed greater virulence on all test hosts followed by the isolate *Xac4*, while the response of other three isolates was intermediate. Among the test hosts grapefruit gave the highest degree of susceptibility to all the strains, while lime and lemon were ranked second and third, respectively. The results of the study suggested variation in the pathogenic nature of these isolates. Hence, it is essential to evaluate the diverse genetic population.

2.4 Genetic variability and diversity for plant growth and fruit quality traits

Until recent years, pomegranates were selected according to the demands of local consumers and not for export. Most of the pomegranate cultivars grown today are the result of human selections from naturally occurring germplasms. Therefore, the majority cultivars found today reflect the local priorities of each country or region e.g., the traditional Indian and Spanish cultivars that are characterized by their soft seeds and low-acid taste. Increased world demand and economic importance of pomegranate export constantly influences pomegranate selection criteria, which will have an increasing role in pomegranate breeding. Pomegranate is having a wide germplasm pool extending from Asia, Europe, North Africa and North America including wild and domesticated accessions (Mars, 2000).

Singh *et al.* (2006) reported that pomegranate germplasm from India might include highly genetically diverse pomegranate varieties that needs to be conserved and characterized. Crop improvement is based on characterization of germplasm for assessing the genetic diversity for any breeding program and further, knowledge of it and its relationships among the cultivated and wild species is important for recognizing gene pools, identifying pitfalls in germplasm collection and developing effective conservation and management strategies (Iezzoni, 2008). Characterization continues to be the first step for the description and classification of germplasm. The pomegranate cultivars have great diversity in their vegetative, flowering and fruiting behaviour.

Karale and Desai (1998) reported that all the characters were not equally heritable (*viz.*, 32.80 % for TSS:acid ratio to 96.74 % for seed mellowness). Characters having high heritability reveal that breeder can select a genotype on its own phenotypic performance. Characters having medium heritability indicate that these were influenced by environmental conditions based on phenotypic values alone. The characters exhibiting low to moderate heritability accompanied by low genetic gain indicate predominantly non-additive gene action for these characters (*viz.*, juice percentage, length and diameter of fruit). Thus, improvement in these characters through selection will be relatively low. The characters showing low heritability would need further selection

pressure. Higher magnitude of non-additive gene action reveal need to go for developing new hybrids using superior segregates from bi-parental progenies.

2.4.1 Plant growth behaviour and flowering characteristics

Pomegranate has a wide diversity with respect to morphological characters such as plant canopy, plant height, days to flowering, fruit size, colour of the fruit and juice taste (Singh *et al.*, 2006). The assessment of variability based on morphological traits has been observed by several scientists in pomegranate, most of the pomegranate varieties are deciduous. However, there are several ever green pomegranate germplasm in India. Some ever green cultivars shed their leaves in higher elevations and colder climates (Nalawadi, 1973). Pomegranate is a versatile crop with a high degree of amenability for flowering and ability to produce profuse flowers. A good knowledge on different aspects of blossom biology viz., flowering season, flower bud development, weight and number of flower, period of anthesis and its peak time, heterostyly, fruit set due to selfing and crossing *etc.*, provides a pre-hand idea on the breeding mechanism. Induction of flowering and regulations of number of fruits on plant becomes important to reap lucrative returns through optimum quantity. Pomegranate trees are deciduous, but in hot climates many cultivars behave as ever greens. Both vegetative and flower buds open well even after warm winters (Chitale and Deshpande, 1970). Flower buds are lateral or terminal and they are mixed continuing both leaf and flower initiation. They are born on normal shoots of one-two year old spurs developing during the summer season (Singh *et al.*, 1978).

The flowering in pomegranate is several times in a year, in the hot climate of south India (Nalawadi, 1973). Several distinct flushes of flowering on the same tree occur quite frequently in sub-tropical climates of Northern hemisphere (Singh *et al.*, 1978). There are three main season of flowering viz., January- February (*Ambe bahar*), June - July (*mrigbahar*) and September- October (*Hasta bahar*) (Sharma *et al.*, 1996; Prasad and Bankar, 2000). The crop is regulated to one of the bahars by suspending the vegetative growth through bahar treatments and other cultural practices. Sonawane *et al.* (1991) reported that January - February (*Ambe bahar*) cropping was the best for the fruit quality compared to September - October (*Hasta bahar*).

Pomegranate flowers exhibit heterostyly which denotes the presence of 2 or more kinds of flowers with respect to pistil length, pistil is present in all the 3 kinds of flowers including staminate (male flower) but the pistil in staminate flower is rudimentary. The flowers were categorized into pin type (the length of pistil is greater than or equal to or that of stamens e.g., hermaphrodite, intermediate flower and thrum type (the length of pistil is less than that of stamens e.g, staminate flowers (Sheikh *et al.*, 2011). They reported that in ‘Ganesh’ the length of the pistil were 2.00, 1.55 and 0.65 cm in hermaphrodite, intermediate and staminate flowers, respectively. The length of the pistil was comparatively higher in ‘Ganesh’ than that of ‘Bhagwa’ irrespective of the kind of flower. The percentage of male flowers is very important (>60-70 %) depending on varieties and season, male types drops and rarely set fruits leaving the hermaphrodite type to produce the majority of the crop (El Sese, 1988; Chaudhari and Desai, 1993). In some situations, perfect flowers are predominant at the beginning of the season, followed by the development of unfruitful flowers and almost all of the normal flowers are produced between the onset of flowering and the end of full bloom (Hussein *et al.*, 1994 and Abou Aziz *et al.*, 1995).

Balamohan *et al.* (2001) collected and studied germplasm of pomegranate from different location of India and biometrical characters revealed lot of diversity among the collected germplasm. Out of collected the YCD-1 found tallest and Mridula genotype shown highest number of branches per plant with larger canopy. Singh *et al.* (2006) reported deciduous Indian varieties and identified 16 genotypes that behaved as evergreen in Rajasthan India. Mir *et al.* (2010) also described that pomegranate is deciduous in winter temperature areas but in tropical and sub-tropical areas it is evergreen or partially deciduous. Meena *et al.* (2011) reported that fifty per cent of the pomegranate ge notypes were found evergreen in nature.

Meena *et al.* (2004) recorded genetic variability, heritability and genetic advance in a collection of 24 pomegranate genotypes. Considerable genetic variability for yield and its component characters was observed. High heritability coupled with high genetic advance was observed for dormancy duration, maturity duration, number of fruits per plant, flowering duration and yield, indicating additive gene effects and emphasizing the effectiveness of selection for these traits to improve economic yield in pomegranate.

Association of growth characters with yield in 29 diverse genotypes of pomegranate (Desai *et al.*, 1992) indicated that plant height has non-significant correlation with yield characters. Plant spread was positively and significantly correlated with yield attributes and need to be given importance in parent selection and progeny screening, Plant spread was also positively associated with evergreen expression. Most of the undesirable fruit characters had positive association amongst theme selves and negative with desirable characters.

2.4.2 Fruit characters

A wide range of variability based on physical and biochemical fruit characters has been observed in pomegranate. Holland *et al.* (2007) stated that pomegranate cultivars display a wide range of phenotypic differences such as fruit qualities and secondary metabolites content.

2.4.2.1 Fruit colour

The fruit colour of the Dholka variety was yellowish crimson with patches of various colours usually dark pink and purple near the base, lighter near the apex; whereas Japanese Dwarf were greenish yellow with deep pink and purple tinge, while Muscat White was greenish yellow with very small green and light pink patches (Nath and Randhawa, 1959). Studies conducted by Malhotra *et al.* (1983b) showed that colour of the fruits of various cultivars of pomegranate were either pink or rose with varying intensities. The main shades recorded were Venetian pink, French rose and Claret rose. Shulman *et al.* (1984) while working on two cultivars. of pomegranate namely Mules Head and Wonderful showed that the fruit colour developed gradually and served as criterion for picking. The stage at which 70 to 90 per cent of the skin is red usually corresponds with a maturity index suitable for commercial picking.

Khodade *et al.* (1990) observed the fruit colour of P-23 pomegranate changed from greenish purple to deep pink with reddish and yellowish patches at maturity. Sharma and Dhillon (1996) reported that colour of the fruits of Kandhari cultivar of pomegranate was creamy with red blush, while fruits of Ganesh have creamy with red tinge coloured skin. Studies conducted by Prasad *et al.* (1999) showed that the colour of the pomegranate fruit cv. Jalore seedless was changed from greenish to deep pink with

red and yellow patches at the maturity of the fruits. Fruit colour changed from light green to deep red in Kandhari and from light green to yellow green with light red tinge in Nabha and Ganesh at maturity (Dhillon and Kumar, 2004). Fruit colour of Kandhari Hansi was observed red and rest of cultivars had greenish yellow to pinkish yellow (Sharma and Bist, 2005).

2.4.2.2 Fruit size

Bailey (1917) broadly classified the varieties of pomegranate on the basis of size of the fruits. Commercial varieties of pomegranate of Baluchistan were also classified on the basis of size of the fruits (Caius, 1940). Fruits of Kandhari variety were bigger in size than that of Muscut White (Cheema *et al.*, 1949). Nath and Randhawa (1959) showed that average length, diameter and circumference of Dholka variety was 7.0 cm, 8.5 cm, 27.7 cm, respectively, whereas in Japanese Dwarf it was 7.35 cm, 7.5 cm and 24.4 cm, respectively. According to Josan *et al.* (1979), cultivars Kazkai, Shirin Anar and Achikdana had the largest fruits (6.5, 6.32 and 6.26 cm, respectively). Malhotra *et al.* (1983b) found that the fruit length in pomegranate ranged from 3.9 cm as recorded in Afghan Kandhari Seedling to 5.6 cm in Anar Shirin-e-Mohamad Ali whereas in fruit breadth, the cv. Guleshah exceeded others by producing fruits as broad as 6.8 cm followed by Kazkai 6.1 cm. The lowest breadth (4.1 cm) recorded was in cv. Anar Post-e-Safed Shirin.

Godara *et al.* (1989) conducted studies on ten cultivars of pomegranate under Hisar conditions and revealed that maximum length was observed in cv. Achikdana, which was closely followed by Bedana Sedana and Baskha Linski. The minimum length was in cv. Shirin Anar which was at par with Anar Shirin Mohamad Ali and Kali Shirin. The maximum fruit breadth was in cv. Bedana Sedana. The size of pomegranate fruit continuously increased from anthesis till harvest (Khodade *et al.*, 1990 and Khadivi-Khub *et al.*, 2015). They showed that the length of fruit increased from 4.43 cm to 7.82 cm at the time of harvest. Similarly, diameter of the fruit increased from 4.20 cm to 8.33 cm. Prasad *et al.* (1999) showed that length and diameter of fruit of Jalore Seedless pomegranate increased from 4.20 and 4.1 cm to 8.01 cm and 8.32 cm, respectively at maturity. They found linear increase in size and diameter of fruit from set, to till the

harvest of fruit. The length and diameter values were between 73.3 mm (Konjski zub) and 83.1 mm (Pastun) and 79.1 mm (Konjski zub) and 95.3 mm (Pastun) Kazankaya *et al.* (2003).

The studies conducted on fruit size of pomegranate cultivars showed that fruit size ranged from 61 mm to 91 mm and 36 to 104 mm (Yilmaz *et al.*, 1992 and Al-Maiman and Ahmad, 2002). Celik and Erasl (2009) studied physical characteristics of pomegranate cv. Eksinar and found that fruit dimensions varied from 52.9 to 75.0 mm for length and 60.6 to 85.9 mm for width. Varasteh *et al.* (2006) evaluated important fruit characters of five commercial pomegranate cultivars in Iran and reported that Malas-e-yazdi had highest fruit length and diameter (103.55 mm and 109.50 mm, respectively) and Malase-Torsh-e-Saveh had minimum length and diameter (83.60 mm and 91.76 mm, respectively). Wani *et al.* (2012) evaluated 17 accessions and found that the fruit length ranging from 5.9-9.24 cm and diameter ranges from 6.54-9.84 cm. Chandra *et al.* (2013) reported that fruit length and diameter ranges from 6.91-9.83 cm and 5.93-8.86 cm, respectively. Khadivi-Khub *et al.* (2015) evaluated important fruit characters of 87 local accessions of pomegranate in Iran and reported that fruit length and diameter ranging from 43.12 – 83.45 mm and 49.12-90.87 mm, respectively.

2.4.2.3 Fruit weight

Nath and Randhawa (1959) reported that average mean weight of the fruit in Dholka, Japanese Dwarf and Muscat White cultivars of pomegranate was 396.9 g, 6.2 g and 396.9 g, respectively. Malhotra *et al.* (1983b) concluded that fruit weight of pomegranate ranged from the lowest of 56.8 g in cv. Afghan Kandhari seedling to as high as 124.7 g in Kazkai and the cultivars such as Baskhi Kalinski, Dholka, Chola Selection-1 and Chola Selection-II produced fruits of medium weight. Misra *et al.* (1983) found that fruit weight was more in cv. Anar Shirin-e- Mohamad Ali (295.0 g) followed by Shirin Anar (250 g) and Dholka (242 g) and the cultivars Anar Shirin-e- Mohamad Ali had produced fruits of bigger size followed by Dholka, whereas, the Kandhari had smaller fruits.

Shulman *et al.* (1984) studied the fruit growth and development of pomegranate and found that the fruit grows continuously from fruit set until the commercial harvest time. At the end of harvest the average weight of the fruit in Bet Shean Valley was 350

g for Mules Head and 400 g for Wonderful and on the coastal plain, it was 250 g and 350 g, respectively. Godara *et al.* (1989) reported that maximum fruit weight of 224 g was obtained in cv. Bedana Sedana of pomegranate and minimum of 97 g was in Shirin Anar. Khodade *et al.* (1990) found that fruit weight continuously increased from 48 g to 276.51 g from fruit set to harvest. Sharma and Dhillon (1996) reported that average fruit weight in Kandhari and Ganesh cultivars of pomegranate was 250 and 282 g, respectively. Prasad *et al.* (1999) found linear increase in fruit weight of cv. Jalore Seedless from fruit set till the harvest of fruit.

Meena *et al.* (2003) conducted a variability study on 24 indigenous and exotic genotypes. Analysis of variance for 10 different characters viz., average fruit weight, seed content, 100 aril weight, weight of 100 seeds, Juice content, total soluble solids (TSS), acidity, total sugars, ascorbic acid and tannin content. High variability, genotypic coefficient of variation, heritability and genetic advance were significantly higher for characters like fruit weight, seed content and weight of 100 arils, juice acidity and weight of 100 seeds. Thus, these traits may be effectively used as selection criterion for screening potential genotypes in a breeding programme. Viswanath *et al.* (1999) reported that fruit weight of pomegranate ranged from 141.5 to 543.0 g Prasad and Bankar (2000) reported that Jalore Seedless has maximum fruit weight (216.4 g), followed by G-137 (208.1 g) cv. of pomegranate. Sharma and Dhillon (2002) reported maximum fruit weight of 450.0 g was recorded in Ganesh followed by 400.0 g in PS-77 and 330.5 g in Basse-in-Seedless. However, the minimum (200.0 g) fruit weight was noted in Jodhpur White cultivar. Dhillon and Kumar (2004) reported that weight of the fruit weight increased up to 150 days after anthesis to maturity. The maximum average fruit weight among all the cultivars was recorded as 447.0 g in Kandahri Hansi and minimum in case of Anar Shirin Mohammad Ali i.e., 95.0 g (Sharma and Bist 2005). Ten pomegranate (*Punica granatum* L.) cultivars namely Kabuli Kandhari, Chawla, Ganesh, Mridula, Jyoti, G-137, Dholka, Bedana, Kandhari and Local check were evaluated for fruit weight and was found significantly more in cv. Bedana compared to rest of the cultivars under study (Mir *et al.*, 2007a and Mir *et al.*, 2007b). Varasteh *et al.* (2006) evaluated important fruit characteristics of five commercial pomegranate cultivars in Iran and reported that Malas-e-Yazdi had the highest fruit weight 699.94 g and Malase-Torsh-e-Saveh had minimum fruit weight (408.23 g).

2.4.2.4 Aril colour and aril weight

Malhotra *et al.* (1983b) reported that arils had different shades like Claret rose, Dawn pink, Venetian pink and French rose, *etc.*, while working with different pomegranate cultivars. Khodade *et al.* (1990) showed that percentage of arils on fruit weight basis, increased with the advancement of maturity. They also reported that the colour of arils was milky initially and turned to creamy pearl white from 120 days onwards which intensified with advancement of fruit maturity. Sharma and Dhillon (1996) noted that arils develop light pink colour in Kandhari, while Ganesh cv. of pomegranate developed white aril colour with light pink tinge.

Jalikop and Kumar (1998) studied 18 genotypes of pomegranate (*Punica granatum* L.) and found significant variations within soft types for 100 aril weight within semi-soft varieties for 100 aril weight of fruit. The data recorded by Prasad *et al.* (1999) on qualitative changes during growth and development of pomegranate fruit showed that the percentage of arils and aril/rind ratio increased continuously with advancement of maturity. Percentage of arils increased rapidly from 38.74 to 67.5 up to December 5 and thereafter, it was almost constant. As far as colour of aril is concerned, initially it was milky white and later turned to pinkish red to dark red with the commencement of maturity.

Gozlekci and Kaynak (2000) found that aril weight increased during development of the fruit. Sharma and Bist (2005) studied red colour aril in Kandhari Hansi and PS-75-K-5 whereas in all other cultivars it was light pink to deep pink.

Gadze *et al.* (2012) studied three main pomegranate cultivars in the Neretva valley in south Dalmatia of Croatia in October 2009 and observed light pink aril colour in cv. Konjskizub while red in cultivars Pastun and Ciparski.

2.4.2.5 Rind weight and thickness

Nath and Randhawa (1959) reported that rind thickness in cvs. Dholka, Japanese Dwarf and Muscat White of pomegranate were 0.7, 0.2 and 0.3 cm, respectively. Malhotra *et al.* (1983b) while working with different pomegranate cultivars observed that the rind thickness ranged from 0.13 cm in Afghan Kandhari seedling to 0.27 cm in

Bedana. According to Misra *et al.* (1983) in Srinagar special pomegranate cultivar exhibited more rind thickness (0.69 cm) than cv. Kandhari, which had rind thickness of 0.27 cm. Godara *et al.* (1989) recorded maximum rind thickness in cv. Russian Seedling (3.93 cm) followed by Achik Dana (3.83 cm) and Surkh Anar (3.80 cm). The cv. Baskha Linski had minimum thickness (1.20 cm) and rest of the cultivars had medium thickness. Studies conducted on fruit development of pomegranate by Khodade *et al.* (1990) showed that percentage of rind and rind thickness decreased gradually from anthesis till the maturity of fruit. Similar results with regard to rind thickness and rind weight were found by Prasad *et al.* (1999). Singh *et al.* (2011) reported maximum rind weight in Kandhari (102 g) and minimum in Ganesh (82 g).

2.4.2.6 Juice content

Siddappa (1943) reported that in one good variety of pomegranates the juice was 40.1 per cent of entire fruit. While, Malhotra *et al.* (1983b) reported that the juice percentage varied from 62.5 per cent in Surakh Anar to 80.8 per cent in Anar Shirin-e-Mohamad Ali. The other cultivars with high juice content were Guleshah (78.7 %), Achikdana (77.1 %), Suni Bedana (76.5 %) and Ak Anar (76.4 %). Shulman *et al.* (1984) reported that initially juice content of pomegranate was less than 25 per cent of the total fruit weight. As the fruit matured, the juice content continued to increase up to harvest time. The juice content in Mules Head reaches 35 and 40 per cent and in Wonderful 40 and 45 per cent in coastal plains and Bet Shean Valley, respectively. Godara *et al.* (1989) recorded highest juice percentage in cv. Russian Seedling (68.37) followed by Bedana Sedana, while maximum juice was obtained in cv. Surkh Anar and Kali Shirin. The juice content in pomegranate cv. P-23 increased from 24.07 to 55.66 per cent with advancement of maturity of fruit (Khodade *et al.*, 1990).

Fruits of two pomegranate cultivars (Jalore Seedless and Khog) were harvested from five years old trees in December and January. Jalore Seedless cv. contained more juice. The fruits harvested in January contained high juice content than those harvested in December (Singh, 1994). Prasad *et al.* (1999) noted that juice percentage in Jalore Seedless pomegranate increased from 24.83 to 51.03 with the advancement of fruit maturity. Similarly, Viswanath *et al.* (1999) found juice percentage between 44.96 to 68.55 in pomegranate. Sharma and Bist (2005) recorded maximum juice percentage as

75.00 per cent in G-137. The juice percentage of pomegranate cultivars varied from 29.55 per cent (Rabbab-e-Fars) to 42.57 per cent (Faroogh), Fadavi *et al.* (2006).

2.4.2.7 Total soluble solids (TSS)

Malhotra *et al.* (1983b) reported that TSS content in the juice ranged from 9.2 per cent in cv. Afghan Kandhari Seedling to 12.9 per cent in Suni Bedana. Shulman *et al.* (1984) reported that TSS increased gradually during fruit development. At the beginning of harvest in mid-August, the TSS of Mules Head was 11 to 14 per cent and reached 14 to 15 per cent later in September. In cv. Wonderful the TSS reached 13 to 14 per cent in mid-August and 15 to 16 per cent by end of September. The data on chemical changes during growth and development of pomegranate fruit showed that TSS increased from 11.75 to 17.70 per cent with the advancement of maturity (Khodade *et al.*, 1990). Sharma and Dhillon (1996) recorded that juice of Kandhari contained 14.0 per cent, while Ganesh contained 13.8 per cent TSS. Whereas, Prasad *et al.* (1999) reported that TSS increased with the advancement of maturity in Jalore Seedless pomegranate. Similar results were obtained by Gozlekci and Kaynak (2000) in pomegranate. Prasad and Bankar (2000) reported that the TSS in juice of different cultivars of pomegranate ranged from 16.2 °Brix in Jodhpur Red to 18.8 °Brix in Bassin Seedless. Sharma and Bist (2005) described that total soluble solids varied from 10.0 °B in PS-75-K-5 to 14.4 °B in Anar Shirin Mohammad Ali. Gadze *et al.* (2012) reported variability in TSS in different pomegranate genotypes with maximum 15.2 and 15.6 in cv. Pastun followed by Ciparski as 14.8 and 15.1 per cent and Konjski zub (13.1 and 15.0 %).

2.4.2.8 Acidity

In general the acidity ranged from 0.81 to 1.23 per cent in pomegranate varieties for table purposes (Siddappa, 1943). Hayes (1957) reported that acids in pomegranate ranged from 1.5 to 2.5 per cent. Nath and Randhawa (1959) recorded 0.4, 0.6 and 0.38 per cent acidity in Dholka, Japanese Dwarf and Muscat White varieties, respectively. Malhotra *et al.* (1983b) reported that total organic acid content as citric acid ranged from the lowest of 0.49 per cent in Anar Shirin-e-Mohamad Ali to as high as 2.30 per cent in Kandhari cvs. of pomegranate. Shulman *et al.* (1984) reported that acid content of juice in pomegranate decreased with maturation. Acidity in pomegranate cv. Mules

Head was very low (< 0.5 %) even at early stages of fruit development. In cv. Wonderful the acidity was very high in June and then decreased considerably.

Godara *et al.* (1989) found that the acidity ranged from 0.71 per cent in Ak Anar and Anar Shirin Mohamad Ali to 6.46 per cent in Russian Seedling cvs. of pomegranate. Developmental studies in pomegranate conducted by Khodade *et al.* (1990) showed that the acidity in P-23 pomegranate decreased from 0.785 per cent at initial stage to 0.273 per cent at maturity. The juice of Kandhari and Ganesh cultivars of pomegranate contained 0.67 and 0.43 per cent acidity, respectively (Sharma and Dhillon 1996). Jalikop and Kumar (1998) reported that juice of Ganesh pomegranate contained 0.78 per cent acidity, while that of cv. Nabha contained 0.934 per cent. However, Prasad *et al.* (1999) noted that acidity in pomegranate juice decreased from 0.761 to 0.30 per cent at maturity. Melgarejo *et al.* (2009) found that citric acid was predominant in pomegranate juice with the range of 0.142 to 2.317 g/100 g. Malic acid was the second most abundant with a range of 0.135 to 0.176 g per 100 g. Prasad and Bankar (2000) showed that acidity ranged from 0.182 per cent in Jodhpur Red to 1.784 per cent in Basse-in Seedless pomegranate cultivars. Sharma and Bist (2005) reported that acidity was minimum *i.e.*, 0.15 per cent in G-137 and maximum in Kandhari Hansi *i.e.*, 0.77 per cent. Acidity of fruit juice was significantly higher in Pastun (4.3 %) compared to cv. Ciparski (0.9 %) and Konjski zub (1.4 %) reported by (Gadze *et al.*, 2012).

2.4.2.9 Maturity Index (TSS:acid ratio)

Khodade *et al.* (1990) reported that TSS: acid ratio in pomegranate juice increased from 14.96 to 64.83 with advancement of maturity. The increase in TSS acid ratio was because of increase in sugar content in juice and reduction in acidity with maturity (Prasad *et al.*, 1999). Viswanath *et al.* (1999) observed that TSS: acid ratio in pomegranate ranged from 29.09 to 93.33. Sharma and Bist (2005) reported TSS/acidity ratio ranged between 15.01 in Kandhari Hansi to 95.16 in G-137. A significant variation in general characters of fruit in fourteen cultivars of pomegranate was assessed by Khosla and Kumar (2009). The physico-chemical composition of 25 pomegranate cultivars grown in Iran has been characterized by Barzegar *et al.* (2004). Sarkhosh *et al.* (2005) studied the correlation between quantitative and qualitative characteristics of pomegranate in different regions of Iran.

Singh and Meena (2003) conducted an experiment to assess the quality of pomegranate cultivars (Eichakdana, Jalore seedless, Ganesh, Khog, Muskut seedless and Basin seedless) and their crosses (Richakda.na $\times$ Jalore seedless, Jalore seedless $\times$ Ganesh, Khog $\times$ Jalore seedless, Ganesh $\times$ Khog, Muskut seedless $\times$ Ganesh and Khog $\times$ Ganesh), grown under Jodhpur (Rajasthan, India) conditions. Observations were recorded for fruit colour, weight, aril colour, aril weight and rind per cent. Juice content and total soluble solids (TSS), acidity, anthocyanin content, maturity index. Khog $\times$ Jalore seedless gave the highest fruit weight (200.4 g), rind thickness (0.39 cm) and rind percentage (39 %). Maturity index (16.39 %). Khog $\times$ Ganesh gave the highest 100-aril weight (29.40 g). Both Muskut seedless $\times$ Ganesh and Basin seedless gave the highest aril percentage (68 %), while Khog $\times$ Jalore seedless gave the lowest aril percentage (61 %). Basin seedless gave the highest juice percentage (65 %), acidity (2.91 %) and anthocyanin content (77.85 mg/ 100 g). Eichakdana $\times$ Jalore seedless and Muskut seedless $\times$ Ganesh gave the highest total soluble solids content (18.0 °Brix).

Singh (2004) studied the performance of nine cultivars: Ganesh, P-23, P-26, G-137, Dholka, Jodhpur Red, Basein Seedless, Jalore Seedless and GICVK-1.

Sarkhosh *et al.* (2009) studied relationships among quantitative and qualitative characters of pomegranate. In this study 28 qualitative and quantitative traits of pomegranate seeds and fruits were evaluated. Analysis of variance revealed that all of the investigated characters between examined genotypes were significant, showing variability of each character between genotypes. Results of simple correlation analysis showed the existence of significant positive and negative correlations among characters. Factor analysis performed for determining the main characters showed that the fruit juice traits acted as a main factor and distributed to about 32.3 per cent of total variance. Cluster analysis using the seven factors divided genotypes into 5 sub-clusters at a distance of 9. This study showed that multivariate analysis could be a useful method for the discrimination of pomegranate genotypes.

Singh *et al.* (2010) studied 16 genotypes to estimate the genetic variability in respect of various quantitative and qualitative traits and their association with each other. Wide range of phenotypic variability and genotypic variability was observed in almost all the tree and fruit characters, particularly plant height, plant spread, fruit

weight, aril percentage, TSS and acidity. High variability exists for fruit yield-contributing traits, offering opportunity for improvement through selection. Individual plant selection for yield, aril, percentage, fruit weight, number of arils and aril/rind ratio would be satisfactorily effective in pomegranate as these characters exhibited high heritability as well as high genetic advance.

Mir *et al.* (2010) evaluated ten genotypes of pomegranate for genetic variability. The range of variability was high for plant height, plant spread, days to maturity, fruit weight and number of arils per fruit. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters under test. High heritability and high magnitude of genetic advance was recorded for plant height, fruit weight and number of arils per fruit. Whereas, low heritability was recorded in days to maturity, juice content, number of arils per fruits.

Zamani *et al.* (2013) conducted an experiment to characterize the pomegranate genotypes based on Principal Component Analysis (PCA). The results revealed that the first three components explained 61.64 per cent of the total morphological variation. Anthocyanin content, TSS, juice percentage, pistil length and rind thickness were predominant in the first component and contributed most of the total variation. Fruit characters such as acidity were negatively correlated with TSS ($r = -0.56$) and pH ($r = -0.86$). Clustering from morphological data allocated individuals into two main clusters with high variation.

Caliskan and Bayazit (2013) conducted experiment on morpho-pomological and chemical characteristics of pomegranate. The results showed that there was significant diversity among the accessions in terms of fruit quality parameters. And also demonstrated that there was a great morpho-pomological variability among the local pomegranates grown in eastern Mediterranean region of Turkey making them a valuable genetic source for incorporation into potential breeding programs especially for different fruit quality characteristics.

Faten and Messaoud (2014) evaluated 28 accessions of Tunisian pomegranate for fruit quality traits by considering the effects of variety, clone and year and their interactions. The results indicated that variety contributed most to the variance in fruit size and colour, while the largest variances in rind thickness, aril and juice yields were

associated with year. Clones differed in most traits, reflecting differences in their genetic background. Also, clone $\times$ year interactions were significant for all traits, indicating the influence of environment on the expression of attributes of pomegranate fruit. High heritability along with genetic advances in fruit weight, fruit colour and acidity suggested that there were significant additive gene effects for such traits. Fruit weight and fruit diameter were unstable over years. In general, the genotypic correlations were lower in magnitude than the corresponding phenotypic correlations for all pairs of traits. Interestingly, the positive genotypic correlations between fruit colour and acidity and between aril and juice yields, confirmed the possibility of selecting clones with red-coloured and acid fruit and others with high aril and juice yields.

Ismail *et al.* (2014) characterized six pomegranate (*Punica granatum* L.) cultivars for fruit quality traits. The fruit weight ranged between 185.0 to 479.4 g of Nab El Gamal and Assuity, The rind thickness varied from 0.6 mm in Araby, Hegazy and Wardi to 0.3 mm in Assuity. The arils weight ranged from 87.5 to 275 g 'Assuity' and 'Nab El Gamal' cvs., respectively. The juice volume ranged from 62.41 to 71.81 ml/100 g arils in 'Wardi' and 'Nab El Gamal' cvs., respectively. The TSS content ranged between 16.01 'Hegazy' and 12.55 per cent 'Assuity'. The anthocyanin content ranged from 1.47 to 10.03 for 'Araby' and 'Hegazy'. The pH values varied from 2.9 (Araby) to 3.3 (Wardi). The Egyptian cultivars of pomegranate have a wide variation in the morphological and chemical characteristics for many uses of fresh fruit and of industry purpose.

Nicolas *et al.* (2016) conducted an experiment to characterize the Spain genotypes of pomegranate based on ANOVA, principal component analysis (PCA) and cluster analysis for economic traits. Genotypes were categorized in to three principle component explaining 53.75 per cent total variation. A first component accounted for about 27.77 per cent of the total variation followed by PC2 (17.49 %) and PC3 (8.49 %). The most important variables integrated by PC1 were fruit weight (FW), length, diameter and arils. The weights of PC1 for leaf and flowers characteristics were less important. PC1 mainly separated the cultivars by the size of their fruits and number of arils. PC2 explained overall, the rind weight (and therefore aril yield), the woody portion index of the arils (seeds), the leaf area and the length of the fruit, flowers, petals and sepals, as well as acidity. Overall, this component differentiated the varieties by the

acidity of their juice. PC3 integrated characters related with the size of the flowers (FD, FL), leaf shape (LL, LB), rind thickness (Ec) and the maturity index (MI), Although, this component was less significant than PC1 and PC2.

2.5 Molecular characterization and diversity in pomegranate

The unprecedented advances in the field of molecular markers are due to the rapid development of population genomics in the last few years. Use of molecular markers has become the need of the hour. Marker is a differentiating or inferring agent. Molecular markers consist of specific molecules, which show easily detectable differences among different strains of a species or different species and whose inheritance is monitored (Spooner *et al.*, 2005). Molecular or DNA based markers are most recently developed and have many advantages over morphological and biochemical markers, may be Non PCR based RFLP (Restriction Fragment Length Polymorphism) or PCR based like RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), SCAR (Sequence Characterized Amplified Regions), CAPS (Cleaved Amplified Polymorphic Sequence), ISSR (Inter-Simple Sequence Repeats), AFLP (Amplified Fragment Length Polymorphism) *etc.* Molecular markers have been proving a pivotal role in different aspects of plants, their relationships, genome mapping (Grattapaglia *et al.*, 2009), gene tagging, diversity studies, marker aided selections, construction of genome libraries, quantitative trait loci (QTL) analysis, *etc.* (Karp *et al.*, 1997).

Random Amplified Polymorphic DNA (RAPD) refers to polymorphism (variations) found within a species in the randomly amplified fragments of DNA generated. This system is based on the polymerase chain reaction and involves the use of short arbitrary oligonucleotides that amplify random segments of the genome of the tested plant. This technique was proposed by Williams *et al.* (1990). The RAPD markers are dominant. RAPD polymorphisms are produced by the presence or absence of PCR amplicons. Specific regions of the DNA molecule are amplified for analysis of variation by the PCR, using primers (usually 10 bases). The number of amplified fragments generated by PCR will depend on the length and sequence of the primer and the genome size.

Simple Sequence Repeats (SSRs) were originally developed as probes consisting of synthetic dinucleotide or trinucleotide motifs to screen numerous clones in DNA libraries. Based on the DNA sequence of screened candidates, PCR primers are designed to identify the motifs that may be dispersed throughout the genome. These PCR primers can discriminate DNA sequences that share the same motifs, but contain a different number of repeats. The development cost of these markers is usually high. Once SSR markers are developed, their ability to identify high polymorphic loci distributed throughout the entire genome sequence gives them significant advantages over other marker techniques (Sakai *et al.*, 2005).

DNA polymorphisms can be detected in nuclear and organelle DNA, which is found in mitochondria and chloroplasts. Molecular markers concern the DNA molecule itself and, as such, analyze variation at the genetic level directly. Molecular markers reveal neutral sites of variation at the DNA sequence level. These variations do not necessarily show themselves in the phenotypes. They can also provide information about a specific character such as resistance to a disease, tolerance *etc.* They are not subject to environmental influences and are potentially unlimited in number unlike morphological and protein based markers, covering the entire genome. They are the feasible markers to use for studying linkages with quantitative trait loci and have greater versatility and resolving power (Spooner *et al.*, 2005).

Koohi *et al.* (2005) evaluated the level of polymorphism in pomegranate cultivars. Four primer pairs were designed based on flanking regions of the microsatellites using the Oligo software. Among the primer sets, two pairs amplified cleanly and produced an easily interpretable PCR product in the pomegranate cultivars. A₁₈ primer pairs displayed polymorphism among 29 pomegranate cultivars. Gorche-Shahvar and Nabati-Ardakan cultivars appeared to be homozygote in this locus while the other cultivars were heterozygote. A₁₀ primer was monomorph and did not show polymorphism among cultivars.

Koohi-Dehkordi *et al.* (2006) in their study constructed microsatellite- enriched libraries from pomegranate and identified microsatellite-containing sequences. Two genomic libraries enriched for AG and GT repeats were constructed from 'Malase Yazd'. The enrichment was efficient and was 51 per cent in the GA library and 47 per

cent in the GT library. Primer pairs were designed for 15 microsatellite-containing sequences. Five out of 15 primer pairs amplified the expected size fragment and were polymorphic in 29 pomegranate individuals.

Curro *et al.* (2010) assessed the level of genetic diversity among cultivars and wild genotypes. Nine Simple Sequence Repeat (SSR) markers were obtained using the Microsatellite-AFLP technique and were successfully amplified in 34 genotypes belonging to Italian, Spanish and Turkish germplasm collections. The number of alleles per locus ranged from 1 to 5 and the total number of alleles was 22.

Ebrahimi *et al.* (2010) reported the development of simple sequence repeat (SSRs) in pomegranate using an enrichment method that makes use of magnetic beads. Enriched genomic libraries with AG and ATG microsatellite motifs were constructed and 60 positive clones were detected by a colony PCR technique.

Hasnaoui *et al.* (2010) reported the development of 11 microsatellite markers (SSR) for *Punica granatum* L. Evaluated on a set of 27 pomegranate accessions sampled in Tunisia, they displayed 25 alleles, with number of alleles per locus ranging between 1 to 4 and an observed heterozygosity from 0.037 and 0.592. This set of SSR markers could be very useful for studies dealing with genetic diversity, assessment of germplasm with cultivars and varieties, fingerprinting and pedigree analysis of this economically important crop species.

Pirseyyedi *et al.* (2010) reported the isolation and characterization of 12 polymorphic microsatellite markers from a repeat-enriched genomic library of *Punica granatum* L. The genetic diversity of these loci was assessed in 60 genotypes of *Punica granatum* L.

Basaki *et al.* (2011) used microsatellite markers to identify informative markers associated with traits sunburn sensitivity, hull cracking sensitivity, fruit height, fruit diameter, fruit shape index (fruit height/diameter ratio), calyx height, calyx diameter, calyx height/diameter ratio, fruit shape, calyx shape, calyx type, fruit taste, flower height, flower diameter, flower height/diameter ratio and style height. From 30 primers that were used, 7 pairs were polymorph and products 23 alleles in 202 pomegranate genotypes.

Soriano *et al.* (2011) reported the development of 117 microsatellite loci from a CT/AG-enriched pomegranate genomic library. In order to check their utility, 11 accessions were analyzed and checked their utility with the polymorphism information content (PIC) value across all loci ranged between 0.09 and 0.71 with an average of 0.37.

Alamuti *et al.* (2012) evaluated the genetic background of 738 pomegranate (*Punica granatum* L.) by using a set of twelve simple sequence repeat markers from 23 provinces of Iran. Genetic diversity among the accessions was analyzed using several variability parameters. Forty-three alleles were detected with a mean of 3.59 alleles per locus. The highest levels of polymorphism were found for the locus ABR11-MP26. The mean values of expected heterozygosity and polymorphic information content were 0.521 and 0.458, respectively. Neighbour-Net analysis based on uncorrected P distances and a model-based method separated the accessions into 8 main groups and several subgroups, with a tendency to cluster by geographical origin.

Gadaleta *et al.* (2012) applied molecular characterization on a set of 15 pomegranate accessions collected from different areas of Apulia region. A total of 20 SSRs were amplified in the pomegranate collection and the polymorphic fragments visualized by capillary electrophoresis. The obtained SSR profiles allowed to specifically characterize each genotype, which may represent a useful tool for the screening of pomegranate germplasm to adopt in breeding programs and as a perspective of further development of pomegranate cultivation in Apulia region.

Jbir *et al.* (2012a) used specific microsatellites (SSRs) markers to characterize a set of 32 Tunisian pomegranate (*Punica granatum* L.) cultivars. Using 13 SSR primers, a total of 40 alleles and 46 genotypes had been identified. As a result, data proved that a high level of polymorphism characterizes the Tunisian pomegranate germplasm at the DNA level. The derived Neighbour-joining (NJ) dendrogram constructed using DAS genetic distances exhibited a genetic diversity structured independently from the geographical origin of cultivars and their denomination. This result suggested that a common genetic basis may characterize Tunisian pomegranate cultivars despite their phenotypic divergences.

Jbir *et al.* (2012b) in their study used microsatellite markers in molecular characterization of 18 Tunisian pomegranate cultivars. The DNA was analysed using 4 pre-selected SSR primers that revealed 13 alleles, which allowed each genotype to be identified. An UPGMA dendrogram was generated using Cavalli-Sforza and Edwards genetic distances. This result was compared to the one based on AFLPs. For AFLPs study, four primers' combinations tested for their ability to generate dominant markers. A total of 192 bands were scored. A genetic distance matrix according to Nei and Li's formula was calculated to construct an UPGMA phylogram. In addition, correlation between distance matrices was assessed using the Mantel test. The results proved a high level of molecular polymorphism in the local pomegranate germplasm and showed efficiently of these molecular markers in the surveying or in the differentiation between cultivars.

Jian *et al.* (2012) selected 28 primer pairs as representative samples for evaluating usability and 18 (64.3 %) were usable markers. 15 of the 18 markers revealed polymorphism among 42 pomegranate accessions, detecting 2-5 alleles (mean = 2.80). The observed heterozygosity (*Ho*) and polymorphic information content (PIC) values ranged from 0.119 to 0.619 (mean = 0.381) and from 0.091 to 0.656 (mean = 0.402), respectively. Genetic diversity of the accession set was elucidated using the newly-developed markers, revealing a distinctive genetic background of the accessions from east-central China.

Norouzi *et al.* (2012) investigated the genetic diversity of Iranian pomegranates, efficiency of universal chloroplast microsatellite loci on 51 cultivated, wild and ornamental Iranian genotypes and five genotypes from outside of Iran as out groups was assessed. The results showed that 16 loci out of 25 loci were polymorphic and revealed 39 alleles with an average of 2.44 alleles per locus. The range of polymorphism information content (PIC) was 0.02-0.39 (average, 0.24), which showed that the majority of loci were informative.

Raina *et al.* (2013) assessed genetic diversity in pomegranate genotypes using 47 SSR markers. A total of 3,154 alleles were amplified with an average of 105 alleles per

genotype. Number of bands amplified ranged from as low as 92 in Russian Seedling to as high as 114 in Shirin Anar. The number of alleles detected for 47 SSR primers ranged from 1 to 4 with an average of 2.15 alleles per primer pair. The polymorphism information content (PIC) of SSR markers ranged between 0.0 and 0.66 with an average value of 0.43 per locus. Genetic similarities among 30 pomegranate genotypes varied from 0.78 to 0.95. The UPGMA dendrogram cluster analysis grouped the pomegranate genotypes into three major clusters (I, II and III). Maximum number of (21) genotypes were clubbed in cluster III and further subdivided into three different sub-clusters IIIA, IIIB and IIIC. UPGMA dendrogram generated showed some genotypes from same province clustered in one group like genotypes Kali Shirin, Anar Shirin, Shirin Anar, Anar Shirin Mohamad Ali Anar Moherab Shirin and Panipat Selection collected from Hisar (India) clustered in cluster IIIA and genotypes Moga Local, P-26, Khog, Ps-75-K3 and Kandhari Ganga Nagari grouped in cluster II were collected from Punjab. SSR markers as an excellent genetic marker system revealed a considerable variation and provided accurate genetic information for allelic profile of genotypes in pomegranate germplasm.

Ravishankar *et al.* (2015) developed and characterized microsatellite markers using Roche 454 GS FLX Titanium pyrosequencing technology. The average G+C content was 41.3%. Primer pairs were designed for total of 171 loci, out of which 167 produced reproducible polymorphic bands in 12 genotypes of pomegranate. A total of 951 alleles were identified, ranging from 1 to 14 per locus (average of 5.56). The polymorphism information content (PIC) value ranged between 0.00 and 0.910 (mean of 0.5427).

Sinjare (2015) revealed that 25 alleles were detected with average of 2.08 alleles per locus. The mean values of expected (H_e) heterozygosity was 0.33. The mean polymorphic information content was 0.26. Genetic distance among the 11 varieties ranged from 0.06 to 0.27. Results of overall data and cluster analysis suggested that this set of primers can be very useful for studying genetic diversity and pedigree analysis of this important economical important fruit species.

2.6 Marker trait association (MTA) and association mapping for plant growth, fruit quality traits and bacterial blight disease resistance in pomegranate

There is a tremendous interest in using marker trait association (MTA) and association mapping to identify genes responsible for quantitative variation of complex traits with agricultural and evolutionary importance. Recent advances in genomic technology, impetus to exploit natural diversity and development of robust statistical analysis methods makes association mapping appealing and affordable to plant research programs. Although traditional QTL-mapping will continue being an important tool in gene tagging of crops, it is now a classical approach and overall is very costly and has low resolution with simultaneous evaluation of only a few alleles (Garcia *et al.*, 2003). Limitations of the QTL approach have led to a change in research focus towards identifying variations in the DNA sequence of genes directly controlling phenotypic variation known by some as Gene Assisted Selection. One of the main advantages of association genetics is the high resolution of marker-trait associations. As natural populations are used in association studies, recombination's that accumulate over many generations of the population break, any long range associations between marker and trait leaving short stretches of the genome associated with the trait. If alleles or SNPs can be found that are strongly associated with superior phenotypes, they can be used for selection across breeding populations. This methodology is better suited to fruit breeding programmes, which aim to maintain a broad genetic base. In contrast, the QTL approach is used for within-family selection. Spurious associations may be observed in association studies where there is undetected genetic structure in the breeding population that invalidates standard statistical tests. Strategies for dealing with population stratification have been developed to avoid these problems (Pritchard *et al.*, 2000 and Wu and Zeng, 2001).

Performing designed crosses for linkage studies, which may sometimes lead to near-isogenic lines is not always possible in fruit trees, which is a serious limitation on the use of molecular markers. In such cases, where the mapping population under study are too small, Linkage disequilibrium based association analysis has received great attention from last few years. This approach has the potential not only to identify and map QTLs, but also to identify polymorphism within a gene that is responsible for the

difference in two alternate phenotypes (Gupta *et al.*, 2005). In linkage mapping the major limitation, is associated with the availability of only a few meiotic events to be used that occurred since experimental hybridization in a recent past. These limitations, however, can be reduced with the use of "association mapping" (Ibarra *et al.*, 2007). According to Goldstein and Weale (2001), turning the gene-tagging efforts from biparental crosses to natural population of lines (or germplasm collections) and from traditional QTL-mapping to linkage disequilibrium (LD)-based association study became a powerful tool in mapping of the genes of interest.

Vijayan *et al.* (2006) has also worked out molecular characterization and identification of different markers associated with yield traits in mulberry using ISSR markers using stepwise multiple regression analysis taking traits as dependent variable and ISSR markers as independent variable. A particularly important variable when considering the prospects of genomic selection in fruit trees is the extent of LD. Due to outbreed nature and recent domestication of fruit trees, LD has been reported to extend to short ranges,. In such populations, prohibitive number of markers would be required to perform genomic selection with appreciable selection accuracy. Elite population with population size 20-100 provide substantially higher LD that would warrant the application of genomic selection (White *et al.*, 2007).

Martinez *et al.* (2008) studied 46 single nucleotide polymorphisms (SNPs) from 41 disease and abiotic stress-inducible genes for their genetic association with carbon isotope discrimination (CID), a time-integrated trait measure of stomatal conductance. A family-based approach to detect genotype/phenotype genetic association was developed for the first time in plants by applying the quantitative transmission disequilibrium test on an association population of 961 clones from 61 families evaluated for phenotypic expression of CID at two sites. Association mapping identifies quantitative trait loci (QTLs) by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm. General understanding of association mapping has increased significantly since its debut in plants (Zhu *et al.*, 2008). Association genetics and population genomics are promising ways to find genes controlling growth in fruit trees. Adopted from human, animal and crop genetics, the association or linkage disequilibrium (LD) mapping paradigm was proposed in forest

trees. It was pioneered in *Eucalyptus* (Thumma *et al.*, 2005) and applied in a number of studies as sequencing information has become available. The approach quickly gained interest because it could be readily applied to large random mating tree populations potentially overcoming the logistic and time limitations and poor resolution of QTL mapping in fruit trees. Because of the limited LD, high nucleotide diversity and lack of reference genome sequences, association genetics has relied on SNP genotyping of candidate genes.

Association mapping is based on LD and used for finding statistical associations between genetic markers and phenotypes. After many generations of recombination and random mating, only alleles of tightly linked loci can keep their association in the population, which allows association mapping that is finer than QTL mapping. Moreover, if candidate genes are used as markers, individual alleles directly involved in genetic control of the phenotype can be detected (Gonzalez *et al.*, 2007). Krutovsky *et al.* (2009) developed methods that take into account the population structure or use family data in populations with known pedigrees. Thus, studying the genetic structure of the population used for association mapping is of particular importance. Neutral markers, such as highly polymorphic microsatellites are usually used for this. In addition to subdivision of the population, its size and origin are of significance in association mapping. It is also necessary to achieve sufficient precision in measuring phenotypic traits. The parameter of genetic differentiation was standardized to take into account high within-population variation in the SSR loci. They also found that the lack of pronounced population subdivision in the studied area should facilitate association mapping in this experimental population and recommended taking the STRUCTURE analysis and population assignments for individual fruit trees (Q-matrix) into account in association mapping.

Sounigo *et al.* (2009) conducted association mapping to identify the genome regions controlling Cocoa traits of agronomic interest for Cocoa and molecular markers usable for a marker assisted selection program on a population composed of 263 Cocoa trees planted in a farm and using 12 commercially released full- sib progenies. These trees were analyzed using 125 SSR markers defined in expressed genes and mapped along the ten Cocoa chromosomes. STRUCTURE software was used to determine the

number of sub-populations in the chosen population and the percentage of admixture of each tree. TASSEL software was used to perform association mapping taking into account the admixture percentages, in order to identify molecular markers linked to flowering intensity, yield, mean number of beans per pod, mean weight of one bean, tolerance to *Phytophthora megakarya* and tolerance to mirids. Several significant associations between molecular markers and these traits were identified. The results were used in the MAS program on cocoa in Cameroon.

Besides the current limitation of scanning only a few hundred genes, association studies in tree species have explored almost exclusively the coding regions of genes, while largely ignoring the regulatory regions. Owing to the rapid decay of LD commonly observed in natural populations of forest trees, mutations that alter the gene expression may not be detected by indirect associations with SNPs in coding regions and could be overlooked in the candidate gene approach (Grattapaglia *et al.*, 2009). They also appreciated that although population genomics may represent a promising approach to identify genes for growth and adaptive traits, limitations still exist especially regarding the selection of candidate genes. There is an ascertainment bias associated with SNP discovery and functional information about the genes' role is needed to validate candidates. According to Dillon *et al.* (2010), forest trees are ideally suited to association mapping due to their high levels of diversity and low genomic linkage disequilibrium. Using an association mapping approach, they identified single-nucleotide polymorphism (SNP) markers influencing quantitative variation in wood quality in a natural population of *Pinus radiata*. Of 149 sites examined, ten demonstrated significant associations with one or more traits after accounting for population structure and experiment wise error. Without accounting for marker interactions, phenotypic variation attributed to individual SNPs ranged from 2 to 6.5 per cent. They did not observe any undesirable negative correlations between wood quality and growth, indicating potential to break negative correlations by selecting individual SNPs in breeding programs. Markers that yielded significant associations were validated in an Australian land race. SNPs from three genes (PAL1, PCBER and SUSY) yielded significant associations. Importantly, associations with two of these genes validated associations with density previously observed in the discovery population. In both cases, decreased wood density was associated with the minor allele suggesting that

these SNPs may be under weak negative purifying selection for density in the natural populations. These results demonstrates that the utility of LD mapping to detect associations, even when the power to detect SNPs with small effect is anticipated to be low.

Wahida *et al.* (2010) used nuclear microsatellites (ncSSRs) to compare genetic structure and diversity estimates of natural populations of Moroccan maritime pine (*Pinus pinaster*). Samples were collected among 10 natural populations distributed in three biogeographically different regions, the Rif Mountain, the Middle and the High Atlas. Forty-five nuclear alleles at seven variable loci were found with a mean of 6.4 alleles per locus. A number of private alleles (17.1 %) were shown in populations from Rif and Middle Atlas. Moreover, in Morocco, *P. pinaster* showed a lower genetic diversity than in other parts of its geographic range. Results showed a correlation between genetic and geographic distances with an R-squared of 0.436. Two clusters were found using STRUCTURE, whereas three main clusters can be distinguished based on genetic distances of phylogenetic tree. Genetic relationships among maritime pine populations in Morocco appear to be related to historical, ecological as well as anthropogenic factors, suggesting the need for conservation strategies at the population level.

Basaki *et al.* (2011) used microsatellite markers to identify informative markers associated with traits, Fruit height, Fruit diameter, Calyx height, Calyx diameter, Flower height, Flower diameter and Style height. From 30 primers that were used seven pairs were polymorphic and produce 23 alleles in 202 pomegranate genotypes. The mean number of alleles was 3.28 alleles for each microsatellite locus. Polymorphic information content ranged from 0.01 (Locus MP07) to 0.56 (Locus MP39) with an average of 0.34. Stepwise regression analysis between molecular data as independent variables and morphological data as dependent variables was performed to identify informative markers associated with the traits. Among the 14 traits, eight traits showed significant association on a total of 14 of the 23 polymorphic SSR bands. The association markers explained 2 to 29 per cent of the variation for individual traits. The most variation of calyx height (0.29) was accounted by MP30-11, MP51-13, MP51-16 and MP07-21. SSR loci associated with fruit diameter and size was the same. The results showed there is a significant and positive correlation among these traits. MP26

marker was linked with most traits studied. Since all the used SSR loci particularly MP26 showed significant association with the studied traits, therefore, it is possible to use these markers along with morphological traits in pomegranate breeding programs for identification of suitable parents to produce mapping populations and hybrid varieties.

Basaki *et al.* (2013) conducted a study to evaluate the relationships between simple sequences repeat (SSR) markers and important traits using the multiple stepwise linear regressions in pomegranate. Initially, they used 30 SSR molecular markers, seven of which were amplified polymorphic products, while the remaining were monomorphic. Among the studied traits, seed softness and aril size had the most adjusted total R^2 ; hence, the associated markers were probable close to coding regions of these traits and could be used for future breeding programs after supplementary precise mapping. Significant regressions of 6 out of the 9 traits (excluding 100-aril weight, leaf length and leaf width) were observed on a variable number of different SSR markers and therefore may be proved useful for marker-assisted breeding after further and necessary validation.

Singh *et al.* (2015) aimed to estimate the population structure and explore the use of association mapping strategies to identify linked markers for bacterial resistance, growth and fruit quality in pomegranate collections from India. In total, 88 accessions including 37 cultivated types were investigated. A total of 112 alleles were amplified by use of 44 publicly available microsatellites for estimating molecular genetic diversity and population structure. Neighbour-joining analysis, model-based population structure and principal component analysis corroborated the genetic relationships among wild-type and cultivated pomegranate collections from India. Identified a cultivated clade of pomegranates in close proximity to Daru types of wild-type pomegranates that grow naturally near the foothills of the Himalayas. Admixture analysis sorted various lineages of cultivated pomegranates to their respective ancestral forms and able to identify four linked markers for fruit weight, titratable acidity and bacterial blight severity. PGCT001 was found associated with both fruit weight and bacterial blight.

3. MATERIAL AND METHODS

The present investigation was aimed at evaluation of large number of γ -rays irradiated seed derived progenies of pomegranate for plant growth, fruit quality traits and bacterial blight disease resistance. The experiments were carried at laboratory, green house and field during 2014-2015 and 2015-16 at Horticulture Research and Extension Station, Vijayapur (Tidagundi), University of Horticultural Sciences, Bagalkot (Plate 1). It is located on the Northern Dry zone of Karnataka state at 16° 59' North latitude, 75° 45' East longitude and at an altitude of 513 m above mean sea level with an average annual rainfall of about 569 mm. The mean monthly maximum and minimum temperature during crop growth period obtained from the Indian Metrological Department (IMD) observatory of the Horticulture Research and Extension Station, Vijayapur (Tidagundi), University of Horticultural Sciences, Bagalkot are presented in Appendix I. The soil type of the experimental block is medium black (Vertisol) with a pH in the range of 7.0 to 7.5. The material and methods used for the present study are mentioned in the present chapter.

Experiment-I

3.1 To Isolate and assess the variability of the pathogen causing bacterial blight (*Xanthomonas axonopodis* pv. *punicae*) of pomegranate

3.1.1 Isolation of the *Xanthomonas axonopodis* pv. *punicae*

The diseased sample of *Xanthomonas axonopodis* pv. *punicae* were collected from different locations of Karnataka (Table 2). The infected plant parts such as leaves showing typical symptoms of pomegranate bacterial blight was cut into small bits and surface sterilized with 0.1 per cent sodium hypochlorite (NaOCl_3) for 10 sec followed by three washes using sterilized distilled water. After drying with sterilized filter paper, these bits were crushed in sterilized distilled water. A loop full of suspension was streaked on nutrient agar (Yeast extract 5 g, peptone 10 g, agar 20 g and distilled water 1000 ml, pH 7.00) medium petri plate and incubated at 28 ± 1 °C for 5-6 days.

Table 2: Collection of bacterial isolates from different locations of Karnataka

Isolate	Place of collection	District	Plant part	Variety
<i>Xap</i> 1	Tidagundi	Vijayapur	Leaf	Bhagwa
<i>Xap</i> 2	Kaladagi	Bagalkot	Leaf	Bhagwa
<i>Xap</i> 3	Munirabad	Koppal	Leaf	Bhagwa
<i>Xap</i> 4	Hiriyur	Chitradurg	Leaf	Bhagwa

Table 3: Characterization of *X. axonopodis* pv. *punicae* with specific marker genes 16s RNA and *gyrB*

Sl. No.	Primers	Sequence (5'-3')	Annealing temperature (°C)	Size (bp)
1	16s rRNA	Xsp16SRFWD2- CTTACGCTAATACCGCATATACG Xsp16SREV1- CTGATCTGCGATTACTAGCGA	55	753
2	<i>gyrB</i>	Forward 5'GTTGATGCTGTT CACCAGCG3' Reverse 5'CATTCATTT CGCCCAAGCCCC3'	55	491

Observations were taken for the development of clearly separated typical bright yellow colonies. Such pure colonies were further sub-cultured onto the agar slants containing the nutrient agar (NA) medium and incubated at $28 \pm 1^{\circ}\text{C}$ for 5- 6 days and then cultures were stored in the refrigerator at 4°C , which served as stock culture for further studies. The four pure cultures obtained after isolation from the diseased samples of all the regions were designated as different isolates from *Xap1* to *Xap4* and maintained further use.

3.1.2 Pathogenicity test

The susceptible varieties Bhagwa and Ganesh were selected for examining pathogenicity on pomegranate plants, which were grown in earthen pots of 30 cm size filled with clay loam soil and FYM in 1:1 ratio in the polyhouse. The temperature and relative humidity in the polyhouse was maintained to $25 \pm 5^{\circ}\text{C}$ and 70 - 80 per cent, respectively. The pathogen cell suspension of all four cultures was independently (2.5×10^8 cfu/ml) sprayed on five weeks old plant that attained 15 - 20 cm height with 5-10 leaves per shoot. The characteristic symptoms observed on pomegranate leaves after 15 to 18 days of inoculation as small, water-soaked, brown to black colored circular to irregular lesions, surrounded by yellow halo. Re-isolations were carried out from these lesions with the original culture to confirm the identity of the pathogen.

3.1.3 Characterization of the *Xanthomonas axonopodis* pv. *punicae*

The isolated four strains of *Xanthomonas axonopodis* pv. *punicae* from pomegranate were further confirmed with morphological, biochemical and molecular level as per standard procedures.

3.1.3.1 Morphological characterization

The morphological characteristics of the *X. axonopodis* pv. *punicae* such as microscopic observation viz., cell shape, appearance and colony size, naked eye observations viz., colony colour and bacterial staining with gram reaction were studied as per the standard procedures described by Schaad (1992).

3.1.3.2 Molecular characterization

However, the morphological and biochemical reactions confirmed and characterized the pathogen but it is essential to confirm at genomic level. Hence, bacteria were characterized with specific marker genes viz., 16s rRNA and *gyrB*.

Genomic DNA extraction

Single colony of *X. axonopodis* pv. *punicae*, bacteria was inoculated in NA broth and incubated at 28 °C for 72 hrs. Cells were harvested by centrifuging at 12,000 rpm for 5 min. Cells were lysed by treating with 550 µL buffer (10 mM EDTA + 10 mM Tris base, pH 8) and 20 µL of freshly prepared lysozyme (0.01 mg/ml of buffer) and incubated in a micro centrifuge tube for 30 min at 35 °C. Cell suspension was treated with 100 µl 5 M NaCl + 76 µl of 10 per cent SDS and 80 µl of CTAB/NaCl and tubes were incubated at 65 °C for 20 min. Tubes were cooled down to room temperature and to remove protein contaminants equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed thoroughly and centrifuged at 12,000 rpm for 10 min at room temperature. Supernatant was carefully transferred to fresh sterile 1.5 ml centrifuge tubes and 1.5 volume of ice-cold isopropanol was added to precipitate DNA and incubated at -20 °C overnight. Tubes were then centrifuged at 12,000 rpm for 10 min at 4 °C and the pellet was washed twice with 70 per cent ethanol. Tubes were air dried completely at room temperature and the DNA was dissolved in sterile nuclease free water and stored at 4 °C. Quantification of DNA was performed by measuring the absorbance at 260 nm in Nanodrop (Eppendorf), quality of the DNA was analysed in 0.8 per cent (w/v) agarose gel and DNA was diluted to uniform concentration of 100 ng/µl for further use.

Polymerase chain reaction

The four strains of *X. axonopodis* pv. *punicae* were confirmed with species specific marker genes viz., 16s rRNA and *gyrB* (Table.3). Hence, the PCR was performed with the standard protocol. The PCR amplification of 16s rRNA and *gyrB* primers were performed using 100 ng DNA template, 1X PCR Buffer, 200 µM dNTPs

mix, 0.4 μ M primer and 1 U *Taq* DNA polymerase (Merck, Bangalore, India) and sterile distilled water to make final volume of 20 μ l. PCR amplification was conducted with a thermo cycler (Eppendorf Vapo Protect, Germany). The amplification were carried out with an initial denaturation at 94 $^{\circ}$ C for 4 min, followed by 30 cycles of 30 sec denaturation at 94 $^{\circ}$ C, 45 sec of primer annealing at 55 $^{\circ}$ C, 1 min extension at 72 $^{\circ}$ C. The final extension step was for 10 min at 72 $^{\circ}$ C.

3.1.4 Variability studies of four strains

The cultures were evaluated to know the variability of the four isolates at molecular level and disease infestation ability.

3.1.4.1 Pathogenic variability

One month old susceptible and resistance varieties of 14 pomegranate genotypes viz., Tobesta, Jyothi, P-13, G-137, P-26, P-23, BT skin, P-16, Patna-5, Kalipathiya, Nana, Ganesh, Bhagwa and Ruby were transplanted into the pots containing sterilized soil amended with necessary nutrients. The seedlings were regularly watered and exposed to sufficient sunlight. To prove the pathogenicity of *X. axonopodis* pv. *punicae*, it was mass multiplied in NA broth. Two months old pomegranate plants were sprayed with prepared bacterial suspension (2.5×10^8 cfu/ml) and for the control plant the water was sprayed with an atomizer. The inoculated plants were kept in polyhouse for further observations.

3.1.4.2 Molecular variability

All four isolates were screened with three different markers viz., REP, ERIC and BOX (Table 4) to distinguish among them at genetic level (Arshiya *et al.*, 2014). The PCR amplification of all three primers were performed independently using 100 ng DNA template, 1X PCR Buffer, 200 μ M dNTPs mix, 0.4 μ M primer and 1 U *Taq* DNA polymerase (Merck, Bangalore, India) and sterile distilled water to make final volume of 20 μ L. PCR amplification was conducted with a thermo cycler (Eppendorf Vapo Protect, Germany). The amplification were carried out with an initial denaturation 94 $^{\circ}$ C for 4 min, followed by 30 cycles of 30 sec denaturation at 94 $^{\circ}$ C, 45 seconds of primer annealing at 55 $^{\circ}$ C, 1 min extension at 72 $^{\circ}$ C. The final extension step was for 10 min at 72 $^{\circ}$ C.

Table 4: Primer sequence for molecular variability studies of *X. axonopodis* pv. *punicae*

Sl. No.	Primer	Nucleotide sequence	Annealing temperature
1	REP1R-I	5' IICGICGICATCIGGC 3'	44°C.
	REP2-I	5' ICGITTATCIGGCCTAC 3'	
2	ERIC1R	5' ATGTAAGCTCCTGGGGATTCA3'	52°C.
	ERIC2	5' AAGTAAGTGACTGGGGTGAGCG 3'	
3	BOXA1R	5'CTCCGGCAAGGCGACGCTGAC3'	53°C.

(Arshiya *et al.*, 2014)**Table 5: Disease reaction scale for bacterial blight of pomegranate on leaves**

Numerical rating	Percentage blighted area on leaves	Description	Reaction
0	Disease free	Disease not seen	Resistant
1	1-10	Disease not easily visible, very few units/plant found diseased after careful search	Tolerant
2	11-25	Disease visible easily in each direction but most (75%) of the units look healthy	Moderately susceptible
3	26-50	Both disease and healthy units are equally observed	Highly susceptible
4	51-75	Disease seen easily with only some healthy units	Highly susceptible
5	76-100	Almost all units are diseased, with few healthy units seen on careful search	Highly susceptible

(Singh *et al.*, 2015)

3.1.5 Screening of pomegranate germplasm bacterial blight disease resistance on leaves

Fourteen pomegranate germplasm were screened out for the resistant and susceptibility of bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae*. These germplasm were planted in polyhouse at a distance of 30 cm × 30 cm apart and when they attained a height of 15-20 cm the bacterial suspension of 2.5×10^8 cfu/ml was sprayed on these plants. The data were recorded using the disease severity scale with reaction of the disease (Table 5).

Experiment-II

3.2 Evaluation of g-ray irradiated seed derived progenies and germplasm of pomegranate for plant growth, fruit quality traits and bacterial blight disease resistance

The disease on leaves was scored for 475 genotypes (γ-rays irradiated seed derived progenies (cv. Bhagwa and Ganesh) and 23 popular germplasm (Appendix II) under polyhouse (*in vitro*) as well in field level (*in vivo*) during *mrigbahar* 2014-15 and 2015-16. Whereas, disease severity was scored on fruit and stem in field level during *Ambe bahar* during 2014-15 and 2015-16. The protocol followed is mentioned as below.

3.2.1 Disease severity on leaves

3.2.1.1 *In vivo*

The 475 pomegranate lines (γ-ray irradiated seed derived progenies of pomegranate and 23 released popular genotypes mentioned in Appendix II) were evaluated including checks over two seasons (2014-15 and 2015-16) during June-July (*mrigbahar* flowering) in an Augmented Block Design. Each genotype was grown as single plant with a spacing of 210 cm between rows and 90 cm between plants. The plants were 3 years old. A general view of pomegranate field is given in Plate 1. The experiment was conducted under irrigated conditions following all the recommended agronomic practices to raise the good crop. The *X. axonopodis* pv. *punicae* was separately multiplied in NA broth (200 ml) as well as in petriplates using NA medium,



a) General view



b) Glasshouse experiment



c) Closeup view of glasshouse experiment

Plate 1: General view of the experiment

later inoculated with a loop full of active stage bacterial culture. The inoculated flasks and plates were incubated for 72 h at 30 °C. The plants were inoculated by spraying with prepared colony suspension (4.5×10^6 cfu/ml) of the bacteria with sprayer and observations recorded subsequently.

3.2.1.2 *In vitro*

One month old saplings of 475 pomegranate lines (23 released popular genotypes and 452 γ -rays irradiated seed derived progenies of pomegranate mentioned in Appendix II) were planted in the pots containing sterilized soil amended with necessary nutrients. The seedlings were regularly watered and kept in polyhouse for further establishment. *X. axonopodis* pv. *punicae*, was separately multiplied in NA broth (200 ml) as well as in petriplates using NGA medium, later inoculated with a loop full of active stage bacterial culture. The inoculated flasks and plates were incubated for 72 hour at 30 °C. The inoculum solution (4.5×10^6 cfu/ml) was prepared. Two months old pomegranate plants were sprayed with water and kept in polyhouse. The leaves of the pre-incubated pomegranate plants were inoculated by spraying with prepared colony suspension of the bacteria with an atomizer. The inoculated plants were kept in polyhouse for further observations.

3.2.2 Disease severity on fruits and stem

The 475 pomegranate lines (23 released popular genotypes and 452 γ -rays irradiated seed derived progenies of pomegranate mentioned in Appendix II) were evaluated including checks over two seasons (2014-15 and 2015-16) during February-July cropping season (Based on *Ambe bahar* flowering that occur during February-March) in a Augmented Block Design. In 2014-15 the crop was pruned during January-2015. Each genotype was grown as single plant with a spacing of 210 cm between rows and 210 cm between plants. The plants were 3 years old. A general view of pomegranate field is given in Plate 1. The experiment was conducted under irrigated conditions following all the recommended agronomic practices like weeding and plant protection measures to raise the good crop. Irrigation was given at weekly interval during summer months and fortnightly in the other months. Cleaning the basin and removal of weeds was carried out at fortnightly interval followed by irrigation. The

following observations were recorded on each genotype in both the seasons. Both qualitative and quantitative characters were recorded on selected 475 genotypes.

3.2.2.1 Quantitative characters

1) Days to maturity of fruit

The number of days required from bud formation to maturity of fruit were measured by tagging of the randomly selected five buds in each genotype was recorded.

2) Plant height

The height of the plant was measured in centimetre (cm) from ground level to the top of the canopy at the time of harvest.

3) Plant spread

The plant spread in East-West and North-South direction was measured in centimetre (cm) during active growth period.

4) Leaf thickness

The third leaf from the basal end of mature shoots of current season growth was taken for recording leaf thickness in millimetre (mm) by averaging thickness of ten randomly selected matured leaves from all sides of plants and measured with vernier caliper.

5) Leaf length

The third leaf from the basal end of mature shoots of current season growth was taken for recording leaf length in centimetres (cm) by averaging length of ten randomly selected matured leaves from all sides of plants.

6) Leaf breadth

The third leaf from the basal end of mature shoots of current season growth was taken for recording leaf breadth in centimetres (cm) by averaging width of ten randomly selected matured leaves from all sides of plants.

7) Flower length

Fully opened hermaphrodite flower was used for measuring the length in centimetres (cm). Measurement was done with the help of vernier caliper.

8) Flower breadth

Fully opened hermaphrodite flower was used for measuring the breadth in centimetres (cm). Measurement was done with the help of vernier caliper.

9) Pistil length

Fully opened hermaphrodite flower was used for measuring the pistil length in centimetres (cm). Measurement was done with the help of vernier caliper.

10) Fruit length

Fruit length from stalk end to persistent calyx end was measured with the help of vernier caliper (Mars and Marrakchi, 1999).

11) Fruit diameter

With the help of vernier calipers, the diameter of the fruit was measured at maximum thickness and recorded in centimetres as depicted in Fig. 1 (Mars and Marrakchi, 1999).

12) Fruit weight

Average weight of the fruit was calculated on the basis of a five fruits with the help of electronic balance (Sartorius) and expressed in grams (g).

13) Calyx length

Calyx length was measured with the help of vernier callipers and expressed in centimetres (cm).

14) Calyx diameter

With the help of vernier calipers, the diameter of the calyx was measured at maximum thickness and recorded in centimetres (cm).

15) Total aril weight

Total arils from one fruit were taken and weight of arils was recorded on electronic balance and expressed in grams (g).

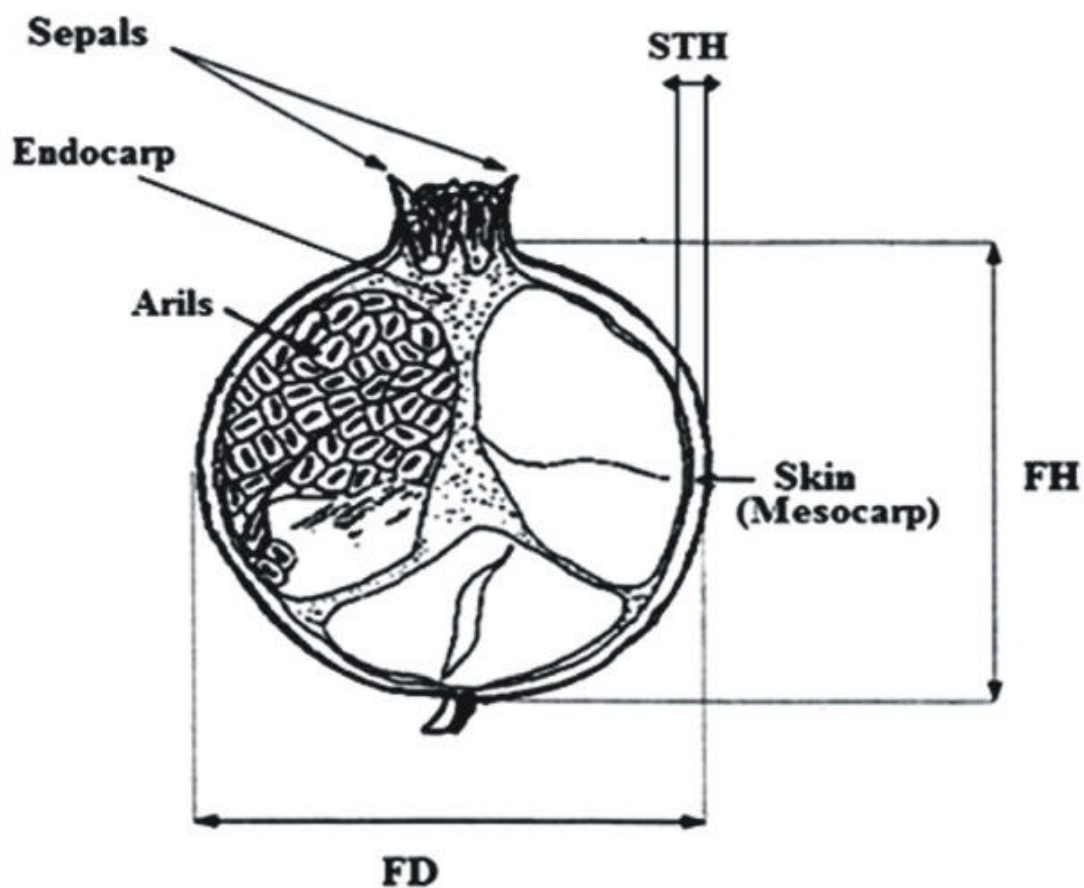


Fig. 1. Longitudinal section of a pomegranate fruit showing the different parts (Mars and Marrakchi, 1999). Parameters measured here were: FH, fruit height (mm); FD, fruit diameter (mm); and STH, skin thickness (mm)

16) Number of arils per fruit

Total number of arils per fruit was counted from five randomly selected fruits and average values were recorded.

17) Weight of 100 arils

A sample of 100 arils after separating the rind from fruit in each replication was taken and weighed on electronic balance and expressed in gram (g).

18) Single aril weight

A sample of 100 arils after separating the rind from fruit in each replication was taken and weighed on electronic balance and average weight of aril was expressed in grams (g).

19) Aril percentage per fruit

Random fruits were taken and peel was removed and kept aside for weighing. The arils were taken out. The aril percentage was counted by dividing the total aril weight with total fruit weight and expressed in percentage (%).

$$\text{Aril percentage} = \frac{\text{Total aril weight (g)}}{\text{Total fruit weight (g)}} \times 100$$

20) Juice percentage per fruit

Random fruits were taken and peel was removed and kept aside for weighing. The arils were taken out and juice extracted with electric juicer (Bajaj). The juice percentage was counted by dividing the total juice weight with fruit weight and expressed in percentage (%).

$$\text{Juice percentage} = \frac{\text{Total weight of juice (g)}}{\text{Total fruit weight (g)}} \times 100$$

21) Volume of juice

All of the juice from pomegranate fruits extracted and measured with the help of measuring cylinder and expressed in millilitre (ml).

22) Total soluble solids (°B)

The total soluble solids (TSS) was recorded by hand held refractometer (Erma Tokyo A032) by taking a drop of fresh juice (degree brix) on prism of the refractometer and observing it against the light.

23) pH of juice

The pH was recorded by using electronic pH meter.

24) Acidity

The juice acidity was determined by titrating 5 ml of juice against 0.1 N NaOH solution using phenolphthalein as an indicator. The end point was noted at the appearance of light pink colour and results were expressed in terms of maleic acid. The per cent acidity was calculated by following formula (AOAC, 1990).

$$\text{Acidity (\%)} = 0.0067 \times \frac{\text{0.1 N NaOH used}}{\text{Juice taken}} \times 100$$

(1 ml of 0.1N NaOH = 0.0067 g maleic acid)

25) Maturity Index (TSS/Acidity)

The ratio was calculated by dividing the value of TSS with that of the corresponding titratable juice acidity.

26) Phenolic content

The total phenolic content was determined with the Folin Ciocalteu Reagent (FCR) (Singleton *et al.*, 1999) and expressed as mg gallic acid per 1000 ml using automated spectrometer (Eppendorf).

27) Anthocyanins

Total anthocyanin content in juice was evaluated spectrophotometrically using the pH differential method (Giusti and Wrolstad, 2001).

28) Rind thickness

Thickness of rind was measured with the help of vernier caliper avoiding the placental joints and measured in millimeters (mm).

29) Rind weight

Rind of five fruits were taken and weighed with the help of electronic balance after separating the arils. Average weight of rind was taken in grams (g).

30) Rind percentage

Random fruits were taken and peel was removed and kept aside for weighing. The arils were taken out and juice extracted with electric juicer (Bajaj). The rind percentage was counted by dividing the total peel weight with fruit weight and expressed in percentage (%).

$$\text{Rind percentage} = \frac{\text{Total weight of rind (g)}}{\text{Total fruit weight (g)}} \times 100$$

31) Seed length

Seed length was measured with the help of vernier calipers and expressed in centimetres (cm).

32) Seed width

With the help of vernier calipers the diameter of the seed was measured at maximum thickness and recorded in centimetres (cm).

33) Hundred dry seed weight

A sample of 100 seeds after separating from aril in each replication was taken, dried and weighed on electronic balance and expressed in grams (g).

34) Disease observations

34.1 Bacterial blight on leaves/fruit/stem (*Xanthomonas axonopodis* pv. *punicae*)

The reaction of each genotype for *Xanthomonas axonopodis* pv. *punicae* was scored, when the crop was at physiological maturity. The disease severity recorded in two seasons as given below.

Observations and grading

The observation on intensity of disease on each of the genotype from top, middle and bottom was recorded from fifteen randomly selected leaves per plant. The average of forty five random leaves is taken as the score of each plant (Table 6). Data on the disease severity was assessed on each genotype using 0-5 Scale (Singh *et al.*, 2015). From disease severity scores PDI was calculated by using the formula as given below (Mayee and Datar, 1986).

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of grade of all observations}}{\text{No. of observations} \times \text{Maximum in the scale grade value}} \times 100$$

34.2 Disease severity on tree

The disease severity on tree including leaf, stem and fruit was calculated as per the below formula (CROPSAP, 2013).

$$\text{Severity (\%)} \text{ on a tree} = 0.10x + 0.70y + 0.20z,$$

Whereas,

x is severity on leaves,

y on fruits

z on stems and

0.1, 0.7 and 0.2 are constants; the weight given to severity on various units based on the economic damage due to infection on these parts.

Table 6: Scoring of pomegranate bacterial blight severity on leaf, fruit and stem

Numerical rating	Percentage blighted area on leaf/fruit	Stems (No. of cankers/tree)	Description	Reaction
0	Disease free	0	Disease not seen	Resistant
1	1-10	1	Disease not easily visible, very few units/plant found diseased after careful search	Tolerant
2	11-25	2-3	Disease visible easily in each direction but most (75%) of the units look healthy	Moderately susceptible
3	26-50	4 – 6	Both disease and healthy units are equally observed	Highly susceptible
4	51-75	7-10	Disease seen easily with only some healthy units	Highly susceptible
5	76-100	≥ 11	Almost all units are diseased, with few healthy units seen on careful search	Highly susceptible

(Singh *et al.*, 2015)

3.2.2.2 Qualitative characters

The following qualitative characters were recorded on each of the genotype in both seasons by following standard procedure of pomegranate descriptor (Mahajan *et al.*, 2002).

i) Flower colour

- (1) Yellow
- (2) Red
- (3) Deep red
- (4) Crimson
- (5) Scarlet
- (6) Light red

ii) Young leaf colour

- (1) Light green
- (2) Green
- (3) Dark green
- (4) Green with pink tinge
- (5) Pinkish brown

iii) Leaf colour

- (1) Green
- (2) Pale green
- (3) Dark green

iv) Thorniness

- (1) Less
- (2) Medium
- (3) High

v) Aril colour

- (1) Creamy white
- (2) Yellow
- (3) Light pink
- (4) Pink
- (5) Red
- (6) Blood red

vi) Fruit skin colour

- (1) Creamy white
- (2) Yellow
- (3) Dull red
- (4) Red
- (5) Deep red
- (6) Pink

3.2.2.2.1 Scoring of varieties based on quality parameters

The acceptability of the fruits to the consumer and processor depends on a combination of several quality attributes that are related to the physico-chemical properties of fruits. However, among various physico-chemical traits, fruit weight,

percentage of arils and juice, hundred-aril weight, TSS, per cent acidity, the maturity index and rind and aril colour are found to be the important fruit quality parameters in pomegranate and were thus considered for scoring of pomegranate varieties; index scores of 1 to 5 were assigned to these traits (Chandra *et al.*, 2013) based on market and consumer preferences (Table 7).

3.2.2.3 Statistical analysis

The statistical analysis on the individual characters was carried out on the mean values of each genotype using statistical package GenStat version 13.1 (VSN International, 2010) available at ICRISAT, Hyderabad.

3.2.2.3.1 Analysis of variance

The analysis of variance for different characters was carried out using the mean data in order to partition the variability due to different sources by following procedure/formula/method given by Panse and Sukhatme (1964). The structure of ANOVA is given in Table 8 and 9.

3.2.2.4 Estimation of genetic parameters

In order to assess and quantify the genetic variability among the genotypes for the characters under study, the following parameters were estimated as given below.

i) Estimation of variance components

Phenotypic and genotypic variance were estimated using following formulae

$$\text{Genotypic variance } \sigma_g^2 = \frac{\text{MSS (genotypes)} - \text{MSS (error)}}{\text{Number of replications}}$$

$$\text{Phenotypic variance } \sigma_p^2 = \sigma_g^2 + \text{MSS error}$$

ii) Coefficient of variability

Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and Devane (1953).

Table 7: Selection index scoring criteria for fruit quality traits in pomegranate genotypes

Score	Fruit weight (g)	Aril (%)	Juice (%)	100 aril weight (g)	TSS ($^{\circ}$ B)	Acidity (%)	Maturity index	Rind color	Aril color
1.	< 200	< 55	< 45	< 20	<13.5	> 2	< 20	Yellow/ yellowish green	Yellowish white / pale yellow / pale yellowish pink
2.	200-250	55-60	45-50	20-25	13.5-14.0	1.5-2.0	20-25	Reddish yellow/ orange-yellow/ yellowish pink	Pink
3.	250-300	60-65	50-55	25-30	14.0-14.5	1.0-1.5	25-30	Reddish orange	Deep pink
4.	300-350	65-70	55-60	30-35	14.5-15.0	0.5-1.0	30-35	Deep red	Deep red
5.	= 350	= 70	= 60	= 35	= 15	= 0.5	= 35	Vivid red	Dark red / vivid red

(Chandra *et al.*, 2013)

Table 8: The structure of ANOVA

Source of variation	d.f.	MSS	F ratio
Blocks (Ignoring accessions and check)	b-1	MSS (B)	MSS (B)/ EMSS
Genotypes (checks +accessions)	e-1	MSS (E)	MSS (E)/ EMSS
Checks	c-1	MSS (C)	MSS (C)/ EMSS
Accessions (Ignoring blocks)	g-1	MSS (G)	MSS (G)/ EMSS
Accessions vs Chek Varieties	(g-1) (c-1)	MSS (GC)	MSS (GC)/ EMSS
Error (e)	(c-1) (b-1)	EMSS	

Where,

d.f. = degree of freedom

MSS = mean sum of squares

MSS (B) = Mean Sum of squares due to blocks

MSS (G) = Sum of squares due to genotypes

MSS (C) = Mean sum of squares due to Checks

MSS (E) = Mean sum of squares due to error

g = Number of genotypes/Entries

Table 9: Analysis of variance (pooled)

Source	d.f.	SS	MSS	F ratio
Genotypes (g)	g-1	S_g	$M_g = S_g / (g-1)$	M_g / M_e
Year (y)	y-1	S_y	$M_y = S_y / (y-1)$	M_y / M_e
Replications (r)	r-1	S_r	$M_r = S_r / (r-1)$	M_r / M_e
Genotype $\times$ year	(g-1) (y-1)	S_i	$M_i = S_i / (g-1) (y-1)$	M_i / M_e
Pooled error (e)	(r-1) (g-1)	S_e	$M_e = S_e / (r-1) (gy-01)$	

Where,

d.f. = degree of freedom

Y = year

R = replication

S_i = sum of squares due to interaction of genotypes and years

$$\text{Genotypic coefficient of variability (GCV)} = \frac{\sigma_g}{\bar{X}} \times 100$$

$$\text{Phenotypic coefficient of variability (PCV)} = \frac{\sigma_p}{\bar{X}}$$

Where,

σ_g = Genotypic standard deviation,

σ_p = Phenotypic standard deviation,

$\bar{X}$ = Mean of the characters.

iii) Heritability

Heritability in broad sense was computed as the ratio of genotypic variance to the total phenotypic variance as suggested by Hanson *et al.* (1956) and expressed as percentage.

$$\text{Heritability (h}_{bs}^2) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

iv) Genetic advance (GA)

Genetic advance was estimated by using the formula given by Johnson *e. al.* (1955).

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

Where, h_{bs}^2 = heritability estimate,

K = Selection differential which is equal to 2.06 at 5 %
intensity of Selections (Lush, 1949)

σ_p = Phenotypic standard deviation

v) Genetic advance expressed as percentage over mean (GAM)

$$GAM = \frac{GA}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = General mean of the character.

3.2.2.5 Principal component analysis

Principal component analysis or canonical (vector) analysis was carried out to study the amount of variation along different axis of differentiation. It is a multivariate statistical technique to reduce the data with large number of correlated variables into a substantially smaller set of new variables through linear combinations of variables that account most the variation present in the original variables. PCA is a function of Eigen values and Eigen vectors covariance matrix. The variability study of the different genotypes was done by Principal Component Analysis (PCA) using GenStat version 13.1 (VSN International, 2010), computer software. PCA is a method of data reduction that transforms the original variables into a limited number of uncorrelated new variables. The technique is thus a useful device for representing a set of variables by a much smaller set of composite variables that account for much of the variance among the set of original variables. It allows visualization of the differences among the individuals, identification of possible groups and relationships among individuals and variables (Martinez-Calvo *et al*, 2008). The numerical data were analyzed by PCA using the computer software GenStat to get an overview of the similarity in the genotypes studied. This statistical procedure was applied to create a correlation matrix from which standardized principal component (PC) scores were extracted. Scatter plots of the first 2 PC scores were created. To determine which of the PC scores accounted for the greatest amount of variation for each trait, the Eigen values of the 6 PC scores were compared for each trait.

3.2.2.6 Genetic diversity

Genetic diversity analysis was carried out to evaluate the genetic divergence among the 475 population. The analysis was done by different methods mentioned below.

1. Mahalanobis D^2 analysis

Mahalanobis's (1936) D^2 - statistic analysis was used for assessing the genetic divergence among the test entries. The generalized distance between any two populations is given by formula,

$$D^2 = SS \sum_{ij} s^{ai} s^{aj}$$

Where,

D^2 = Square of generalized distance

$\sum_{ij}$ = Reciprocal of the common dispersal matrix $s^{ai} = (\mu_i - \mu_1)$

$s^{aj} = (\mu_j - \mu_2)$

μ = General mean

Since the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized character mean (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computational procedure. The D^2 values were obtained as the corresponding uncorrelated (Ys) values of any two accessions (Rao, 1952).

Experiment–III

3.3 Genotyping of g-ray irradiated seed derived progenies and germplasm of pomegranate with SSR markers

3.3.1 DNA isolation of plants

Young leaves of plants were collected from field and DNA was isolated by Qiagen DNAeasy plant genomic DNA extraction kit (Cat. No. 69104) as per the instructions given in the manual. The young leaves were ground in to a fine powder by

liquid nitrogen in a mortar and pestle and 400 µl of lysis solution (AP1) and 4.0 µl of RNase A were added to the tube; mixed it thoroughly by vortexing and inverting. The tube was incubated at 65 °C for 10 min with occasional inversion to dissolve the precipitate. To this mixture, 130 µl of precipitation solution (P3) was added and mixed completely by inversion and placed the samples on ice for 5 min. The samples were centrifuged at 14,000 rpm for 5 min. The supernatant was carefully pipetted on to a QIA shredder spin filtration column placed in a 2 ml collection tube and centrifuged at 14,000 rpm for 2 min. The flow through was transferred to a new centrifuge tube and 1.5 volumes of binding solution (AW1) was mixed by inversion. The 650 µl mixture was transferred into DNeasy Mini spin column which was placed in a collection tube and centrifuged at 8,000 rpm for 1 min. The flow through liquid was discarded. The remaining 700 µl of the mixture was also transferred to their respective DNeasy Mini spin column and centrifuged at 14,000 rpm for 1 min. Later, the flow through liquid was discarded and prior to first time use, ethanol was added to the wash solution concentrate. Binding column was placed into a fresh 2 ml collection tube and applied 500 µl of the diluted wash solution to the column. The tube was centrifuged at 8,000 rpm for 1 minute and discarded the flow through liquid and retained the collection tube. Added another 500 µl of diluted wash solution to the column and centrifuged at 14,000 rpm for 2 minutes to dry the column. In the next step, the binding column was transferred to a fresh 1.5 ml collection tube and applied 100 µl of pre-warmed (65 °C) elution solution (AE) to the column, incubate for 5-10 minutes at room temperature (15-25 °C) and centrifuged at 8,000 rpm for 1 minute. Elute was collected in the same collection tube. Alternatively, a second collection tube was used for the second elution to prevent dilution of the first elute.

3.3.2 Quantification of DNA

Quality of the sample DNA stock was checked and quantified in 0.8 per cent agarose gel with known concentration of uncut lambda DNA of 50 ng/µl, 100 ng/µl and 200 ng/µl and in Nanodrop (Eppendorf). The DNA stocks of the samples were diluted accordingly to make it to required 5.0 ng/µl. Then the diluted DNA was confirmed with 5.0 ng/µl and 10 ng/µl uncut lambda in 0.8 per cent agarose gel.

3.3.3 Genotyping using SSR primers

A set of 125 SSR molecular markers (Hasnaoui *et al.*, 2010, Pirseyedi *et al.*, 2010 and Singh *et al.*, 2015) were used for PCR amplification (Table 10).

3.3.4 SSR amplification

Polymerase chain reaction (PCR) was employed by using a touchdown PCR profile and an amplification protocol appropriate for each pair of primers (Table 10). DNA amplification was performed in 5 µl reaction mixture in Gene Amp® PCR system 9700 (Applied Biosystems). The recipes for PCR reaction mixture for all the labelled and unlabelled primers were common except the primer concentration and Taq polymerase. Reaction mixture consisted of 25 mM MgCl₂ (Bioline), 2 mM dNTPs, 10X PCR buffer (Bioline for unlabelled and Amplitaq Gold for labelled primers) and 5 U/µl Taq polymerase (1U Bioline Taq for unlabeled and 5U Amplitaq gold for labelled primers). Concentration of the primer for unlabeled and florescent labelled primers was 10 pm/µl (Forward and Reverse primers mixture) and for M13 tailed primers, the primer concentration of 1pm/µl of forward + 2 pm/µl of reverse primers mixture was used (Table 11, 12,13 and 14).

3.3.5 Electrophoresis

PCR products were confirmed for amplification on 1.2 per cent agarose gel before loading them in the sequencing gel. For separation of amplified DNA fragments, capillary electrophoresis (ABI 3700) was employed.

3.3.6 Capillary electrophoresis

Amplified products of M13-tailed primers and florescent labelled primers were separated by capillary electrophoresis (ABI 3700). After the amplification, ABI run plate, a total of 11 µl containing a mixture of 1.2 µl of PCR product, 2.8 µl of deionized water, 7.0 µl of Hi-diformamide, 0.2 µl of LIZ 500 size standard was prepared. Then the plate containing this mixture was centrifuged and kept for denaturation at 95 °C for 5 minutes followed by chilling on ice for 5 min. Then again the plate was centrifuged at

Table 10: Nucleotide sequence of SSR primers used in the study

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
1	PGCT009	F: TTTACTTTACCCTCTTCCCC R: TAAACCAAAGCTACCAAGGA	55	(CT) ₁₇	112	Singh <i>et al.</i> , 2015
2	PGCT030	F: CCTCATGTCAGATTGTTTGG R: GTTATGAGAGGGAGGCAGGA	56	(CT) ₁₅	152	Soriano <i>et al.</i> , 2011
3	EPS07	F: CATGGGAGAACAGACAACCTCAA R: AGCCAGTCTGTAACTATCGCC	55	(AGA) ₆	252	Jian <i>et al.</i> , 2012
4	ABRII-MP07	F: GATTAACAGCAAAGCCTAGAGG R: AGTAGCTGCAACAAGATAAGG	55	(AT) ₉ (GT) ₇		Basaki <i>et al.</i> , 2013
5	PGCT002	F: AAACCCACCATCTCTCACTC R: CCTCTCTTCTTCAGTACTCCTCT	55	(CT) ₁₆	116	Singh <i>et al.</i> , 2015
6	PGCT090	F: TACAGGCTACCACAGGTTGA R: ATTGCCACCACATCACTGT	56	(AG) ₂₁	155	Singh <i>et al.</i> , 2015
7	EPS04	F: AAAGGGGAAAAAGACGAAGAAG R: CCCTGTCCTTAAGTCTGAGTGG	55	(CT) ₁₀	257	Jian <i>et al.</i> , 2012
8	ABRII-MP12	F: TTGAGTCCCGATCATATCTC R: TCAATCTGTCAGGAACAACA	55	(CA) ₁₁	262	Basaki <i>et al.</i> , 2013
9	PGCT025	F: AGTAGCCCGTTTAAGATGCT R: GCAGAGGAAAGAGAAAGAGG	55	(CT) ₁₄	130	Soriano <i>et al.</i> , 2011
10	PGCT057	F: ACCCATAGTCTCACCCATTC R: GCGATCCCTAGAGAGAGAAAA	56	(CT) ₈	161	Soriano <i>et al.</i> , 2011
11	EPS03	F: CGCTGGTCACACTACTTACTCG R: TTGTAGTGGAAGACACAGCAGC	55	(AT) ₁₂	263	Jian <i>et al.</i> , 2012
12	ABRII-MP28	F: ATCCTCTGTCTTTGTGTTCG R: T GAGTAATTCCGGTCAGAAG	55	(GAGG) ₃ (GA) ₁	350	Basaki <i>et al.</i> , 2013
13	POM_AAC12	F: TAAAGTTGTGAAATTTGTGATGAG R: TCTGAATTTGCCCAAAGACG	55	(CA) ₅	136	Curro <i>et al.</i> , 2010
14	EPS08	F: TTCCCGAGAAAGTTGCATATCT R: TAGTCCGTGAGGATTTTGTCTCT	56	(AG) ₁₈	204	Jian <i>et al.</i> , 2012
15	ABRII-MP30	F: CCCAGTTTGTAGCAAGGTA R: AAGCTGACATTCTTTGAAGC	55	(TGAGC) ₃	170	Nafees <i>et al.</i> , 2015
16	PGCT097	F: TCCCATAAACATAGCAGGAA R: GGCATCTCAGGGGTAATAAA	55	(CT) ₁₇	152	Singh <i>et al.</i> , 2015
17	PGCT062	F: CATTTTCTGTTACCCCTTGG R: TCTCGACATGCTTAGTCTCG	56	(CT) ₁₄	206	Soriano <i>et al.</i> , 2011

Contd.....

Table 10 contd....

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
18	pg17	F: CAT CAG ACT ACG ATG GCA CT R: GCA TAA TAG CCT TCA ATT TAC A	55	(TCA) ₁₄	140-145	Parvaresh <i>et al.</i> , 2012
19	ABRII-MP33-	F: TCTGTTTATTGCTGAAAGGG R: TCTTCTTCTTCTCCACCGTA	55	(AG) ₁₂	112	Pirseyedi <i>et al.</i> , 2010
20	PGCT070	F: TAACAACCATGCCCCCTTAAT R: CCAATTAACGCGCTCATCT	56	(CT) ₂₀	210	Soriano <i>et al.</i> , 2011
21	ABRII-MP42	F: GAGCAGAGCAATTCAATCT C R: AACAATTTCCCATGTTTGAC	55	(GA) ₉	194 / 200-220	Pirseyedi <i>et al.</i> , 2010
22	ABRII-MP39	F: AGTCTCTGAAGTTTGTCTCGGA R: CTGAGTAAAGCATCTCACTG	55	(GA) ₈ (TTTTCT) ₂	251	Pirseyedi <i>et al.</i> , 2010
23	PG13	F: AGGCTGAGAGTCTCCCAAC R: TTGAGATCGAACGAATCGAC	55	(AG) ₃₂	193	Singh <i>et al.</i> , 2015
24	POM_AGC11	F: CGTCATCCCTTATGTTCTTC R: CTGGGGAAGTCGACGAAG	55	(CT) ₁₀ TTCT (CA) ₆	196 – 200	Curro <i>et al.</i> , 2010
25	ABRII-MP51	F: CGCACTTTGTAGCTTGTA R: TT GCTGACATTCAAAGAAGC	55	(TGTGC) ₃	172	Basaki <i>et al.</i> , 2013
26	EPS10	F: TAGCACAGGGGAAATCTGAAAT R: GGAAGAGTTTGGTTCAGGATTG	56	(GAT) ₆	130	Jian <i>et al.</i> , 2012
27	EPS05	F: TTGTTGGGTATTCCTCTTCTC R: ACATCATAACCTTGCCCTC	55	(CTT) ₁₁	197	Jian <i>et al.</i> , 2012
28	PGCT043	F: ACCACCGCACATAATAACTTC R: TTGAATACGCTGTGTTCT	56	(CT) ₉	232	Singh <i>et al.</i> , 2015
29	POM_AAC2	F: TGTGTATCCCATCTTCTTCC R: TTTCCACCGCCATTTACTTC	55	(CT) ₁₂	196 – 207	Curro <i>et al.</i> , 2010
30	PGCT058	F: GGGAACCGTTTTTCATTGTT R: TTTTGGTTGAAAGTTTAGAGAGA	56	(CT) ₁₇	144	Singh <i>et al.</i> , 2015
31	PGCT022	F: GCGCGTTATTCGATAATTC R: GGCTCGAACATCATTACATC	55	(CT) ₈	220	Soriano <i>et al.</i> , 2011
32	ABRII-MP04	F: CAGGTGATTGACTACTTGG R: CAGATCTACAATAACATCAC	55	(GT) ₇	201 / 195-215	Pirseyedi <i>et al.</i> , 2010
33	pg22	F: CCC CGC ACT TAG AAT CTA TTA R: TCC AGT TCC AAT CGA CAG AC	55	(ACAT) ₃ (AT) ₈ (AG) ₂₂ (AT) ₃	230-240	Parvaresh <i>et al.</i> , 2012

Contd.....

Table 10 contd....

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
34	PGCT024	F: GATGGACTGCTCGTTTAGAA R: TTCCTCGTCATAGCAGAAAAG	55	(CT) ₅	170	Soriano <i>et al.</i> , 2011
35	PGCT031A	F: AGTTTGATCGACTGAGGAATG R: CACTCGAGAAGCTCTGTGAA	56	(GA) ₂₀	208	Singh <i>et al.</i> , 2015
36	POM_AAC1	F: GGGTCTTCCTAATTCTCTGG R: TACAACCTTCGGACTCACTTGC	55	(CT) ₉ (TA) ₈	174 – 178	Curro <i>et al.</i> , 2010
37	PGCT014	F: ATCTTCACTTAATCCCATGA R: AATCAAAACTTCCATTTCTC	51	(GA) ₁₇	103	Singh <i>et al.</i> , 2015
38	PGCT003	F: CATTAGTCTGGAAAGATGAG R: GAACTTCCAGTATAATATATCAGAG	50	(TC) ₁₆	150	Singh <i>et al.</i> , 2015
39	ABRII-MP26	F: TTTCTCGAAGAATTGGGTAA R: CT GAGTAAGCTGAGGCTGAT	50	(AG) ₂₅	135	Basaki <i>et al.</i> , 2013
40	EPS06	F: AAATCGCATCCCTCCGTCT R: CTGTTCCGCCAGGGTAAAGA	56	(TC) ₁₃	147	Jian <i>et al.</i> , 2012
41	POM_AAC7	F: GCCTGGACATCT AACGCTCTC R: GCCGAACAAAGTCTGAAAC	55	(TC) ₃ (TTTCT) ₃	240	Curro <i>et al.</i> , 2010
42	pg23	F: ACC ACT CCC ACC ATT ATT GC R: GGA GGG AAG AGA CGA GCA TT	56	(TC) ₁₆	227-235	Parvaresh <i>et al.</i> , 2012
43	ABRII-MP46	F: AGTTGATCTGATGGACAAGG R: CAGTACGGTGCTCAATACAA	55	(GTT) ₄	270 / 250-300	Pirseyedi <i>et al.</i> , 2010
44	pg24	F: GAAAGAGTGTGTGAGAGAATCTGC R: GGACGGATGCAAGAAATGG		(TCA) ₈	91	Soriano <i>et al.</i> , 2011
45	pg7	F: CTTCCATTCCCCTAGCAACC R: CCCCCGAACTTATCCT TC		(AG) ₂₀	195	Soriano <i>et al.</i> , 2011
46	pg16	F: CTGCATATCCCAAGTCAAGGTG R: GGCTTGATTGATGGGCTAT		(AT) ₁₁	242	Soriano <i>et al.</i> , 2011
47	POM046	F: CTTCTCTTACCGAACTATG R: CCACTTTGACACTTCTACC		(TC) ₁₆	205-300	Singh <i>et al.</i> , 2015
48	PG20	F: AGTCCACCCTTTACCACATCC R: CTATCCCATACAACGGACCA	58	(AG) ₁₂	119	Singh <i>et al.</i> , 2015
49	PGCT039	F: TCCGACGATATAATCCCAAT R: ATTGCTTTCTTTTGCACCTC	57	(TC) ₁₆	158	Singh <i>et al.</i> , 2015
50	PGCT016	F: ACATTGCCATAGCTGTTTT R: GGAATCAGGAAGATCGAGTAGAGA	57	(CT) ₄	187	Soriano <i>et al.</i> , 2011

Contd.....

Table 10 contd....

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
51	PGCT087	F: TCCTCCGACCCCTTTCTTATC R: CCCTATCATCCTTCCCATTC	58	(CT) ₂₂	237	Soriano <i>et al.</i> , 2011
52	PGCT110	F: GAGCCATTGTAGAGACAAGA R: GACTGCTGACAACTTTCTTT	52	(GA) ₂₂	103	Soriano <i>et al.</i> , 2011
53	PGCT008	F: ATTCAGCAGATTTTCAGGTC R: GATGAGGTGTGAGTTTGATG	53	(CT) ₁₆	206	Singh <i>et al.</i> , 2015
54	pg21	F: CAA GAC AGA AGC ACC ATC CA R: TCT CCC AAA TCA GAC CAA CC	54	(AG) ₇	210-225	Parvaresh <i>et al.</i> , 2012
55	POM_AAC13	F: TCTCCCGACAACAAATCAC R: CCCGACACAACACATACTTCAG	53	(AC) ₉ (AT) ₆ (AC) ₆ ... (TA) ₁₁	275 – 277	Curro <i>et al.</i> , 2010
56	EPS01	F: TCTATTCCACATAGAAAAGAGGGG R: ATGATGTCTATGCAATTGGCTG	57	(GGA) ₇	121	Jian <i>et al.</i> , 2012
57	PGCT102	F: AACCTATGGTGCCTTCCTTC R: AAATCGCAGACACACTCACA	57	(CT) ₁₇	159	Singh <i>et al.</i> , 2015
58	EPS09	F: TAATCCCATTCCAAACAAGTCC R: ATATTGACGGAGGCTTCACTGT	57	(AG) ₁₅	199	Jian <i>et al.</i> , 2012
59	PGCT069	F: TCTTGTTACGCCTTGTTGGT R: CACAAATCCTCAAGCAGACC	58	(CT) ₁₁	238	Nafees <i>et al.</i> , 2015
60	pg19	F: GCATAGAATGACCTGAGTCGTG R: T GACAGCCCCCTCTTGATTC	58	(TCA) ₁₅	139	Soriano <i>et al.</i> , 2011
61	PGCT082	F: CCACTCATCAACCTCACCAC R: CAATGCAGAGAAGCTGGAAC	58	(TC) _{7N21} (TC) ₁₀	201	Singh <i>et al.</i> , 2015
62	PGCT038b	F: CGTGCCAAATGGGTAAATAA R: AGAACTCCACGACCCATAAA	57	(GA) ₂₁	262	Soriano <i>et al.</i> , 2011
63	PGCT076	F: TTATCGCCTTCCTCTTCTCC R: CCCGAGAAATGCTAGACAGA	58	(CT) ₁₃	148	Soriano <i>et al.</i> , 2011
64	PGCT035B	F: AAAACGACACCACCAATTCT R: CATTTGAAGGGAGAGAGGAA	57	(CT) ₁₅	180	Singh <i>et al.</i> , 2015
65	PGCT065	F: GAAACAGAAGAAGCGCAAAA R: AGGCGATCACACTCAACAAG	58	(CT) ₂₀	201	Soriano <i>et al.</i> , 2011
66	PGCT111	F: TATCTGTGCGCAGGAAGGATG R: GAAGCCAATTCTCAAAGATG	58	(AG) ₁₉	235	Soriano <i>et al.</i> , 2011
67	PGCT010	F: ACCGACACACAAACCCGC R: AGGAGAGGTGGAGGAGGAT	57	(CT) ₁₇	153	Soriano <i>et al.</i> , 2011

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Table 10 contd....

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
68	PGCT112	F: CAGCCAATTACGGCAACTAA R: GTCCCTCGCAAACACCTAAA	58	(TC) ₁₉	181	Soriano <i>et al.</i> , 2011
69	PGCT028	F: AAAAGCTGGCACTCAAACTC R: GGCATTACTTCCAGGACAAC	57	(CT) ₅	215	Singh <i>et al.</i> , 2015
70	PGCT061	F: GAATAAGGCGTCCCTCTCTC R: CTCCTCCTCGTAATCCCAAC	58	(CT) ₁₈	150-200	Nafees <i>et al.</i> , 2015
71	PGCT085	F: GCACAGATGTTTTGGAGTCA R: ATTGTTGGAAAGGAAAGCTG	57	(CT) ₁₀	154	Parvaresh <i>et al.</i> , 2012
72	PGCT047	F: TTCAGCTTCCTTAGCTTCTCC R: AGCAGAATCACCATCCTCAC	57	(GA) ₁₆	184	Singh <i>et al.</i> , 2015
73	PGCT105	F: AGGAGGTGAGGTTGGTGATG R: TTGCGTCGTTTTGAATTTTT	58	(CT) ₂₄	220	Singh <i>et al.</i> , 2015
74	PGCT086b	F: TGGTGATTCTGTGTTGTTTC R: CAACAACCTCCTCTGCTCTC	57	(AG) ₁₁	150-250	Nafees <i>et al.</i> , 2015
75	PGCT067	F: GAGCGAAACAGAGGAAAACG R: AACAGGTTGAACACGACAGC	58	(CT) _{8CA} (CT) ₁₂	185	Soriano <i>et al.</i> , 2011
76	PGCT080	F: TGAGTGGAAGGGAATAGGA R: TCACCCTCTCCAAAATCAAA	57	(GA) ₁₇	230	Soriano <i>et al.</i> , 2011
77	PGCT093A	F: GTAGCCACTTTAGGGCGAGA R: CGTCTAAAAGCGACAGCAAG	58	(CT) ₁₈	230	Soriano <i>et al.</i> , 2011
78	PGCT020	F: TTCCTTTTCGCTTTCACCTCATC R: CCCGATCATTAAATCCACAAA	59	(CT) ₁₅	152	Singh <i>et al.</i> , 2015
79	PGCT034	F: ATTTCTGTGCTCTGTGCCTCT R: GTTGGGAAGAACGGAAAAA	60	(CT) ₁₅	195	Singh <i>et al.</i> , 2015
80	PGCT089b	F: TGCATCCTTCCCCTACTCTC R: AGCTCATGTAATGCGTCGTG	59	(GA) ₁₈	150-200	Nafees <i>et al.</i> , 2015
81	PGCT005	F: TCCGTGTGTGAAGAAGACCA R: GGTTTGGATTCTTGGGTTTT	59	(CT) ₁₅	117	Soriano <i>et al.</i> , 2011
82	PGCT109	F: CCACTTCCCTCCTACCTTCC R: ACGTCTGCTTGACCTCTTT	60	(GA) ₁₆	188	Singh <i>et al.</i> , 2015
83	PGCT013	F: TCTCACACACACAGCAGAA R: GAGAAAAGAGGAAACCGCAGA	59	(CT) ₁₇	262	Soriano <i>et al.</i> , 2011
84	pg10	F: TGC TAG ACA GAA CTG GGA GAA C R: AGA GAG TGG GGT TTC CAT TG	60	(AG) _{9GG} (AG) ₁₄	224-245	Parvaresh <i>et al.</i> , 2012
85	PGCT083	F: TTCGGTTGCATTGATTTCTT R: AGCTCAGTGGAGAGGACTGG	59	(CT) ₁₇	127	Soriano <i>et al.</i> , 2011

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Table 10 contd....

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
86	pg15	F: CGAGACCCTTCAGTTCTTCG R: CAGGCTCGTCCTGAATATCG	60	(CT) ₇	189	Soriano <i>et al.</i> , 2011
87	PGCT015	F: GACGCCCTTAGTTTGCTCCA R: CTCGGGACAGGACTTGGAAT	60	(CT) ₁₀	161	Soriano <i>et al.</i> , 2011
88	PG5	F: GCCACCTCTGCAATTCTCTC R: GCAAAGGTTAGGCTCCGAAT	58	(AG) ₄ AC (AG) ₄ AC (AG) ₄	247-252	Singh <i>et al.</i> , 2015
89	PGCT104	F: CGCCCAAAACAGGAAATAGA R: GCTGGGATCTGGAAGAAATG	59	(GA) ₁₈	192	Singh <i>et al.</i> , 2015
90	PGCT088	F: TCTCTCTCTACCCCGACACC R: TAGCGTCAAGATTGTGAAAAGG	59	(CT) ₁₀	110-400	Nafees <i>et al.</i> , 2015
91	pg18b	F: TCTAAGGGCAGAATGGCACT R: TGGCACTAGATCCGTAAATCTC	59	(TCA) ₁₄	335-347	Soriano <i>et al.</i> , 2011
92	pg2	F: CGGGATCTTCGTTCTCGAT R: GGAATCCGTGAGCTGAGAGT	59	(TCCGT) ₂ TTCC (TTC) ₄	151	Soriano <i>et al.</i> , 2011
93	PGCT09	F: CTGCTGCTGCTTCCTATTC R: AAGAGGAGAGACGGTGACGA	60	(CT) ₁₄	193	Soriano <i>et al.</i> , 2011
94	pg8	F: CAC CAT AGA CTT AAA CGA GCA CAA R: GAA GCT CCA TTG CCT CGT CC	59	(AG) ₂₀	115-150	Parvaresh <i>et al.</i> , 2012
95	pg10b	F: TGCTAGACAGAACTGGGAGAAC R: AGAGAGTGGGGTTTCCATTG	60	(AG) ₉ GG (AG) ₁₄	370-385	Soriano <i>et al.</i> , 2011
96	pg3	F: CGAAGAACGGCTAATCAACG R: GATCCACAACCACGTCCAAC	61	TGGC (TGG) ₃	121	Soriano <i>et al.</i> , 2011
97	pg12	F: CTCTCAAAGCCCTGAACCTG R: GAGCTAAGGTAATGGAGCAGAAG	61	(TC) ₁₇	157	Soriano <i>et al.</i> , 2011
98	PG6	F: GGT TGC TCA TCC CTT GAC TC R: GCG TCT GTC AGT GTC TTA GGC	61	(AC) ₁₅	180-210	Singh <i>et al.</i> , 2015
99	pg14b	F: GCA CAT TTC TTC CAC CTT CC R: GGT TAC AAT GCA CAG AGT CCA	61	(AG) ₃₂	210	Parvaresh <i>et al.</i> , 2012
100	pg9	F: CGGTGGAATTGGCATAAGAC R: CTGAACTGGAAGTCGCACAC	61	(AG) ₂₃	154	Soriano <i>et al.</i> , 2011
101	pg25	F: ATCAAGAACTCCCCGAACT R: CCTTCTTCCATTCCCTAGC	64	(CT) ₁₃	193	Soriano <i>et al.</i> , 2011
102	pg14a	F: GCA CAT TTC TTC CAC CTT CC R: GGT TAC AAT GCA CAG AGT CCA	61	(AG) ₃₂	227-245	Parvaresh <i>et al.</i> , 2012

Contd.....

Table 10 contd....

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
103	pg14c	F: GCA CAT TTC TTC CAC CTT CC R: GGT TAC AAT GCA CAG AGT CCA	61	(AG) ₃₂	205	Parvaresh <i>et al.</i> , 2012
104	PGCT037A	F: GTTTCGATTGGCCGCTTTA R: TGTCTTGCCTTCAAGCACCT	61	(CT) ₆	100	Soriano <i>et al.</i> , 2011
105	PGCT021	F: GATGGCGAAGTGTGTCCTCT R: TTGGGACTGTGTT GACTGCT	59	(CT) ₆	158	Singh <i>et al.</i> , 2015
106	PGCT094	F: GGCTTTTGCTTATGACACTGC R: CCTCGATTGGTAGCTTTCG	59	(GA) ₆	198	Singh <i>et al.</i> , 2015
107	PGCT098b	F: ATCAACCAAACCGCACAGAC R: CCATTTCAATTCTCCCCCTCT	60	(AG) ₁₆	150-200	Nafees <i>et al.</i> , 2015
108	ABRII-MP34	F: GGAAGAAGCAGAGCAATAGA R: GFCCTGAGTAACCTGAGCTG	55	(GAA) ₃	240	Pirseyedi <i>et al.</i> , 2010
109	PGCT001	F: AGCTCCGATTGAGAGCAGAT R: TTGGAGCAATTGGAGAGAGA	59	(TC) ₁₈	101	Singh <i>et al.</i> , 2015
110	PGCT051	F: CTAATGGCTGCTTGGCTTGT R: TTTCACCGAAATTCCCAAAC	59	(CT) ₆	204	Singh <i>et al.</i> , 2015
111	PGCT066b	F: CGAGGAGTGGTCCAGGTTAG R: AACAGACGACAAGGGGAATG	59	(AT) ₈	150-430	Nafees <i>et al.</i> , 2015
112	PGCT032	F: TCTGAAGCCGATCTCGAAGT R: GTCAGCCAAGCATTACACAG	59	(CT) ₆	103	Soriano <i>et al.</i> , 2011
113	PGCT060	F: CACGCCAGAGAGAAGAAGA R: CTACCCGACACGACACAAAA	59	(CT) ₁₁	159	Soriano <i>et al.</i> , 2011
114	PGCT004A	F: AACCCAGGGGATACCAAAAAG R: ACGAAATGGGAAATGCAGAG	60	(CT) ₃	204	Soriano <i>et al.</i> , 2011
115	pg18	F: TCT AAG GGC AGA ATG GCA CT R: TGG CAC TAG ATC CGT AAA TCT C	59	(TCA) ₁₄	186-200	Parvaresh <i>et al.</i> , 2012
116	pg11	F: CTCCTTCGGTTCTTGGTCT R: ACGATGGAGTGCTTGTGGAT	60	(ATAG) ₆	172	Soriano <i>et al.</i> , 2011
117	PGCT026	F: TTGGGACCGCATAGAGACTG R: CTCGTCCCCCAAATTAACA	59	(CT) ₄	219	Soriano <i>et al.</i> , 2011
118	PGCT075b	F: GGCGAGCTTCTGCTACTTCT R: TCTGTCCCCAGATCATCAAA	59	(CT) ₆	200-250	Singh <i>et al.</i> , 2015
119	PGCT004B	F: CTCTGCATTTCCATTTCGT R: GGGGACGTAGAACTCAGCAA	60	(CT) ₁₁	116	Soriano <i>et al.</i> , 2011
120	PGCT093B	F: GCCTTTTCCTGCTTTCCTTT R: CATACAGCGGACCACAACAC	60	(CT) ₄	181	Nafees <i>et al.</i> , 2015

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Table 10 contd....

Sl. No.	Primer	Primer pair sequence (5'-3')	Ta (°C)	SSR motif /Repeat	Expected fragment size (bp)	Reference
121	PGCT050	F: GTCGGTCGTTCCAGTGAAGT	60	(TC) ₂₃	250	Singh <i>et al.</i> , 2015
		R: AACACCAGACATCGGCAAAC				
122	PGCT096b	F: CAGACCCTGCGCTCGCT	59	(AG) ₁₈	200-250	Nafees <i>et al.</i> , 2015
		R: TTATGGAGAGCGGGAGAAAC				
123	PGCT007	F: ACTCTGATTGAGCCCTACTG	52	(GA) ₁₅	150	Soriano <i>et al.</i> , 2011
		R: GACTACCTTACACACCTCTCTC				
124	PGCT068	F: ACAGTCTCCTAATACTAATTCCTC	52	(CT) ₁₁	215	Soriano <i>et al.</i> , 2011
		R: TCCTTATTTCTTTTGTACCC				
125	PGCT046	F: ACATCCCTTCTCTCTCTTTC	52	(CT) ₁₃	245	Soriano <i>et al.</i> , 2011
		R: GCATTCCTCCTCGTCTTC				

bp = base pairs

Ta = Annealing temperature

Table 11: Touchdown PCR for labeled and unlabelled primers used for genotyping of pomegranate

Sl. No.	Steps	Unlabelled Primers			Labeled Primers			Cycles
		Temperature (°C)		Time	Temperature (°C)		Time	
		60-55 (56)	65-60 (59)		60-55 (56)	65-60 (59)		
1	Initial denaturation	95	95	3 min	95	95	15 min	5 Cycles
2	Denaturation	94	94	20 sec	94	94	20 sec	
3	Annealing	60	65	20 sec	60	65	20 sec	
4	Primer extension	72	72	30 sec	72	72	30 sec	30 Cycles
5	Denaturation	94	94	20 sec	94	94	20 sec	
6	Annealing	56	59	20 sec	56	59	20 sec	
7	Primer extension	72	72	30 sec	72	72	30 sec	
8	Final extension	72	72	20 min	72	72	20 min	
9	Store at	4	4		4	4		

Table 12: PCR reactions for unlabelled primers

Components	Concentration ¹ /μl	Unlabelled primers		
		Protocol 1	Protocol 2	Protocol 3
		PCR reaction (5 μl)	PCR reaction (5 μl)	PCR reaction (5 μl)
Primers (F+R)	10 pM	0.30	0.50	0.30
Taq buffer (Bioline)	10 X	0.50	0.50	0.50
Mg 2+ (Bioline)	25 mM	0.15	0.20	0.20
dNTP's	2 mM	0.50	0.50	0.50
Template	5 ng/l	1.00	1.00	1.00
Deionised water		2.40	2.15	2.15
Taq polymerase (Bioline)	1 U	0.15	0.15	0.15

Protocol 1: Universal protocol

Protocol 2: Seq19D06, XIP 121

Protocol 3: Seq4E08, XIP407c and XIP295

Table 13: PCR reactions for the M13-tailed primers

Components	Concentration/ μ l	PCR reaction (5 μ l)
Primers (F+R)	1 pM F + 2 pM R	0.50
Taq buffer (AmpliTaq Gold)	10 X	0.50
Mg 2^+ (AmpliTaq Gold)	25 mM	0.50
dNTP's	2 mM	0.25
Template	5 ng/l	1.00
Deionised water		1.73
Taq polymerase (Amplitaq Gold)	5 U	0.02
Dye (FAM, NED, PET, VIC)	2 pM/l	0.50

Table 14: PCR reactions for the Florescent labeled primers

Com pon en ts	Concentration/ μ l	PCR reaction (5 μ l)
Primers (F+R)	10 pM	0.3
Taq buffer (Amplitaq Gold)	10 X	0.5
Mg 2^+ (Amplitaq Gold)	25 mM	0.5
dNTP's	2 mM	0.25
Template	5 ng/l	1
Deionised water		2.43
Taq polymerase (Amplitaq Gold)	5 U	0.02

900 rpm for 1 min and immediately the plate was wrapped with aluminium foil (to protect the mixture from exposing to light as LIZ is sensitive and losses its activity after exposing it to light). Then the plate was kept in ABI 3700 genetic analyzer. The “G5” dye set, “GeneScan Pope” run module and GS 500 LIZ analysis module were employed and the fragments were separated in 36 cm capillary array. After completion of the run, the A and B peaks were sized using Genescan software and scoring was done with the help of Genotyper software (Plate 2).

3.3.7 Statistical analysis

Polymorphic information content (PIC) was obtained using power marker software that provides an estimate of the discriminatory power of a locus or loci, by taking into account, not only the number of alleles that are expressed, but also relative frequencies of those alleles, was estimated using the following equation of Anderson *et al.* (1993).

$$PIC = 1 - \sum_{i=1}^n P_{ij}^2$$

Where,

P_{ij} is the frequency f_t allele in i primer and summation extends over n patterns.

3.3.7.1 Cluster analysis

The molecular data from SSR studies were analyzed with the computer package DARwin ver. 5.0.158 (Perrier and Jacquemoud-Collet, 2006). The distance matrix was developed using Squared Euclidean Distances (SED) that estimated all pair wise differences in the amplification product. The genetic distance was computed as,

$$S_{ij}^2 = 1d_j^2$$

Where,

$$d_j = (X_{ik} - X_{jk})$$

X_{ik} = binary code of i^{th} plant for allele ‘k’ and

X_{jk} = binary code of j^{th} plant for allele ‘k’.

Plate 2: Screening of pomegranate genotypes using capillary electrophoresis (ABI 3700) genetic analyzer

A dendrogram was developed based on Unweighted Pair Group Method (UPGM) with Arithmetic Mean. Confidence limits of different clades were tested by bootstrapping 1000 times to assess the repetitiveness of genotype clustering (Felsenstein, 1985).

Experiment-IV

3.4 Marker trait association (MTA) and association mapping for plant growth, fruit quality traits and bacterial blight disease resistance

Association mapping involved the following basic steps

1. Selection of the population of the species
2. Morphotyping / precise phenotyping of above population for different morphological or other traits of concern.
3. Molecular characterization including structural analysis of the population
4. Association analysis using phenotypic data, genotypic data and the Q-matrix obtained from structural analysis.

3.4.1 Methodology

Association mapping can be carried out by using a variety of software packages. These include STRUCTURE for population structural analysis, TASSEL for association analysis, EIGENSTRAT for PCA and association analysis, SPAGeDi for relative kinship *etc.* In our study; we used STRUCTURE and TASSEL software packages to carryout association analysis.

In our study, marker trait association was carried out for different fruit quality traits and disease resistance.

3.4.1.1 Population structure analysis

The genotypic data based on SSR markers for the 188 genotypes of pomegranate was run for structural analysis and clustering was done. This analysis was done by using

the software STRUCTURE 2.2 (Pritchard *et al.*, 2000), which identified the different γ irradiated seed derived progenies and germplasm with distinct allele frequencies within the population taken under study.

Q matrix was obtained by using STRUCTURE, which is defined as an $n \times p$ population structure incidence matrix where n is the number of individuals assayed and p is the number of sub-populations assumed; Q is inferred from Pritchard's STRUCTURE estimates with p (Pritchard's K) sub-populations. The number of presumed sub-populations (K) was set from 1-10 and the process was repeated 35 times. For each run, burn-in and iterations were set to 50,000 to 100,000, respectively and a model "without admixture and correlated allele frequencies" was used. *Ad hoc* statistics was related to rate changes in the log probability of the data according to the number of K's, as suggested by Evanno *et al.* (2005), with ΔK being used as a predictor of the ideal number of clusters and shows a peak for the true value of K. A genotype was assigned to a specific sub-population, with which it has ≥ 80 per cent affiliation probability; and, genotypes with less than 80 per cent affiliation probability were treated as admixture.

3.4.1.2 Marker trait association analysis

The software TASSEL ver 2.1 (Trait analysis by Association, Evolution and Linkage) (Bradbury *et al.*, 2007) was used for association analysis between the individual markers and the different traits studied for the genotypes from the pomegranate population studied, employing the general linear model (GLM) based on the chosen Q matrix produced by the software STRUCTURE ver 2.2. Further, to identify the common SSR loci in both GLM and single marker analysis (SMA) was performed to determine the significance and the contribution of the marker towards traits under study using WinQTL Cartographer version 2.5 (Wang *et al.*, 2007). Analysis of variance (ANOVA) and linear regression (Haley and Knott, 1992) were used in SMA to check the significance and to work out the coefficient of determination (R^2) of each marker towards the phenotypic variation arising from linked markers.

4. EXPERIMENTAL RESULTS

The results of the investigations on “Evaluation of γ -rays irradiated seed derived progenies and germplasm for fruit quality traits and bacterial blight resistance and analysis of marker-trait association in pomegranate (*Punica granatum* L.)” during 2014-15 and 2015-16 are presented in this chapter under following headings:

- 4.1 Collection of bacterial blight affected pomegranate plant parts from different parts of Karnataka
- 4.2 Evaluation of γ -ray irradiated seed derived progenies and germplasm of pomegranate for plant growth, fruit quality traits and bacterial blight disease resistance
- 4.3 Molecular characterization
- 4.4 Marker trait association (MTA) / association mapping studies in pomegranate

4.1 Collection of bacterial blight affected pomegranate plant parts from different parts of Karnataka

The various parts of the plant viz., leaves, stem and fruit showing typical symptoms of bacterial blight were collected from affected pomegranate orchards in different parts of Karnataka and isolates were designated as mentioned in the Table 2.

4.1.1 Symptomatology

Symptoms were observed on leaves, branches and fruits. Initially small, water soaked, lesions were noticed on the lower surface of the leaves (Plate 3) followed by appearance of small brown to black coloured spots on the upper surface with round to irregular shape. As the disease progressed, these spots also grew, increased in size, coalesced and extended up to midrib in a week's time, covering the major portion of the leaf lamina. Severely infected leaves turned yellow, chlorotic and finally dropped. Stem infection manifested in the form of long, narrow and elongated light brown to black coloured lesions on the main stem and branches. The lesions later on became rough and cankerous. On developing green fruits, symptoms were noticed as small, pin head sized,



a) Initial water soaked symptoms on lower leaf surface



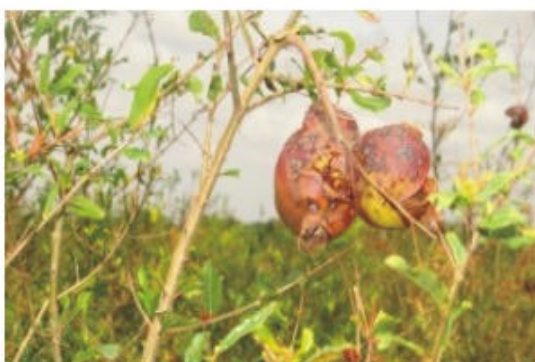
b) Yellow halo, Coalesced and extended lesions upto midrib at later stage



c) Lesions on infected twig



d) Initial water soaked symptoms on fruit at early stage



e) Blighted and fruit cracking symptoms at later stage



Plate 3: Symptoms exhibiting pomegranate bacterial blight on different parts of the plant

black lesions with diffused water soaked margin (oily spots), which later on developed into black coloured, medium to big sized spots. Too many such spots were observed on the single fruit. Severely affected fruits split opened with “L”, “Y” or star shaped cracks within the spot. Infected fruits did not further develop nor suddenly dropped, but dried up and remain hanged on the plant itself.

4.1.2 Isolation and maintenance of pure culture

The causal organism was isolated from the infected leaf, bark of the stem and fruit pericarp showing typical symptoms of bacterial blight (Plate 4). Isolation was carried out by employing the serial dilution plating technique using nutrient agar medium. Repeated isolation from the infected plant parts yielded well separated, yellow colonies of bacterium on nutrient agar medium after 72 hr of incubation at 28 °C. Colonies were purified by streaking the isolated colony on nutrient agar medium and pure colonies (Plate 4) obtained were further streaked on to the slants and kept for incubation at 28 °C for 72 hrs. Cultures so obtained were stored in the refrigerator at 4 °C, which served as a stock culture for further studies.

4.1.3 Pathogenicity

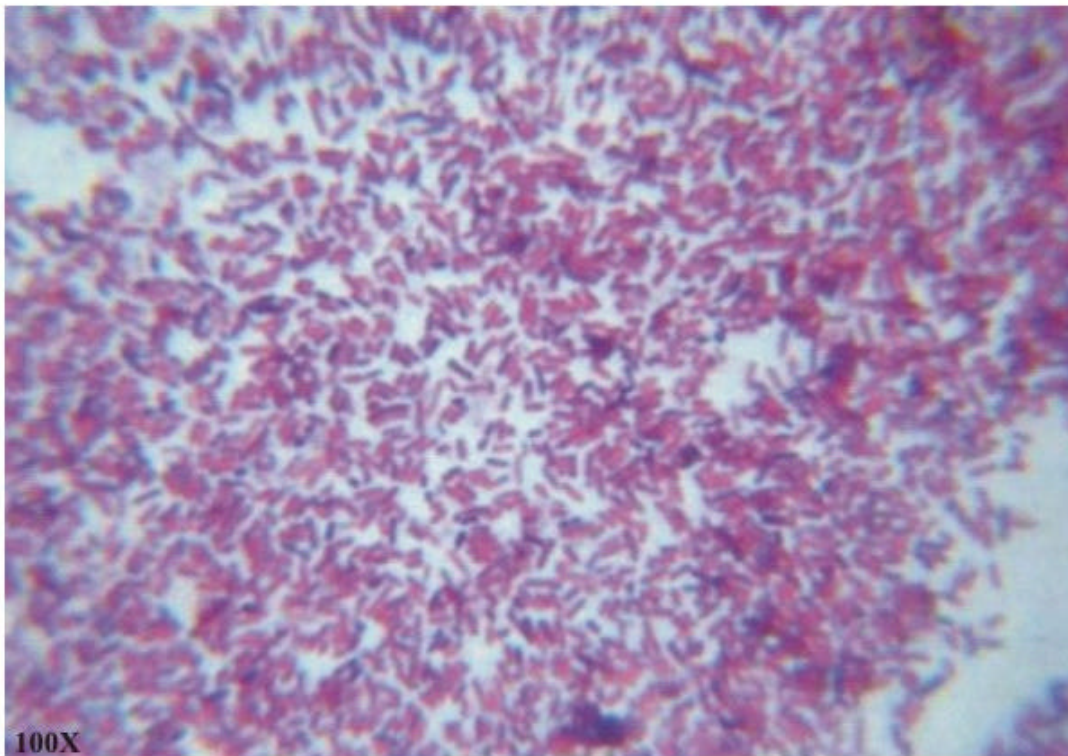
For proving pathogenicity, the bacterial cell suspension (4.5×10^6 cfu/ml) of bacterium collected from leaf of infected plant during collection was sprayed on the susceptible pomegranate plants of Bhagwa and Ganesh varieties. The characteristic symptoms were observed on pomegranate leaves after 15 days of inoculation as small, water soaked, brown to black coloured lesions surrounded by yellow halo, which later on developed into angular to irregular shaped spots along the veins and vein lets of the leaf lamina leading to marginal necrosis (Plate 5). Re-isolations were carried out from these lesions for each isolate and compared with the original culture to confirm the identity of the pathogen. The pathogen from artificially inoculated plants yielded the bacterial colonies similar to the original one.

4.1.4 Identification of pathogen

The identification of pathogen involved in causation of bacterial blight of pomegranate was done by conducting studies on its morphological, cultural, biochemical and molecular characteristics as per standard procedure.



a) Pure culture of *X. axonopodis* pv. *punicae*



b) Gram stained cells of *X. axonopodis* pv. *punicae*

Plate 4. Pure culture of *Xanthomonas axonopodis* pv. *punicae* and Gram staining



a) Initial water soaked symptoms on lower leaf surface



b) Control

Plate 5. Pathogenicity tests of bacterial blight of pomegranate

4.1.4.1 Cultural characters

The cultural variability of four bacterial isolates on nutrient agar medium collected from different locations of Karnataka presented in Table 15 revealed the variations in respect of colony characters such as size, shape, colour, fuscan production and appearance (Plate 6). The isolates differed in respect of size of colony as the isolates *Xap1*, *Xap2* and *Xap4* developed medium to large bacterial colonies. The isolate *Xap3*, produced small to medium colonies. The isolates of *Xanthomonas axonopodis* pv. *punicae* also differed greatly in shape of colony as the isolates *Xap2*, *Xap3* and *Xap4* were having circular shape. *Xap1* was having circular. The colour of the colony also differed greatly as the isolate *Xap1*, was creamish yellow in colour. The isolates *Xap2*, *Xap3*, formed light brown colonies. The remaining isolate i.e., *Xap4*, light yellow colonies. The isolates *Xap2* and *Xap3* produce fuscan (brown pigment) in the medium. The remaining isolates couldn't produce any brown pigmentation in the medium. The number of days taken for the growth of bacterial colonies has also differed to some extent that *Xap1* and *Xap3* were grown five days after streaking. The isolates *Xap2* and *Xap4* were grown four days after streaking.

4.1.4.2 Morphological characters

The results of the various morphological and cultural characters are presented in Table 16. All the isolates had small rod shaped cells and reacted negatively to Gram stain (Plate 4).

The colonies of the all the isolates were circular with smooth margin, light yellow to dark yellow coloured, convex to slightly raised, mucoid, small to medium (1-3 mm) in size, single and paired colonies were recorded on nutrient agar medium (Plate 6).

4.1.5 Morphological Variability among Isolates of *Xanthomonas axonopodis* pv. *punicae*

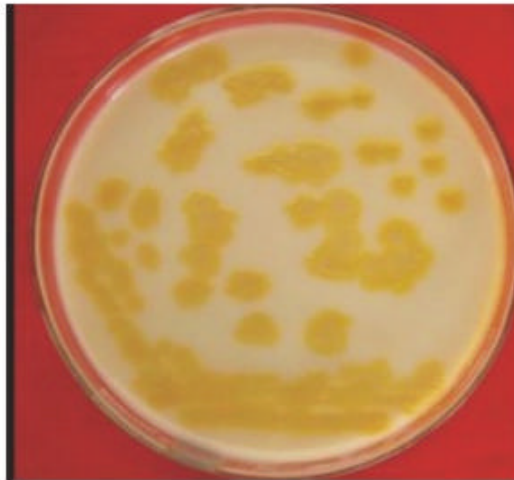
The data in Table 16 indicates the morphological variability among different isolates collected from different locations of Karnataka. The mean length of four different isolates of *X. axonopodis* pv. *punicae* ranged from 1.75-1.91 μm . The maximum mean length was recorded in isolate *Xap3* with 1.91 μm and the minimum

Table 15: Cultural variability among isolates of *Xanthomonas axonopodis* pv. *punicae*

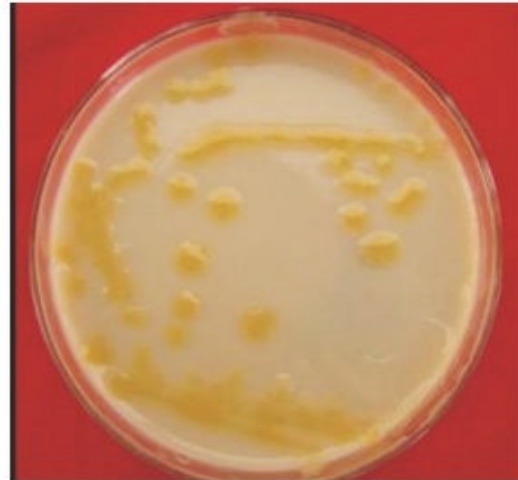
Isolate	Size of colony	Shape of colony	Colour of colony	Fuscan production	Appearance of colony (No. of days)
<i>Xap1</i>	Medium to large	Circular	Creamish yellow	-	5
<i>Xap2</i>	Medium to large	Circular to irregular	Light brown	+	4
<i>Xap3</i>	Small to medium	Circular	Light brown	+	5
<i>Xap4</i>	Medium to large	Circular to irregular	Light yellow	-	4

Table 16: Morphological variability among isolates of *Xanthomonas axonopodis* pv. *punicae*

Isolate	Length (µm)		Breadth (µm)		Flagellar movement	Endospore staining
	Range (µm)	Mean (µm)	Range (µm)	Mean (µm)		
<i>Xap1</i>	1.80-1.94	1.84	0.28-0.33	0.30	+	-
<i>Xap2</i>	1.73-1.82	1.75	0.23-0.26	0.24	+	-
<i>Xap3</i>	1.89-1.96	1.91	0.30-0.34	0.33	+	-
<i>Xap4</i>	1.76-1.85	1.77	0.27-0.33	0.32	+	-



b) *Xap2*



a) *Xap1*



c) *Xap3*



d) *Xap4*

Plate 6. Cultural variability among isolates of *Xanthomonas axonopodis* pv. *punicae*

length was recorded in isolate *Xap2* with a mean of 1.75 μm . The mean breadth of isolates of *X. axonopodis* pv. *punicae* ranged from 0.24 to 0.33 μm . The maximum mean breadth was recorded in isolate *Xap3* with 0.33 μm and the minimum breadth was recorded in isolate *Xap2* with a mean of 0.24 μm . All the isolates showed flagellar movement with no endosperm.

4.1.6 Pathogenic variability

The data in Table 17 revealed that all four isolates of *X. axonopodis* pv. *punicae* developed symptoms after 15 days of inoculation. All the isolates exhibits water soaked, circular to irregular, dark brown symptoms on leaves (Plate 7) of Bhagwa variety.

The data in Table 18 revealed that, there was significant difference within *X. axonopodis* pv. *punicae* isolates with respect to their virulence and reaction with different varieties. Among the test isolates, *Xap1* was found to be virulent (PDI 42.38 %) across the varieties and was superior over other isolates viz., *Xap2*, *Xap3* and *Xap4* which were statistically on par and were rated as moderately virulent (Fig. 3).

Pomegranate varieties expressed variation (Fig. 3) in their susceptibility to *X. axonopodis* pv. *punicae*. The variety Bhagwa recorded highest mean PDI (53.16) followed by P-16 (PDI 52.49), P-13 (51.83) and Ganesh (PDI 49.49) and rated as highly susceptible. Variety 'Patna 5' shown moderately susceptible with mean PDI of 11.00 whereas, Nana as tolerant (PDI 7.00). This study indicates that all leading varieties are susceptible to bacterial blight (Table 18).

It was observed that the interaction between varieties and isolates was statistically significant. Highest PDI (75.99) was recorded in interaction between P-16 $\times$ *Xap1* followed by P-13 $\times$ *Xap1* (63.32) and it was least with Nana $\times$ *Xap2* (6.67), Nana $\times$ *Xap3* (6.67) followed by Nana $\times$ *Xap1* (7.34) and Nana $\times$ *Xap4* (7.34).

There was considerable variation in virulence of isolates and susceptibility of varieties. Isolates *Xap1* (PDI 42.38) and *Xap2* (PDI 40.99) were found more virulent irrespective of varieties compared to other isolates. Varieties Bhagwa, P-13 and Ganesh were found to be highly susceptible irrespective of isolates and recorded maximum mean PDI of 53.16, 51.83 and 49.49 per cent, respectively. Patna-5 and Nana showed uniform resistant reaction against four isolates (Table 18).

Table 17: Pathogenic variability among isolates of *Xanthomonas axonopodis* pv. *punicae*

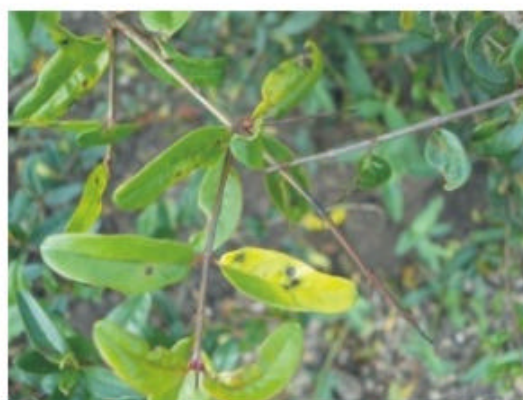
Isolate	Lesion size (mm)		Symptom appearance
	After 15 days	After 18 days	
<i>Xap1</i>	0.2	3.9	Water soaked, circular to irregular, dark brown spots
<i>Xap2</i>	0.3	3.6	Water soaked, circular, light brown spots, dark brown spots
<i>Xap3</i>	1.0	4.0	Water soaked, irregular, light to dark brown spots
<i>Xap4</i>	0.2	3.8	Water soaked, circular, dark brown spots, dark brown spots



a) *Xap1*



b) *Xap2*



c) *Xap3*



d) *Xap4*

Plate 7. Pathogenic infection of *Xanthomonas axonopodis* pv. *punicae* isolates on leaves of Bhagwa variety

Table 18: Reaction of pomegranate varieties against different isolates of *Xanthomonas axonopodis* pv. *punicae*

<i>X. axonopodis</i> pv. <i>punicae</i> isolates	Pomegranate varieties														
	Tobesta	Jyothi	P-13	G-137	P-26	P-23	BT skin	P-16	Patna-5	Kalipathiya	Nana	Ganesh	Bhagwa	Ruby	Mean PDI
<i>Xap1</i>	41.33 (39.99)	34.00 (35.62)	63.32 (52.75)	52.66 (46.52)	55.99 (48.45)	33.33 (35.16)	39.33 (38.76)	75.99 (60.69)	9.34 (17.75)	32.00 (34.43)	7.34 (15.57)	46.66 (43.09)	61.32 (51.57)	40.66 (39.61)	42.38 (40.00)
<i>Xap2</i>	31.33 (33.46)	30.66 (33.62)	55.32 (48.07)	51.33 (45.76)	43.33 (41.16)	39.33 (38.84)	20.00 (26.55)	55.32 (48.09)	10.67 (18.92)	25.33 (30.19)	6.67 (14.89)	50.66 (45.38)	59.99 (50.80)	39.33 (38.82)	40.99 (39.38)
<i>Xap3</i>	15.33 (23.01)	22.00 (27.16)	53.99 (47.29)	45.99 (42.70)	43.33 (41.16)	53.99 (47.29)	41.33 (40.00)	37.33 (37.29)	13.34 (21.39)	26.66 (31.06)	6.67 (14.89)	41.99 (40.37)	43.34 (41.09)	38.66 (38.45)	34.57 (35.23)
<i>Xap4</i>	35.33 (36.46)	36.00 (36.85)	34.66 (35.83)	43.33 (41.12)	45.33 (42.30)	33.33 (35.16)	39.33 (38.76)	41.33 (40.01)	10.67 (18.92)	25.33 (30.19)	7.34 (15.3)	58.66 (50.08)	47.99 (43.85)	56.66 (48.87)	36.81 (36.70)
Mean	30.83 (33.23)	30.66 (33.31)	51.83 (45.98)	48.33 (44.03)	46.99 (43.27)	39.99 (39.11)	35.00 (36.02)	52.49 (46.52)	11.00 (19.25)	27.33 (31.47)	7.00 (15.16)	49.49 (44.73)	53.16 (46.83)	43.83 (41.44)	38.69 (37.82)
For the comparison of	S.E.m. ±								C.D. @ 1 %						
Isolates	0.89								3.37						
Varieties	1.67								6.31						
Isolates × varieties	3.34								12.61						

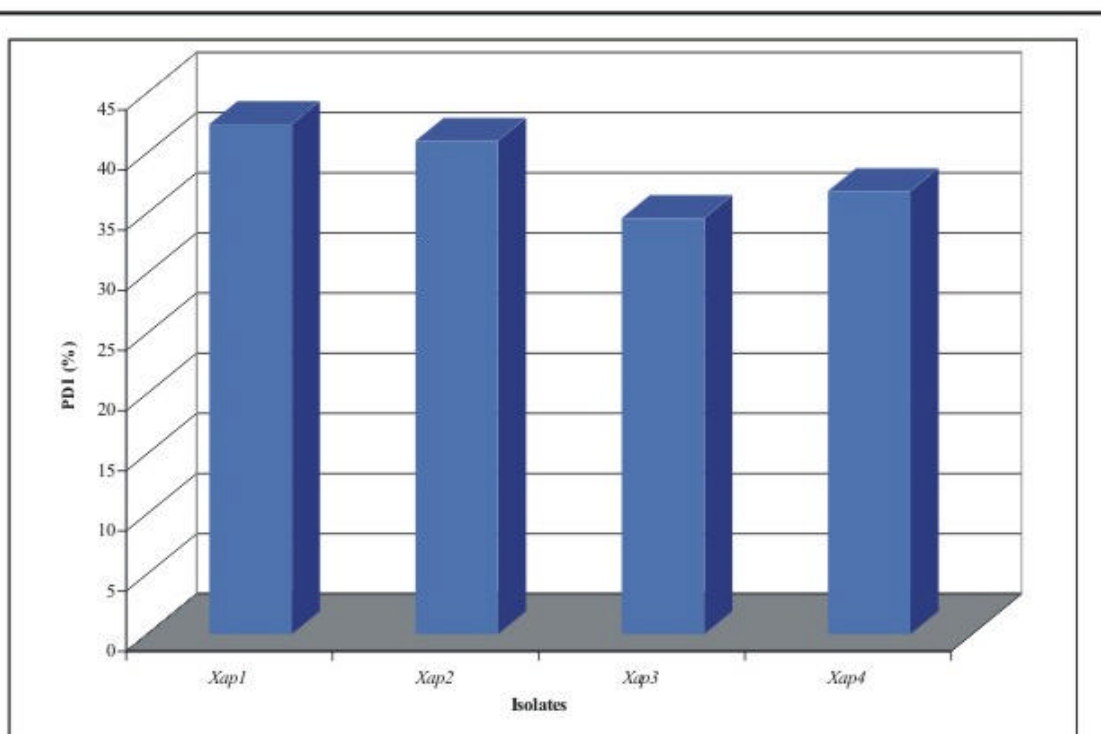


Fig. 2. Variation in virulence of *Xanthomonas axonopodis* pv. *punicae* isolates infecting pomegranate

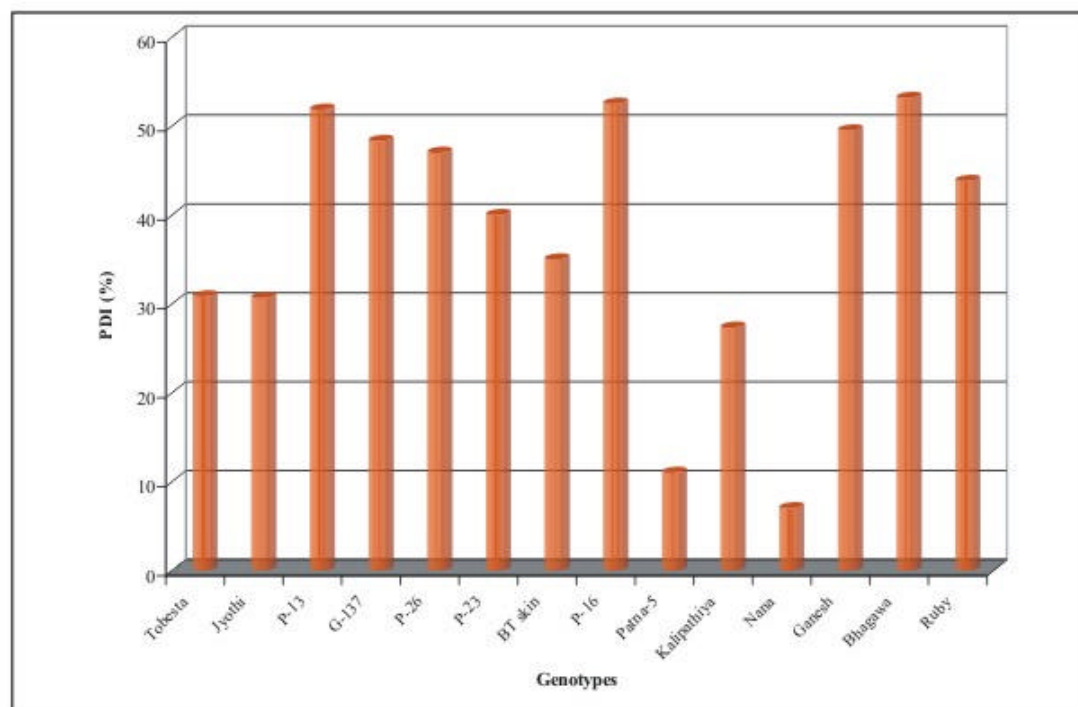


Fig. 3. Variation in varietal susceptibility of pomegranate to *Xanthomonas axonopodis* pv. *punicae* isolates

4.1.7 Molecular variability

4.1.7.1 Characterization of different isolates of *X. axonopodis* pv. *punicae* on the basis of 16s rRNA and *gyrB* gene analysis

The isolates indicated in Table 2 maintained at -80°C in glycerol stock revived from stored culture by streaking on Nutrient agar (NA) (Yeast extract 5 g, peptone 10 g, agar 20 g and distilled water 1000 ml, pH 7.00) agar plate and incubated at 28°C for 72 hrs. Well separated fluidal colony of *Xap* isolates were selected for genomic DNA extraction after growing them at 48 hr at 28°C at 200 rpm in NA broth. The extraction of total genomic DNA was carried out following a modified protocol as described under material and methods.

The genomic DNA was extracted from four isolates of *X. axonopodis* pv. *punicae* from four locations in Karnataka. DNA extracted from bacterial cultures yielded good quality of high molecular weight DNA as visualized by agarose gel electrophoresis (Plate 8). Further the purity of DNA was tested by using nanodrop.

There was significant difference in the concentration of DNA extracted from the isolates of *X. axonopodis* pv. *punicae*. The DNA concentration obtained from these isolates were further diluted so as to get DNA concentration equivalent to 100ng/ μl for PCR amplification.

The four isolates were characterized based on the 16s rRNA and *gyrB* gene analysis. Four isolates were used for the amplification of 753 bp of the 16s rRNA coding region and 491 bp of *gyrB* gene. The amplicon size of around 763 bp and 491 bp were noticed in all the four isolates using 16s rRNA and *gyrB* gene primer and confirms that, all these isolates belong to *X. axonopodis* pv. *punicae* (Plate 9 and 10).

4.1.7.2 ERIC, BOX and Rep PCR generated genomic finger prints and their relationship with pathogenic variability of *X. axonopodis* pv. *punicae*

ERIC PCR has been shown as useful in diversity analysis of bacterial plant pathogens, including the genera of *Xanthomonas* and *Pseudomonas*. In this study, genomic and pathogenic variability of four pomegranate bacterial blight isolates were

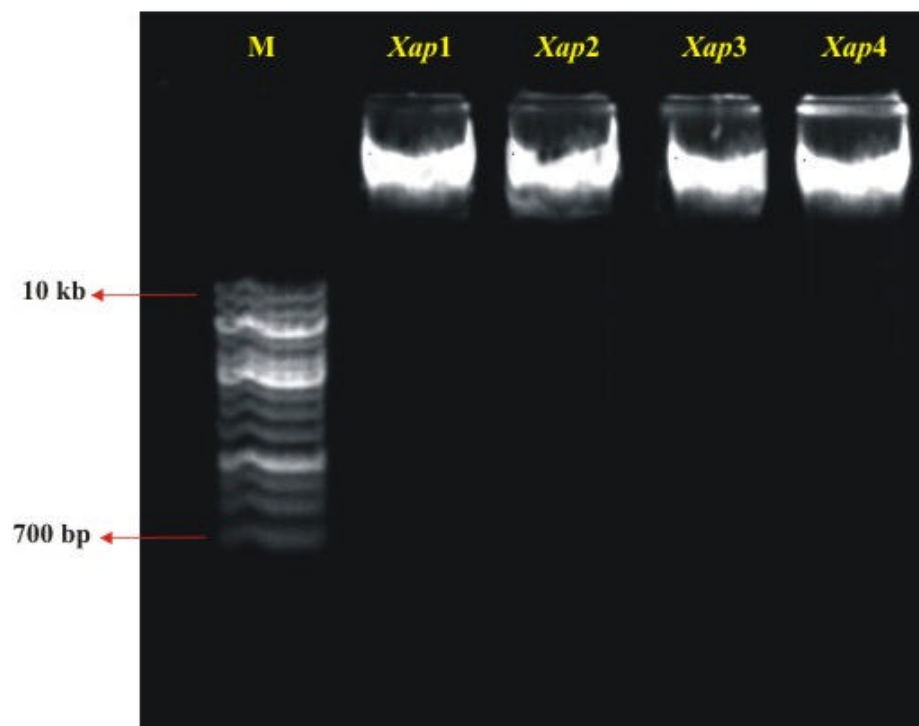


Plate 8. Genomic DNA samples isolated from different isolates of *X. axonopodis* pv. *punicae*

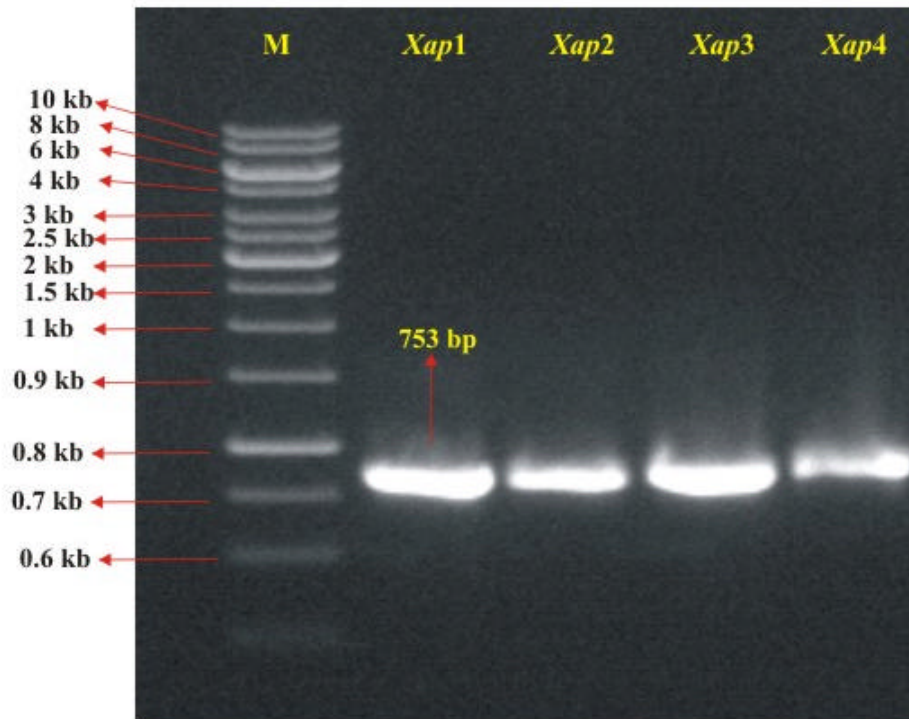


Plate 9. PCR amplification with 16s rRNA primer

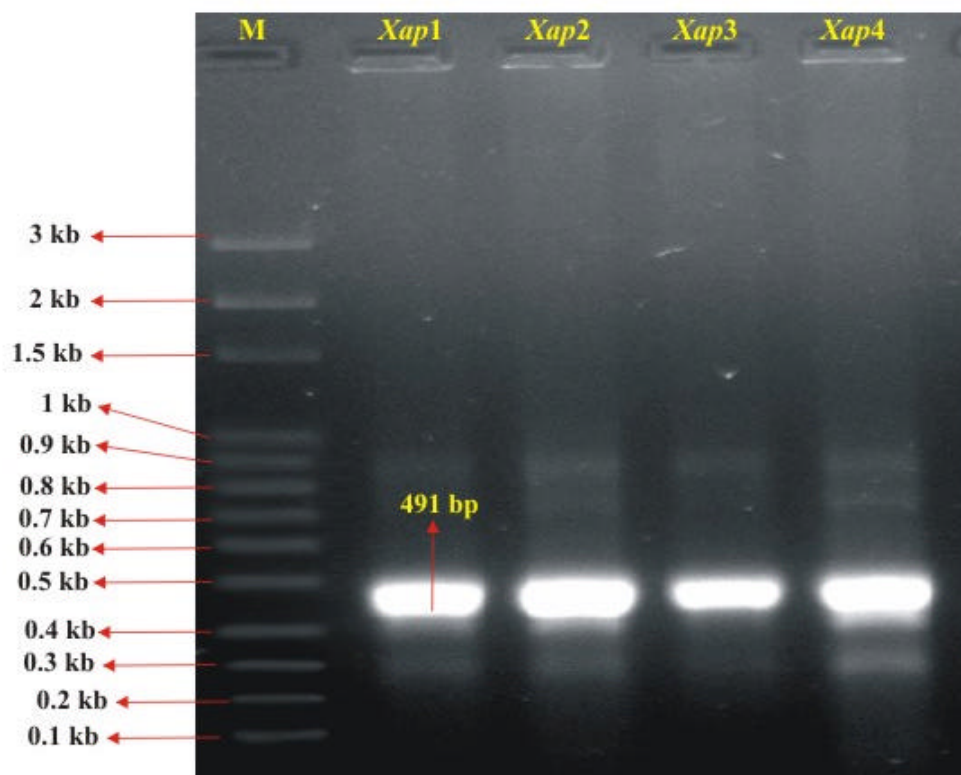


Plate 10. PCR amplification with *gyrB* primer

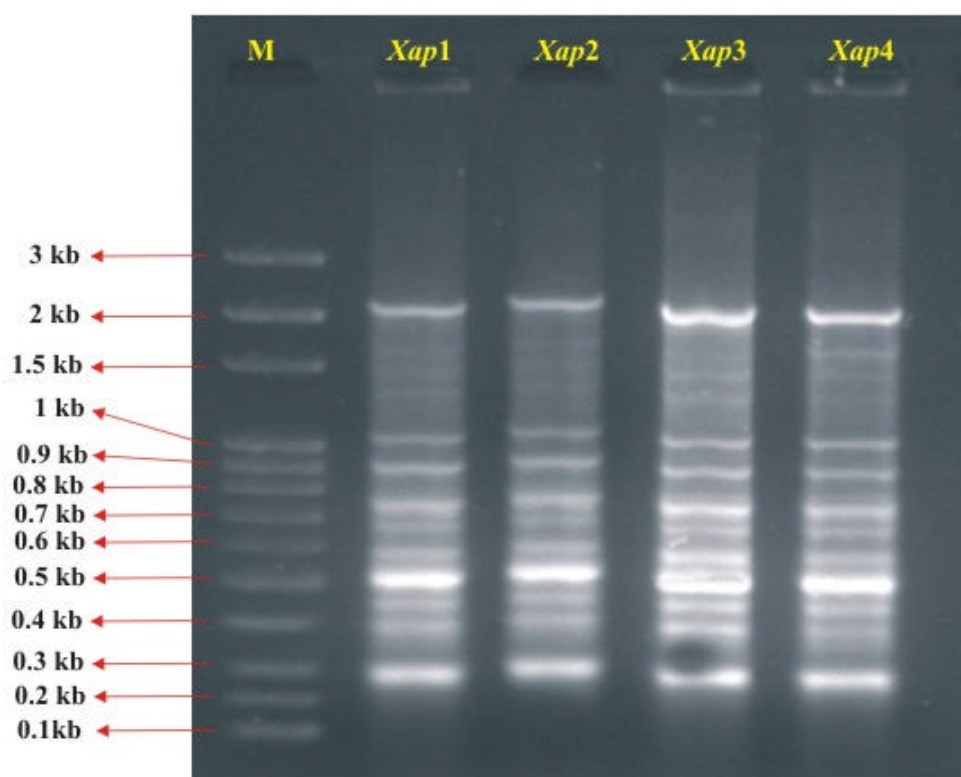


Plate 11. PCR amplification with *Box* primer

collected from different locations of Karnataka viz., Vijayapur, Bagalkot, Koppal and Chitradurga districts and were analysed.

Amplification of ERIC, BOX and Rep region DNA from four isolates of pomegranate bacterial blight from four locations with three different primers yielded a total 28, 20 and 24 bands respectively (Table 19 and Plate 11, 12 and 13) with monomorphic banding pattern in all four isolates and amplicon size ranging from 300 to 2000 bp with BOX and ERIC primers, whereas in case of Rep primers the range is 200 to 1000 bp only. From this, it is confirmed that not much variation is existing in the collected isolates. Hence, isolate from Vijayapur was used for further screening of the germplasm against bacterial blight both *in vivo* and *in vitro*.

4.1.8 Screening of g-ray irradiated seed derived progenies and germplasm of pomegranate for bacterial blight disease resistance

Screening of the genotypes was conducted both under natural condition (*in vivo*) and artificial inoculation conditions (*in vitro*). The mean value of the genotypes tested for bacterial blight disease on leaves for two seasons (2014-15 and 2015-16) is given in Appendix I.

4.1.8.1 Bacterial blight resistance on leaves (*in vivo*)

The Bacterial blight incidence was recorded during *mrig bahar* 2014-15 and 2015-16 using 0-5 Scale as mentioned in the Material and Methods. The results pertaining to reaction of pomegranate genotypes are presented in Table 20 and the means are given in Appendix I. The mean value among all genotypes was 43.39 (PDI) with a range of 9.52 (Nana) to 56.11 (PG25-82). Further, these 475 genotypes were grouped in to five categories. Out of 475 genotypes only one (Nana) was found to be tolerant, only two genotypes Patna-5 and PK20-70 were found to be tolerant with a rating of 1; 12 genotypes were found moderately susceptible, while, 272 genotypes were found highly susceptible with rating of 3. Only one genotype was found highly susceptible. The remaining 188 recorded a rating of 4, with highly susceptibility.

Table 19: Amplicons and their sizes generated in *Xap* isolates from different sources using BOX, ERIC and REP primers

Amplicon size (bp)	BOX PCR				ERIC PCR				REP PCR			
	<i>Xap1</i>	<i>Xap2</i>	<i>Xap3</i>	<i>Xap4</i>	<i>Xap1</i>	<i>Xap2</i>	<i>Xap3</i>	<i>Xap4</i>	<i>Xap1</i>	<i>Xap2</i>	<i>Xap3</i>	<i>Xap4</i>
2000	+	+	+	+	+	+	+	+	-	-	-	-
1500	+	+	+	+					+	+	+	+
1300	+	+	+	+	+	+	+	+	-	-	-	-
1000	+	+	+	+					+	+	+	+
850	-	-	-	-	+	+	+	+	+	+	+	+
750	+	+	+	+					-	-	-	-
680	+	+	+	+					-	-	-	-
600	+	+	+	+	+	+	+	+	-	-	-	-
500	+	+	+	+					+	+	+	+
450	+	+	+	+					+	+	+	+
400	+	+	+	+					-	-	-	-
300	+	+	+	+					-	-	-	-
200	-	-	-	-	+	+	+	+	+	+	+	+
No. of amplicons	7	7	7	7	5	5	5	5	6	6	6	6

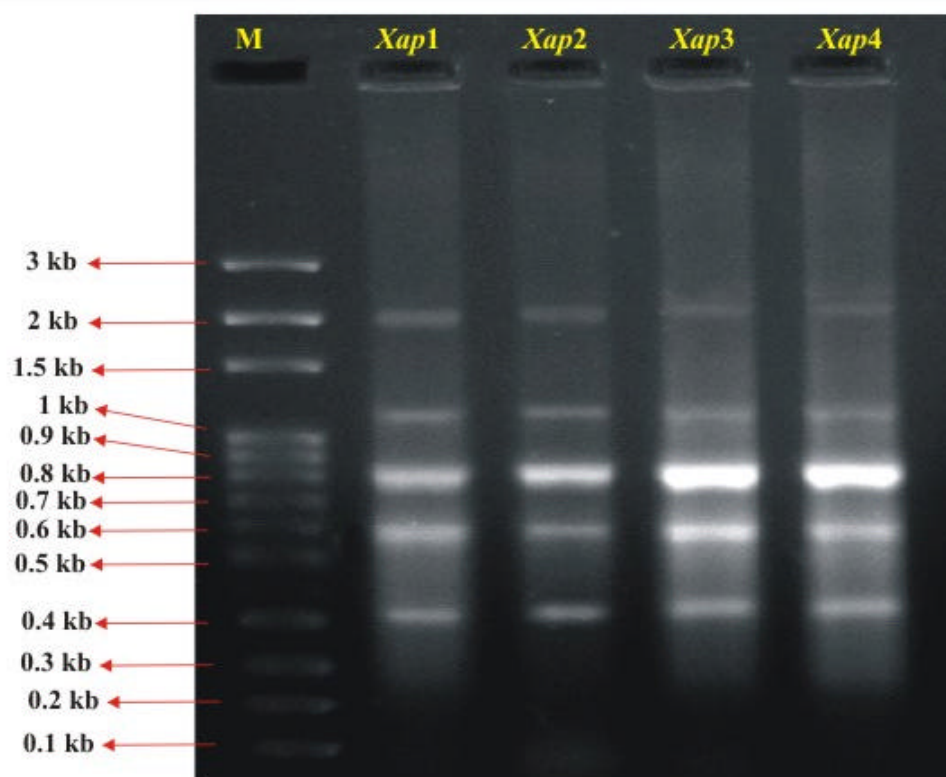


Plate 12. ERIC PCR generated genomic fingerprints

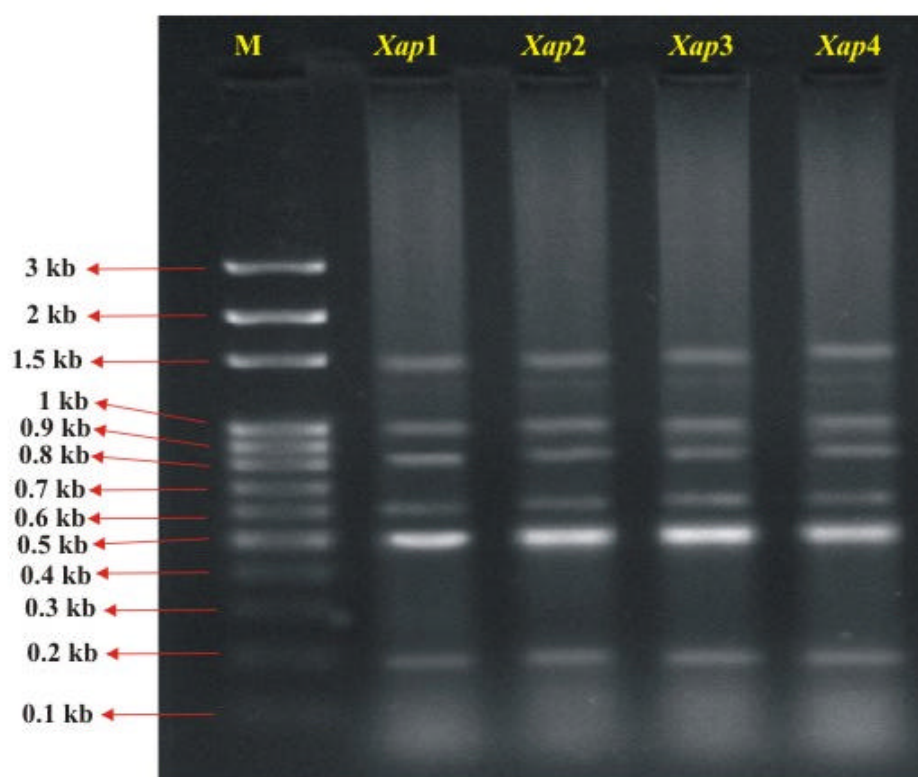


Plate 13. Rep PCR generated genomic fingerprints

Table 20: Reaction of pomegranate genotypes against bacterial blight disease under *in vivo* condition

Rating	Percentage blighted area on leaf (%)	Number of genotypes	Reaction	Genotypes
0	Disease free	01	Resistant	Nana
1	1-10	02	Tolerant	Patna -5, PK20-70
2	11-25	12	Moderately susceptible	PK10-10, PK10-63, PK10-131, PK10-134, PK20-62, PK20-157, PK20-228, PG25-46, PG25-82, G-137, Co-white
3	26-50	272	Highly susceptible	
4	51-75	188	Highly susceptible	
5	76-100	01	Highly susceptible	

Table 21: Reaction of pomegranate genotypes against bacterial blight disease under *in vitro* condition

Rating	Percentage blighted area on leaf (%)	Number of genotypes	Reaction	Genotypes
0	Disease free	01	Resistant	Nana
1	1-10	01	Tolerant	PG20-80
2	11-25	17	Moderately susceptible	PK10-134, PK20-92, PK20-228, PG15-9, PG20151, PG25-4, PG25-46, PG25-82, Early Bhagwa, Yarcaud, Tobesta, Kabuli yellow, P-13, Bedana seedless, G-137, Jalore seedless, Patna-5,
3	26-50	276	Highly susceptible	
4	51-75	179	Highly susceptible	
5	76-100	01	Highly susceptible	

The check varieties Bhagwa, Ganesh and Ruby found to be highly susceptible to Bacterial blight in both the seasons.

4.1.8.2 Bacterial blight resistance on leaves (*in vitro*)

The data regarding reaction of pomegranate genotypes for bacterial blight disease is presented in Table 21 and the mean values are given Appendix-I. The results indicated that the mean value among all the genotypes was 45.93 PDI with a range of 0 (Nana) to 82.65 PDI (PK20-160). Further, these 475 genotypes were grouped in to 5 categories. Out of 475 genotypes, Nana and PG20-80 was found to be resistant and tolerant respectively. Only 17 genotypes were found to be moderately susceptible with a rating of 2. Majority of genotypes were found to be highly susceptible, with 276 in rating 3 and 179 genotypes in a rating of 4. Only one genotype was found highly susceptible. The check varieties Bhagwa, Ganesh and Ruby showed highly susceptible to bacterial blight in both the seasons.

4.2 Evaluation of g-ray irradiated seed derived progenies and germplasm of pomegranate for plant growth, fruit quality traits and bacterial blight disease resistance

4.2.1 Analysis of variance

The mean sum of squares for growth, fruit quality and its component traits in 475 γ irradiated seed derived progenies of pomegranate cv. Bhagwa and Ganesh including germplasm are presented in Table 22. The Analysis of variance pooled across the seasons for different traits indicated the presence of significant amount of high variability among the genotypes and genotype $\times$ season interaction for majority of the traits.

4.2.2 Mean, range and components of variation

The data on mean, range and components of variation for fruit quality traits are presented in Table 23. The mean performance of all germplasm lines is presented in Appendix-II and frequency distribution curves for all traits were depicted in Fig. 4.

Table 22: Analysis of Variance (ANOVA) for plant growth, fruit quality traits and bacterial blight disease resistance in pomegranate genotypes

Sl. No	Characters	Sources of variation			
		Genotypes	Environment	Gen. × Env.	Error
1.	Days to maturity of fruit	731.23**	0.001	26.48**	7.46
2.	Plant height (cm)	326.27**	602.93	293.68**	27.95
3.	Plant spread (cm)	202.69**	207.09	54.82	230.94
4.	Leaf thickness (mm)	0.00001	0.006	0.004	0.011
5.	Leaf length (cm)	0.054**	0.094	0.12**	0.143
6.	Leaf breadth (cm)	0.008*	0.00001	0.048**	0.021
7.	Flower length (cm)	0.007	0.006	0.088**	0.041
8.	Flower breadth (cm)	0.003	0.014	0.029**	0.004
9.	Pistil length (cm)	0.005**	0.000001	0.016**	0.005
10.	Fruit length (cm)	0.144**	0.091	0.111	0.283
11.	Fruit diameter (cm)	0.146**	0.158	0.27**	0.108
12.	Fruit weight (g)	434.27**	355.537	1606.889**	184.27
13.	Calyx length (cm)	0.036**	0.000001	0.064**	0.024
14.	Calyx diameter (cm)	0.023**	0.00001	0.027**	0.022
15.	Total aril weight (g)	134.75**	150.39	261.322**	182.572
16.	Number of arils per fruit	986.75	1896.834	9821.208**	1554.068
17.	Weight of 100 arils (g)	7.08**	1.201	20.31**	1.91
18.	Single aril Weight (g)	0.0001**	0.0006	0.002**	0.004
19.	Aril percentage per fruit (%)	5.20**	0.261	8.966**	12.494
20.	Juice percentage per fruit (%)	15.72**	0.0001	14.598**	8.243
21.	Volume of juice	28.47**	3.951	15.468**	11.841
22.	Total soluble solids (°B)	0.40**	0.094	1.414**	0.61

Contd.....

Table 22 contd.....

Sl. No	Characters	Sources of variation			
		Genotypes	Environment	Gen. × Env.	Error
23.	pH of juice	0.009**	0.038	0.033**	0.019
24.	Acidity (%)	0.031**	0.002	0.007**	0.001
25.	Maturity index (TSS/Acidity)	5.667**	2.071	21.542**	16.21
26.	Phenolic content (mg/1000 ml gallic acid)	80537.59**	43.646	9319.365**	53.01
27.	Anthocyanins (mg/100 ml)	6957.49**	33.367	2286.882**	7.41
28.	Rind thickness (mm)	0.28**	0.007	0.00001	0.15
29.	Rind weight (g)	151.21**	70.917	651.872**	73.31
30.	Rind percentage (%)	11.53**	0.001	0.684**	19.58
31.	Seed length (cm)	0.0002	0.001	0.001**	0.001
32.	Seed width (cm)	0.001**	0.001	0.00	0.00
33.	100 dry seed weight (g)	0.047**	0.047	0.141**	0.014
34.	Bacterial blight on stem (PDI)	64.94**	64.937	17.724**	6.45
35.	Bacterial blight on leaves (PDI)	10.48**	10.48	0.001	41.77
36.	Bacterial blight on fruit (PDI)	93.58**	93.578	29.569**	48.34
37.	Disease severity on tree (%)	51.69**	51.694	12.684**	21.52

*, ** significant at 5 % and 1 % levels respectively.
d.f. (genotypes) = 474

Table 23: The estimates of variability and genetic parameters for plant growth, fruit quality traits and bacterial blight disease resistance in g-ray irradiated seed derived progenies and germplasm of pomegranate

Sl. No.	Characters	Mean	Range	S^2_p	S^2_g	PCV	GCV	Heritability (%)	GA	GAM (%)
1.	Days to maturity of fruit	139.13	74.12- 209.370	746.39	731.23	19.64	19.45	97.97	55.14	39.63
2.	Plant height (cm)	157.64	63.02-228.17	480.29	326.27	13.90	11.46	67.93	30.67	19.45
3.	Plant spread (cm)	122.52	54.29-161.32	289.45	202.69	13.89	11.62	70.03	24.54	20.03
4.	Leaf thickness (mm)	0.51	0.4-0.58	0.00	0.00	13.25	0.02	0.00	0.00	0.00
5.	Leaf length (cm)	4.85	2.88-5.77	0.15	0.05	8.07	4.80	35.45	0.28	5.88
6.	Leaf breadth (cm)	1.63	0.82-3.3	0.04	0.01	11.93	5.60	22.07	0.09	5.46
7.	Flower length (cm)	3.62	2.85-5.71	0.06	0.01	6.85	2.26	10.89	0.06	1.55
8.	Flower breadth (cm)	1.47	0.9-3.04	0.02	0.00	9.33	3.88	17.30	0.05	3.33
9.	Pistil length (cm)	1.26	0.85-2.55	0.01	0.01	9.55	5.84	37.43	0.09	7.38
10.	Fruit length (cm)	5.62	4.35-6.62	0.27	0.14	9.28	6.75	52.88	0.57	10.13
11.	Fruit diameter (cm)	5.57	3.91-7.01	0.31	0.15	9.97	6.86	47.33	0.54	9.73
12.	Fresh fruit weight (g)	107.62	23.19-235.86	1285.07	434.27	33.31	19.36	33.79	24.96	23.19
13.	Calyx length (cm)	1.74	1.04-2.92	0.07	0.04	15.66	10.90	48.46	0.27	15.63
14.	Calyx diameter (cm)	1.64	0.86-2.29	0.04	0.02	12.57	9.23	53.92	0.23	13.90
15.	Total aril weight (g)	55.53	20.13-104.64	312.32	134.75	31.82	20.90	43.14	15.71	28.26
16.	Number of arils per fruit	297.22	115.53-584.62	6296.67	986.75	26.70	10.57	15.67	25.62	8.62
17.	Weight of 100 arils (g)	18.86	8.17-34.03	17.73	7.08	22.32	14.11	39.96	3.47	18.38
18.	Single aril weight (g)	0.19	0.09-0.33	0.00	0.00	21.48	13.44	39.11	0.03	17.37
19.	Aril percentage per fruit (%)	45.83	34.32-54.33	12.89	5.20	7.83	4.98	40.33	2.98	6.51
20.	Juice percentage per fruit (%)	42.00	27.29-70.99	25.14	15.72	11.94	9.44	62.54	6.46	15.38
21.	Volume of juice (ml)	27.42	16.3-57.41	39.24	28.47	22.85	19.46	72.54	9.36	34.14
22.	Total soluble solids (°B)	14.16	11.03-17.16	1.26	0.40	7.92	4.45	31.50	0.73	5.14
23.	pH of juice	3.32	2.86-3.83	0.03	0.01	5.26	2.86	29.56	0.11	3.19

Contd.....

Table 23 contd.....

Sl. No.	Characters	Mean	Range	s^2_p	s^2_g	PCV	GCV	Heritability (%)	GA	GAM (%)
24.	Acidity (%)	0.49	0.34-4.22	0.04	0.03	38.04	35.89	89.01	0.34	70.00
25.	Maturity Index (TSS/Acidity)	30.31	11.9-43.76	20.60	5.67	14.98	7.85	27.50	2.57	8.49
26.	Phenolic content (mg/1000 ml gallic acid)	277.78	18.75-1938.59	85210.90	80537.59	105.09	102.17	94.52	568.35	204.61
27.	Anthocyanins (mg/100 ml)	71.67	0.17-679.97	8102.83	6957.49	125.59	116.38	85.86	159.22	222.16
28.	Rind thickness (mm)	3.30	1.63-4.94	0.32	0.28	17.07	16.00	87.85	1.02	30.88
29.	Rind weight (g)	53.56	10.06-158.03	495.98	151.21	41.58	22.96	30.49	13.99	26.11
30.	Rind percentage (%)	44.73	33.08-56.98	16.90	11.53	9.19	7.59	68.21	5.78	12.92
31.	Seed length (cm)	0.68	0.52-0.76	0.00	0.00	4.41	1.66	14.13	0.01	1.32
32.	Seed width (cm)	0.27	0.21-0.3	0.00	0.00	9.09	8.49	87.18	0.04	16.30
33.	100 dry seed weight (g)	1.83	0.9-3.72	0.12	0.05	19.05	11.85	38.71	0.28	15.19
34.	Bacterial blight on stem (PDI)	51.80	15.31-72.66	75.46	64.94	16.77	15.56	86.06	15.40	29.73
35.	Bacterial blight on leaves (PDI)	41.60	33.33-46.38	21.21	10.48	11.07	7.78	49.41	4.69	11.27
36.	Bacterial blight on fruit (PDI)	48.65	18.22-81.96	120.78	93.58	22.59	19.89	77.48	17.54	36.05
37.	Disease severity on tree (%)	48.45	23.33-70.02	63.57	51.69	16.46	14.84	81.32	13.36	27.57

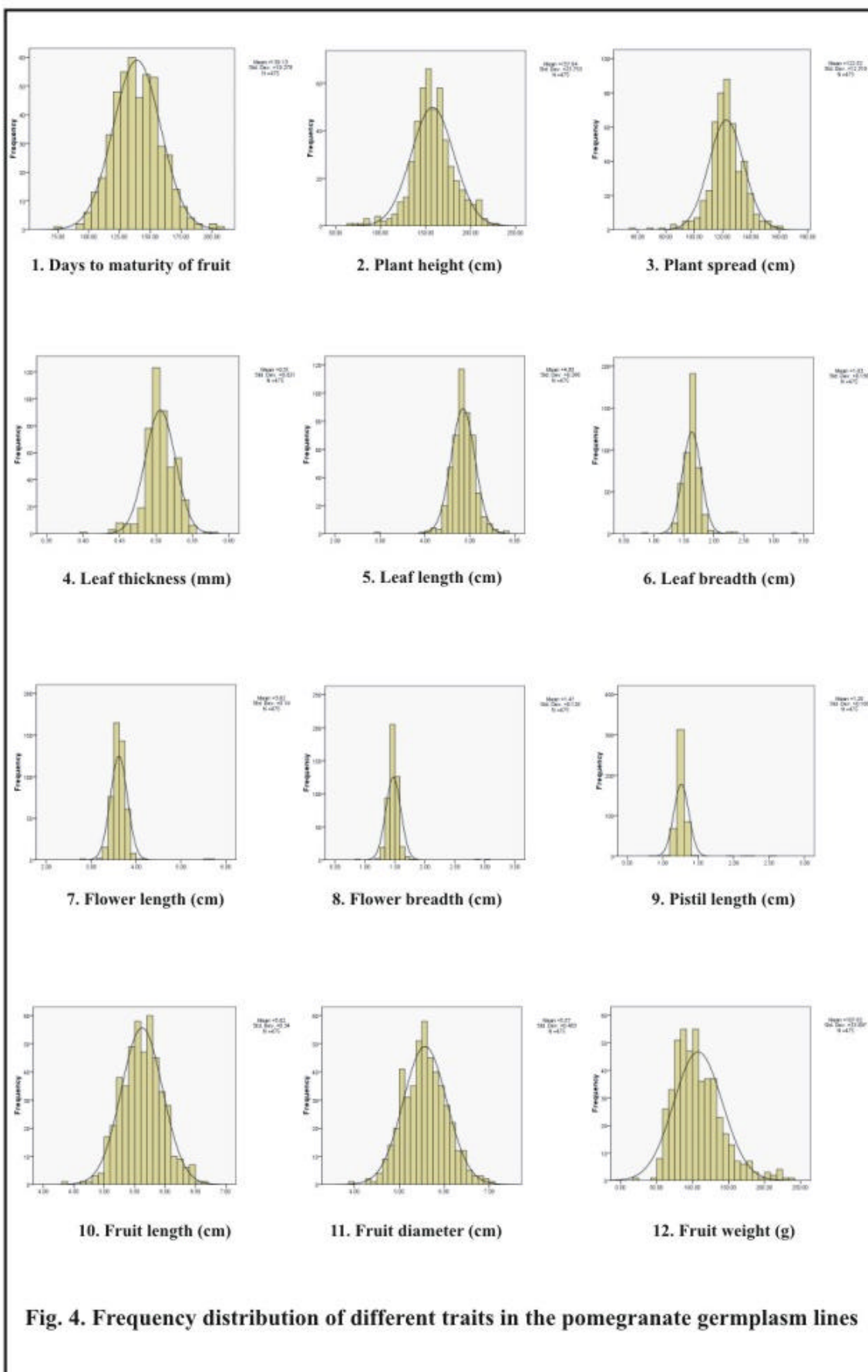
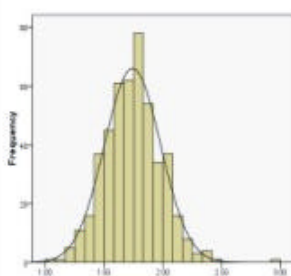
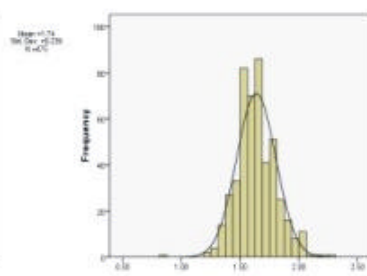


Fig. 4. Frequency distribution of different traits in the pomegranate germplasm lines

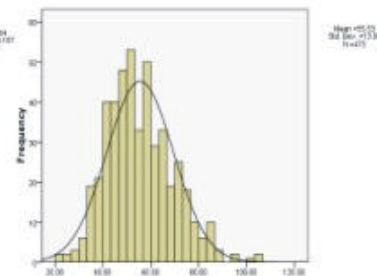
Fig. 4 contd....



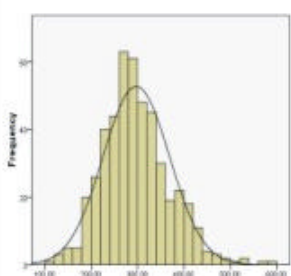
13. Calyx length (cm)



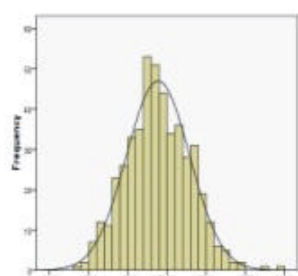
14. Calyx diameter (cm)



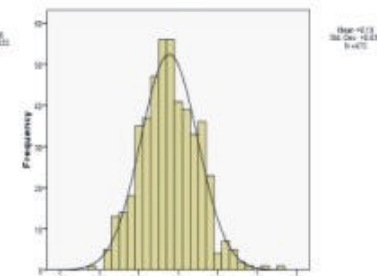
15. Total aril weight (g)



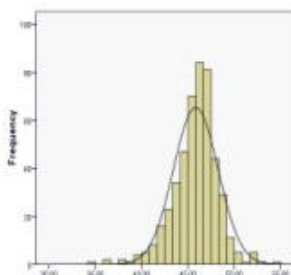
16. Number of arils per fruit



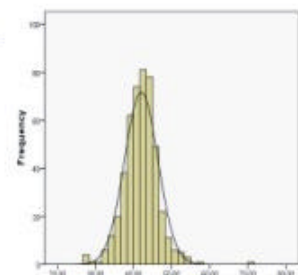
17. 100 aril weight (g)



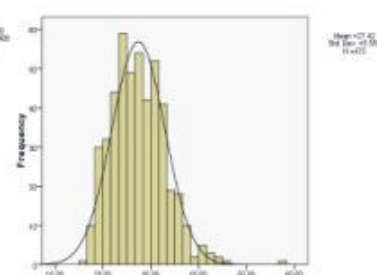
18. Single aril weight (g)



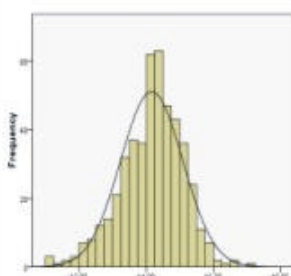
19. Aril percentage per fruit (%)



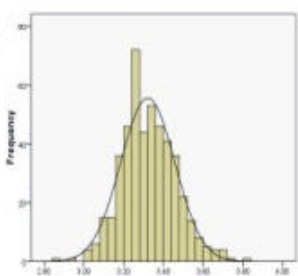
20. Juice percentage per fruit (%)



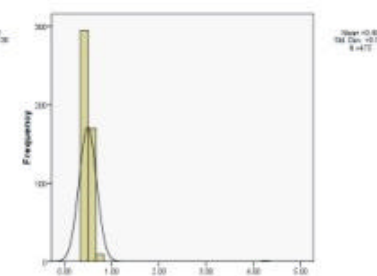
21. Volume of juice



22. Total soluble solids (°B)

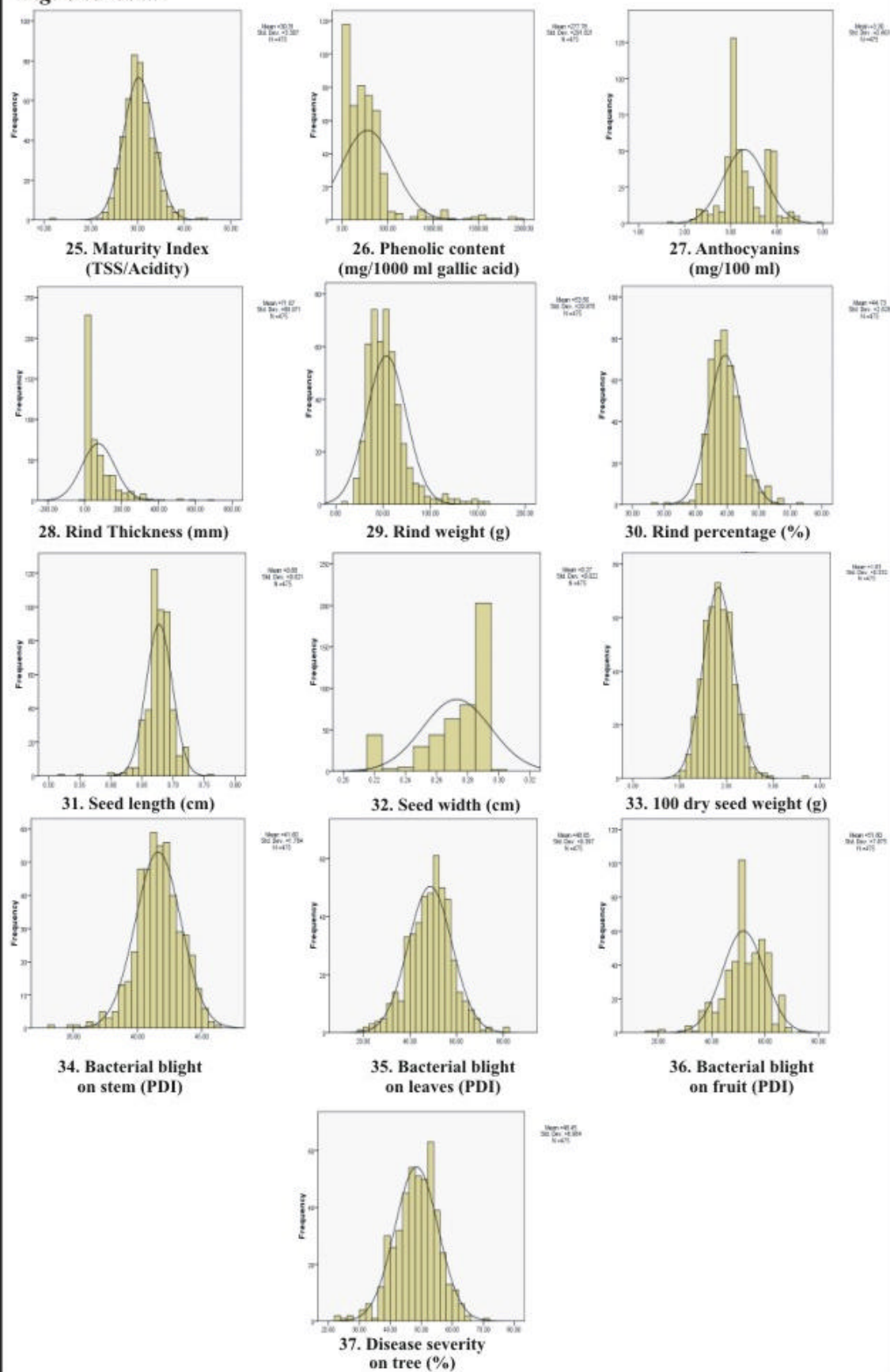


23. pH of juice



24. Acidity (%)

Fig. 4 contd....



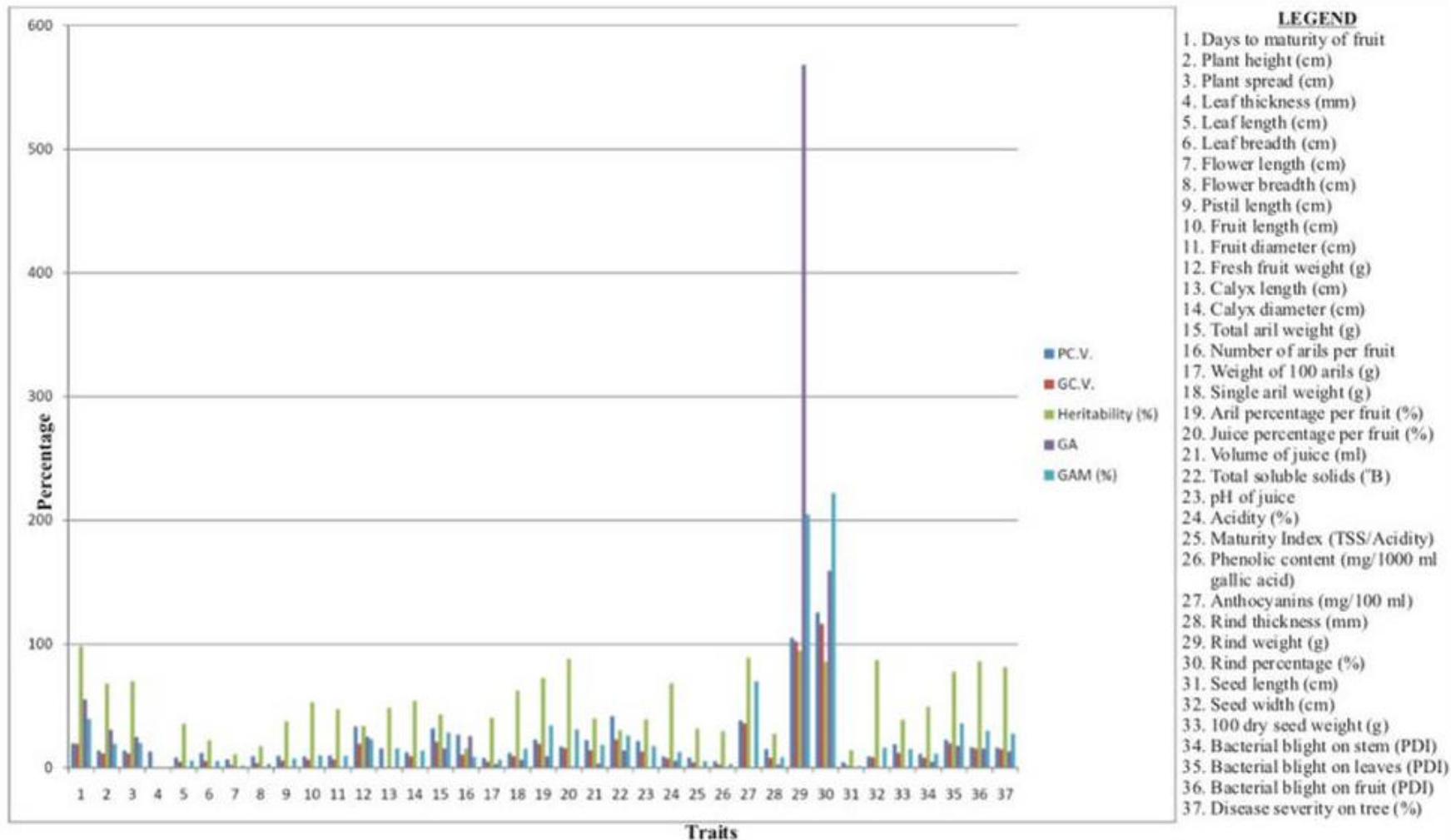


Fig. 5: Genotypic (GCV), phenotypic co-efficient of variation (PCV), heritability (h^2_{bc}) and genetic advance as percent mean (GAM) for characters in pomegranate

4.2.2.1 Days to maturity

On an average for two seasons, Days to maturity was ranging from 74.12 to 209.3 with average mean of 139.13 days. Frequency distribution of germplasm lines was normal for the trait. Genetic variation for days to maturity was moderate as indicated by moderate GCV (19.45 %), PCV (19.64 %), heritability (97.97 %) and GAM (39.63 %) over environments.

4.2.2.2 Plant height

On an average over for seasons, plant height was highest in PG10-31 (228.17 cm) and statistically significant with all checks with wide range of variation ranging from 63.02 to 228.17 with mean 157.64. Frequency distribution of germplasm lines was normal for the trait. Genetic variation for plant height was moderate as indicated by moderate GCV (11.46 %), PCV (13.90 %), with high heritability (67.93 %) and GAM (19.45 %) over two seasons.

4.2.2.3 Plant spread

The pooled variability for plant spread was high which ranges from 54.29 to 169.32 cm with an average of 122.52 cm. The moderate PCV (13.89) and GCV (11.62) were recorded for this trait with high heritability (70.03 %) coupled with moderate genetic advance as per cent of mean (20.03 %).

4.2.2.4 Leaf thickness

The pooled variability for leaf thickness was low with the range from 0.40 mm to 0.58 mm with an average of 0.51 mm. The moderate PCV (13.25) and low GCV (0.02) were observed for this trait with no heritability and genetic advance as per cent of mean (0.00 %).

4.2.2.5 Leaf length

The pooled variability for leaf length was found with a wide range of variation, ranging from 2.88 to 5.77 cm with an average of 4.85 cm. The low PCV (8.07) and GCV (4.80) with moderate heritability (35.45 %) and moderate genetic advance as per cent of mean (10.79 %) was observed for this trait.

4.2.2.6 Leaf breadth

The pooled variability for leaf breadth ranged from 0.82 to 3.30 cm with an average of 1.63 cm. The moderate PCV (11.93) and low GCV (5.6) was observed for this trait. The magnitude of heritability estimated was low (22.07 %) with low expected genetic advance as per cent of mean (5.46 %).

4.2.2.7 Flower length

The pooled variability for flower length ranged from 2.85 to 5.71 cm with an average of 3.62 cm. The PCV and GCV were low with 6.85 and 2.26, respectively. The magnitude of heritability estimated was low (10.89 %) with low expected genetic advance as per cent of mean (1.55 %).

4.2.2.8 Flower breadth

The pooled variability for flower breadth ranged from 0.90 to 3.04 cm with an average of 1.47 cm. The PCV and GCV were low with 9.33 and 3.88, respectively. The magnitude of heritability estimated was low (17.30 %) with low expected genetic advance as per cent of mean (3.33 %).

4.2.2.9 Pistil length

The pooled variability for pistil length ranged from 0.85 to 2.55 cm with an average of 1.26 cm. The PCV and GCV were low with 9.55 and 5.84, respectively. The magnitude of heritability estimated was moderate (37.43 %) with low expected genetic advance as per cent of mean (7.38 %).

4.2.2.10 Fruit length

The pooled variability for fruit length ranged from 4.35 to 6.62 cm with an average of 5.62 cm. The PCV and GCV were low with 9.28 and 6.75, respectively. The magnitude of heritability estimated was moderate (52.88 %) with moderate expected genetic advance as per cent of mean (10.13 %).

4.2.2.11 Fruit diameter

The pooled variability for fruit diameter was high which ranged from 3.91 to 7.01 cm with an average of 5.57 cm. The low PCV (9.97) and GCV (6.86) were recorded for this trait with moderate heritability (47.33 %) coupled with low genetic advance as per cent of mean (9.73 %).

4.1.2.12 Fresh fruit weight

The pooled variability for fresh fruit weight has shown wide range of variation, which ranges from 23.19 to 235.86g with an average of 107.62g. The high PCV (33.31) and moderate GCV (19.36) was observed for this trait. The magnitude of heritability estimated was moderate (33.79 %) with high expected genetic advance as per cent of mean (23.19 %).

4.2.2.13 Calyx length

The pooled variability for calyx length ranges from 1.04 to 2.92 cm with an average of 1.74 cm. The moderate PCV (15.66) and GCV (10.90) were observed for this trait. The magnitude of heritability estimated was moderate (48.46 %) with moderate expected genetic advance as per cent of mean (15.63 %)

4.2.2.14 Calyx diameter

The pooled variability for calyx diameter ranges from 0.86 to 2.29 cm with an average of 1.64 cm. The moderate PCV (12.57) and low GCV (9.23) was observed for this trait. The magnitude of heritability estimated was moderate (53.92 %) with moderate expected genetic advance as per cent of mean (13.90 %)

4.2.2.15 Total aril weight per fruit

The pooled variability for total aril weight was high which ranges from 20.13 to 104.64 g with an average of 55.53 g. The high PCV (31.28) and GCV (20.90) were observed for this trait with moderate heritability (43.14 %) and high genetic advance as per cent of mean (28.26 %).

4.2.2.16 Number of arils per fruit

The pooled variability for total aril weight was high with a range from 115.53 to 584.62 with an average of 297.22. The moderate PCV (26.70) and GCV (10.57) was observed for this trait. The magnitude of heritability estimated was low (15.67 %) with low expected genetic advance as per cent of mean (8.62 %).

4.2.2.17 Weight of 100 arils

The pooled variability for hundred aril weight ranged from 8.17 to 34.03 g with an average of 18.86 g. The moderate PCV (22.32) and low GCV (14.11) was observed for this trait. The magnitude of heritability estimated was moderate (39.96 %) with moderate expected genetic advance as per cent of mean (18.38 %)

4.2.2.18 Single aril weight

The pooled variability for single aril weight was high with a range from 0.09 to 0.33g with an average of 0.19 g. The high PCV (21.48) and moderate GCV (13.44) were observed for this trait. The magnitude of heritability estimated was moderate (39.11 %) with high expected genetic advance as per cent of mean (17.37 %).

4.2.2.19 Aril percentage per fruit

The pooled variability for aril percentage per fruit ranged from 34.32 to 54.33 per cent with an average of 45.83 per cent. The low PCV (7.83) and GCV (4.98) was observed for this trait. The magnitude of heritability estimated was moderate (40.33 %) with moderate expected genetic advance as per cent of mean(6.51 %)

4.2.2.20 Juice percentage per fruit

The pooled variability for juice percentage per fruit was high with a range from 27.29 to 70.99 with an average of 42.00 per cent. The moderate PCV (11.94) and low GCV (9.44) was observed for this trait with high heritability (62.54 %) and high genetic advance as per cent of mean (15.38 %).

4.2.2.21 Volume of juice per fruit

The pooled variability for volume of juice was high with a range from 16.30 to 57.41 ml with an average of 27.42 ml. The high PCV (22.85) and moderate GCV (19.46) were recorded for this trait with high heritability (72.54 %) coupled with high genetic advance as per cent of mean (34.14 %).

4.2.2.22 Total soluble solids

The pooled variability for rind percentage with a range from 11.03 to 17.16 with an average of 14.16. The low PCV (7.92) and GCV (4.45) were observed for this trait. The magnitude of heritability estimated was moderate (31.50 %) with low expected genetic advance as per cent of mean (5.14 %).

4.2.2.23 pH of juice

The pooled variability for juice pH with a range from 2.86 to 3.83 with an average of 3.32. The low PCV (5.26) and GCV (2.86) were observed for this trait with low heritability (29.56 %) and low genetic advance as per cent of mean (3.19 %).

4.2.2.24 Acidity

The pooled variability for acidity of juice was high with a range from 0.34 to 4.22 per cent with an average of 0.49 per cent. The high PCV (38.04) and GCV (35.89) were recorded for this trait with high heritability (89.01 %) coupled with high genetic advance as per cent of mean (70.00 %).

4.2.2.25 Maturity index

The pooled variability for maturity index with a range from 11.90 to 43.76 per cent with an average of 30.31 per cent. The moderate PCV (14.98) and low GCV (7.85) was observed for this trait. The magnitude of heritability estimated was low (27.50 %) with low expected genetic advance as per cent of mean (8.49 %).

4.2.2.26 Phenolic content

The pooled variability for phenolic content was high with a range from 18.75 to 1938.59 mg with an average of 277.78 mg. The high PCV (105.09) and GCV (102.17) were observed for this trait. The magnitude of heritability estimated was high (94.52 %) with high expected genetic advance as per cent of mean (204.61 %).

4.2.2.27 Anthocyanins

The pooled variability for anthocyanins was high with a range from 0.17 to 679.97 mg with an average of 71.67 mg. The high PCV (125.59) and GCV (116.38) were observed for this trait. The magnitude of heritability estimated was high (85.86 %) with high expected genetic advance as per cent of mean (222.16 %).

4.2.2.28 Rind thickness

The pooled variability for rind thickness was high with a range from 1.63 to 4.94 mm with an average of 3.30 mm. The moderate PCV (17.07) and GCV (16.00) were recorded for this trait with high heritability (87.85 %) coupled with high genetic advance as per cent of mean (30.88 %).

4.2.2.29 Rind weight

The pooled variability for total aril weight was high with a range from 10.06 to 158.03 g with an average of 53.56 g. The high PCV (41.58) and GCV (22.96) were observed for this trait. The magnitude of heritability estimated was moderate (30.49 %) with high expected genetic advance as per cent of mean (26.11 %).

4.2.2.30 Rind percentage per fruit

The pooled variability for rind percentage with a range from 33.08 to 56.98 per cent with an average of 44.73 per cent. The low PCV (9.19) and GCV (7.59) were observed for this trait. The magnitude of heritability estimated was high (68.21 %) with moderate expected genetic advance as per cent of mean (12.92 %).

4.2.2.31 Seed length

The pooled variability for seed length ranged from 0.52 to 0.76 cm with an average of 0.68 cm. The low PCV (4.41) and GCV (1.66) were recorded for this trait with low heritability (14.13 %) coupled with low genetic advance as per cent of mean (1.32 %).

4.2.2.32 Seed width

The pooled variability for seed length ranged from 0.21 to 0.30cm with an average of 0.27 cm. The low PCV (9.09) and GCV (8.49) was recorded for this trait with high heritability (87.18 %) coupled with moderate genetic advance as per cent of mean (16.30 %).

4.2.2.33 Hundred dry seed weight

The pooled variability for 100 dry seed weight ranged from 0.9 to 3.72 g with an average of 1.83 g. The moderate PCV (19.05) and low GCV (11.85) were observed for this trait. The magnitude of heritability estimated was moderate (38.71 %) with moderate expected genetic advance as per cent of mean (15.19 %).

4.2.2.34 Bacterial blight disease on stem (PDI)

The pooled PDI variability for bacterial blight on stem ranges from 15.31 to 72.66 per cent with an average of 51.80 per cent. The moderate PCV (16.77) and GCV (15.56) were observed for this trait. The magnitude of heritability estimated was high (86.06 %) with high expected genetic advance as per cent of mean (29.73 %).

The screened genotypes were categorized into five groups based on the reaction to the bacterial blight disease and presented in Table 24. Out of 475 genotypes, none of the genotypes were found resistant and only two genotypes Nana and PK20-50 were having less than 10 per cent disease infection and rated as tolerant. Six genotypes namely PK10-90, PK10-140, PK15-72, PK20-38, PK20-238 and PK25-48 were found to be moderately susceptible. Majority of genotypes (322) were rated as highly susceptible

Table 24: Reaction of pomegranate genotypes against bacterial blight disease on stem during 2014-15 and 2015-16

Rating	Percentage blighted area on stem (%)	Number of genotypes	Reaction	Genotypes
0	Disease free	Nil	Resistant	
1	1-10	02	Tolerant	PK20-54 and Nana
2	11-25	06	Moderately susceptible	PK10-90, PK10-140, PK15-72, PK20-38, PK20-238, PG25-48
3	26-50	67	Highly susceptible	
4	51-75	322	Highly susceptible	
5	76-100	78	Highly susceptible	

Table 25: Reaction of pomegranate genotypes against bacterial blight disease on leaves during 2014-15 and 2015-16

Rating	Percentage blighted area on leaf (%)	Number of genotypes	Reaction	Genotypes
0	Disease free	Nil	Resistant	
1	1-10	01	Tolerant	Nana
2	11-25	10	Moderately susceptible	PK10-10, PK10-88, PK10-201, PK10-206, PK20-64, PK20-157, PG25-48, Early Bhagwa, Yarcaud, Patna5
3	26-50	339	Highly susceptible	
4	51-75	125	Highly susceptible	
5	76-100	Nil	Highly susceptible	

with rating of 4 followed by rating 5 and 3 with 78 and 67 genotypes respectively under the category of highly susceptible. The check varieties Bhagwa, Ganesh and Ruby fall under highly susceptible group with disease rating of 4.

4.2.2.35 Bacterial blight disease on leaves (PDI)

The pooled PDI variability for bacterial blight on leaves ranges from 33.33 to 46.38 per cent with an average of 41.60 per cent. The moderate PCV (11.07) and low GCV (7.78) were observed for this trait. The magnitude of heritability estimated was moderate (49.41 %) with moderate expected genetic advance as per cent of mean (11.27 %).

Further, these 475 genotypes were grouped into 5 categories. Out of 475 genotypes none of them were found to be resistant, only one genotype Nana was found to be tolerant with a rating of 1; ten genotypes showed moderately susceptible, while 339 and 125 genotypes showed highly susceptible with rating 3 and 4 respectively and data are presented in Table 25. The check varieties Bhagwa, Ganesh and Ruby showed highly susceptible to bacterial blight.

4.2.2.36 Bacterial blight disease on fruits (PDI)

The pooled PDI variability for bacterial blight on fruits ranges from 18.22 to 81.96 per cent with an average of 48.65 per cent. The high PCV (22.59) and moderate GCV (19.89) were observed for this trait. The magnitude of heritability estimated was high (77.48 %) with high expected genetic advance as per cent of mean (36.05 %).

The screened genotypes were categorized into five groups based on the reaction to the bacterial blight and presented in Table 26. Out of 475 genotypes, none of the genotypes found resistant and only six genotypes viz., PK10-90, PK10-201, PK10-204, PK15-12, PK20-38 and PK25-18 were having less than 10 per cent disease infection and rated as tolerant. Eighteen genotypes were found to be moderately susceptible. Majority of genotypes (339) were rated as highly susceptible with rating of 3 and 125 genotypes fall under the category of highly susceptible with disease score rating of 4. The check varieties Bhagwa, Ganesh and Ruby fall under the category of highly susceptible.

Table 26: Reaction of pomegranate genotypes against bacterial blight disease on fruits during 2014-15 and 2015-16

Rating	Percentage blighted area on fruit (%)	Number of genotypes	Reaction	Genotypes
0	Disease free	Nil	Resistant	
1	1-10	06	Tolerant	PK10-90, PK10-201, PK10-201, PK15-12, PK20-38, 310, PK25-18
2	11-25	18	Moderately susceptible	36, 45, 110, 131, 152, 154, 157, 160, 205, 214, 230, 306, 367, 387, 399, 455, 470.472
3	26-50	339	Highly susceptible	
4	51-75	125	Highly susceptible	
5	76-100	Nil	Highly susceptible	

4.2.2.37 Bacterial blight disease severity on tree

The pooled variability for bacterial blight on tree ranges from 23.33 to 70.02 per cent with an average of 48.45 per cent. The moderate PCV (16.46) and GCV (14.84) were observed for this trait. The magnitude of heritability estimated was high (81.32 %) with high expected genetic advance as per cent of mean (27.57 %). Further disease severity on tree is calculated by using the formula (Singh *et al.*, 2015) and results are presented in Appendix II.

4.2.3 Characterization of germplasm, accessions for qualitative traits

Germplasm accessions were characterized with respect to flower colour, young leaf colour, leaf colour, thorniness, aril colour and fruit skin colour. The variations for these characters are presented in Table 27.

Majority of the genotypes had light red flowers with 213 accessions (44.84 %), green with pink tinge young leaf with 306 accessions (64.42 %) and dark green leaves with 459 accessions (96.63 %). Majority of the genotypes had medium thorniness with 206 (43.37 %) and red arils with 241 accessions (50.74 %). The colour of the mature fruits was red in majority of the genotypes with 243 accessions (51.59 %).

4.2.4 Performance of superior genotypes for fruit quality traits and bacterial blight disease resistance

All the 475 genotypes were assessed based on nine selected quality parameters; Only 20 genotypes (Table 28, Plate 14 and 15) were found superior to check varieties Bhagwa (25), Ganesh (21) and Ruby (20). Among which only ten genotypes were found superior over best check variety Bhagwa (25) and remaining ten genotypes found superior over Ganesh and Ruby. The genotype PK10-190 and PK15-41 scored the maximum (28 points) out of a total index score of (45 points) followed by PG10-37, PK10-59 and PK20-47 with index score of 27 points. Generally, the superiority of the genotypes were due to high score index over checks. Out of 20 genotypes only two genotypes (PK10-59 and PK15-48) were found to be moderately susceptible to bacterial blight with low disease severity of 32.43 and 30.16 per cent, respectively.

Table 27: Variation for qualitative characters in pomegranate genotypes (475 accessions)

Sl. No.	Characters		Number of accessions	Per cent accessions
1		<i>Flower colour</i>		
	1	Yellow	01	0.21
	2	Red	129	27.16
	3		61	12.84
	4	Deep red	46	9.68
	5	Crimson	25	5.26
	6	Scarlet	213	44.84
		Light red		
2		<i>Young leaf colour</i>		
	1	Light green	55	11.58
	2	Green	36	7.58
	3	Dark green	62	13.05
	4	Green with pink tinge	306	64.42
	5	Pinkish brown	16	3.37
3		<i>Leaf colour</i>		
	1	Green	4	0.84
	2	Pale green	12	2.53
	3	Dark green	459	96.63
4		<i>Thorniness</i>		
	3	Less	114	24.00
	5	Medium	206	43.37
	7	High	155	32.63
5		<i>Aril colour</i>		
	1	Creamy white	128	26.95
	2	Yellow	01	0.21
	3	Light pink	101	21.26
	4	Pink	01	0.21
	5	Red	241	50.74
	6	Blood red	02	0.42
6		<i>Fruit skin colour</i>		
	1	Creamy white	00	0
	2	Yellow	82	17.26
	3	Dull red	142	29.89
	4	Red	243	51.16
	5	Deep red	3	0.63
	6	Pink	5	1.05

Table 28: Selection index score for fruit quality traits of 20 superior pomegranate genotypes

S.No.	Genotypes	Fruit weight	Aril (%)	Juice (%)	100 Aril weight	TSS	Acidity	Maturity index	Rind colour	Aril colour	Total selection index scores	Reaction to bacterial blight			
												Stem	Leaves	Fruits	DS on Tree
1	PK10-190	1	1	1	3	5	5	5	3	4	28	HS	HS	HS	55.04
2	PK15-41	1	1	1	3	5	5	5	3	4	28	HS	HS	HS	33.35
3	PG10-37	1	1	5	3	5	4	4	3	1	27	HS	HS	HS	69.60
4	PK10-59	1	1	2	2	5	5	4	3	4	27	HS	HS	MS	32.43
5	PK20-47	1	1	2	2	4	5	5	3	4	27	HS	HS	HS	56.48
6	PG25-37	2	1	2	4	5	5	5	1	1	26	HS	HS	HS	68.00
7	PK15-40	1	1	1	3	4	5	4	3	4	26	HS	HS	HS	42.40
8	PK10-2	1	1	2	2	4	5	4	3	4	26	HS	HS	HS	56.88
9	PK15-48	1	1	1	2	5	5	4	3	4	26	HS	HS	MS	30.16
10	PK15-85	1	1	1	2	5	5	4	3	4	26	HS	HS	HS	71.20
11	PK20-64	1	1	3	2	3	5	4	3	4	26	HS	HS	HS	44.80
12	PG20-23	1	1	3	2	5	5	4	3	1	25	HS	HS	HS	58.24
13	PG10-35	1	1	1	2	5	5	5	3	1	24	HS	HS	HS	85.95
14	PG10-36	1	1	1	3	5	5	4	3	1	24	HS	HS	HS	62.32
15	PG20-30	1	1	1	3	5	5	4	3	1	24	HS	HS	HS	52.24
16	PK20-92	1	1	1	4	3	5	4	3	2	24	HS	HS	HS	51.04
17	PK10-182	2	1	1	2	4	5	4	3	2	24	HS	HS	HS	76.00
18	PG20-93	1	1	1	2	5	5	5	3	1	24	HS	HS	HS	70.48
19	PK15-78	1	1	1	3	4	5	4	2	2	23	HS	HS	HS	56.72
20	PG10-38	1	1	1	3	5	5	4	1	1	22	HS	HS	HS	52.08

Contd.....

Table 28 contd.....

S.No.	Genotypes	Fruit weight	Aril (%)	Juice (%)	100 Aril weight	TSS	Acidity	Maturity index	Rind colour	Aril colour	Total selection index scores	Reaction to bacterial blight			
												Stem	Leaves	Fruits	DS on Tree
Checks															
1	Ganesh	1	1	2	2	3	4	3	3	2	21	HS	HS	HS	57.63
2	Bhagwa	1	1	2	1	3	5	4	4	4	25	HS	HS	HS	68.95
3	Ruby	1	1	1	1	1	5	4	3	3	20	HS	HS	HS	52.32
4	Nana	1	1	1	1	2	1	1	3	3	14	MS	MS	MS	9.47
5	Patna-5	1	1	1	1	3	4	3	1	1	16	HS	MS	HS	35.34
C.D. @ 5 %															9.84
C.V.															9.58

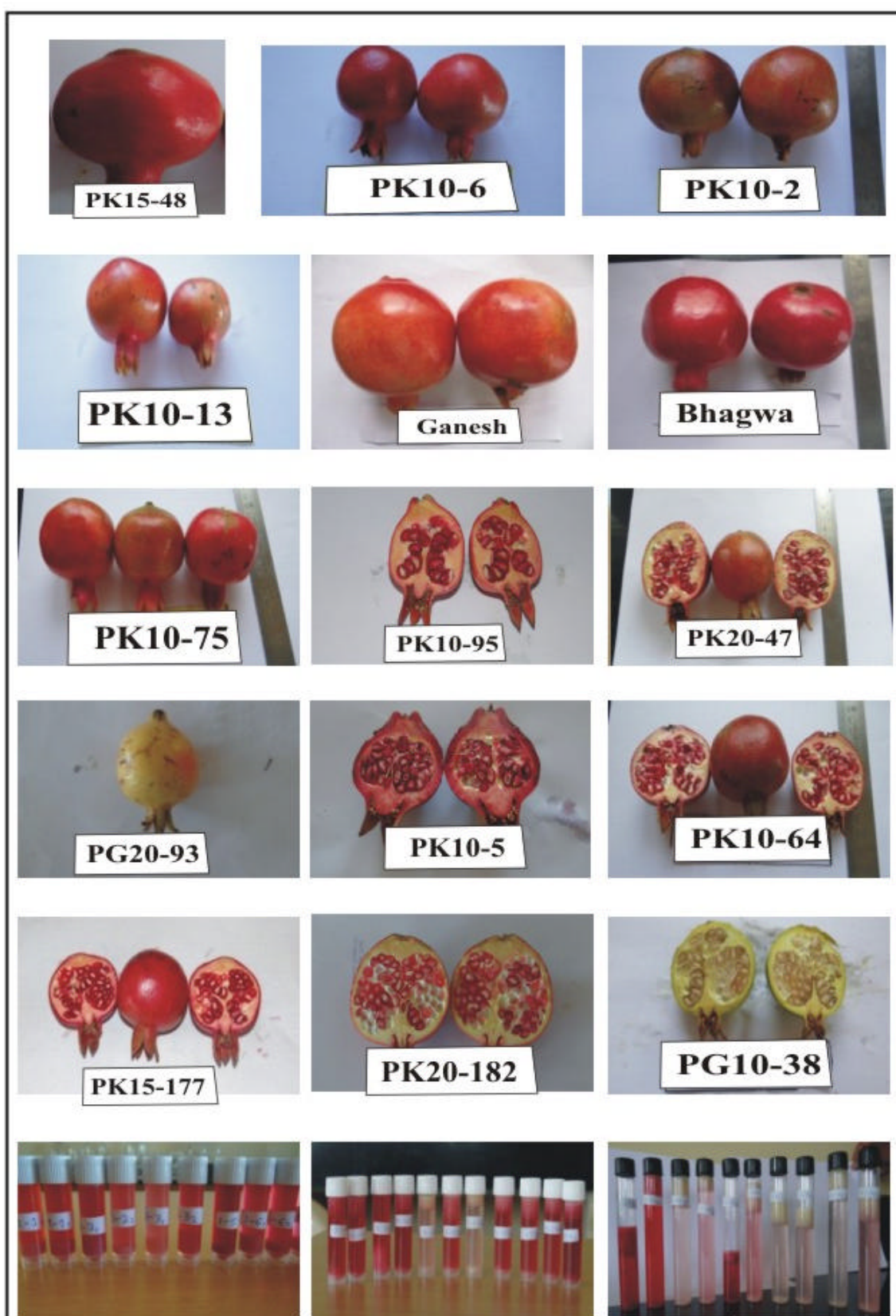


Plate 14. Variability in fruit, aril and juice colour in pomegranate genotypes

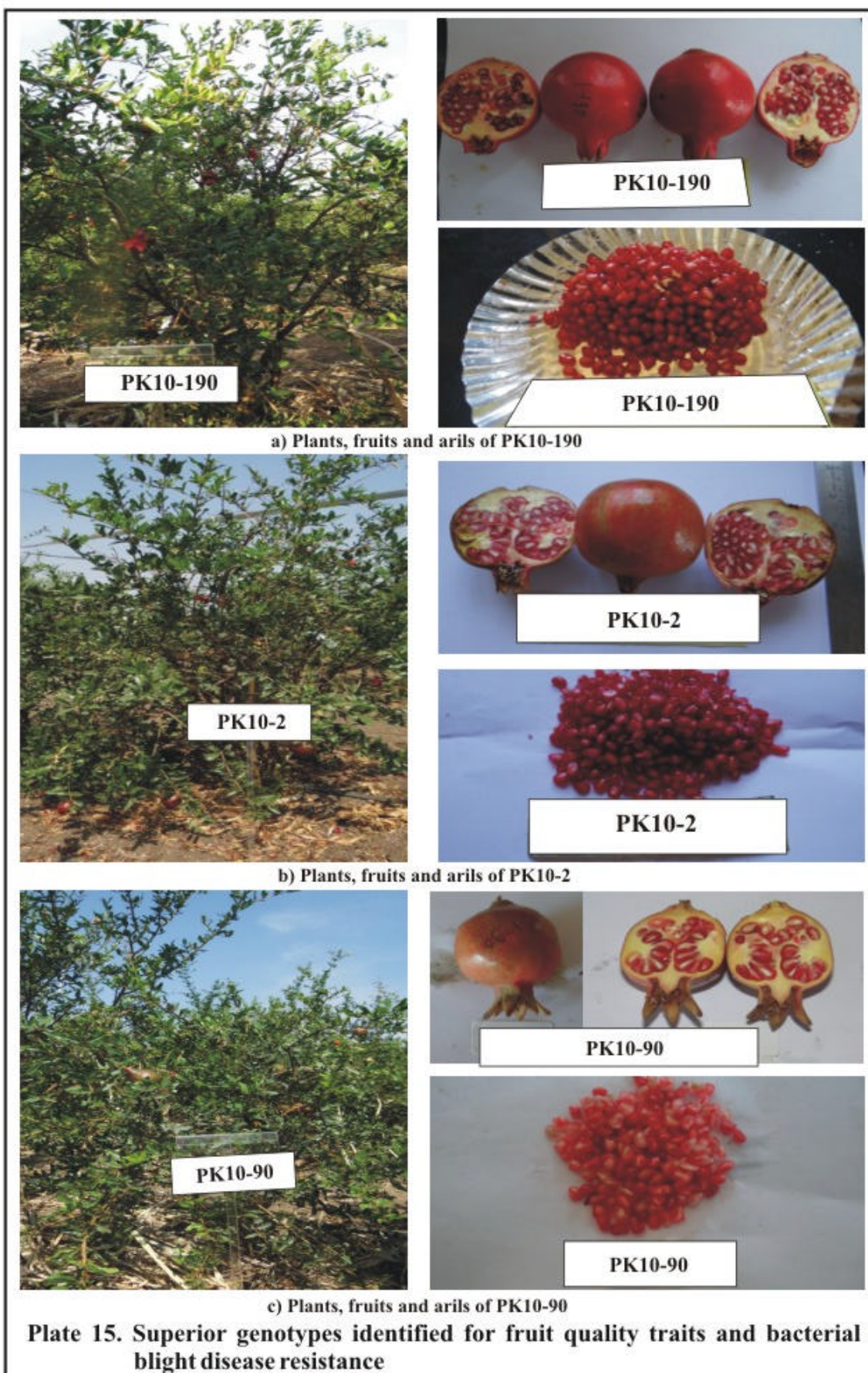


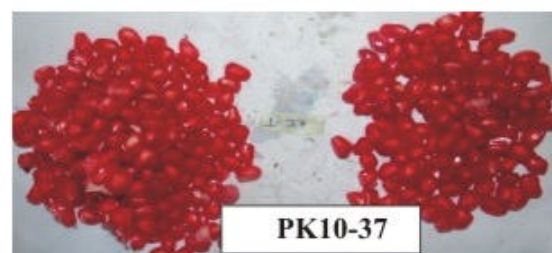
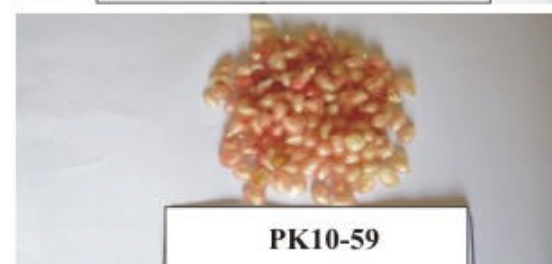
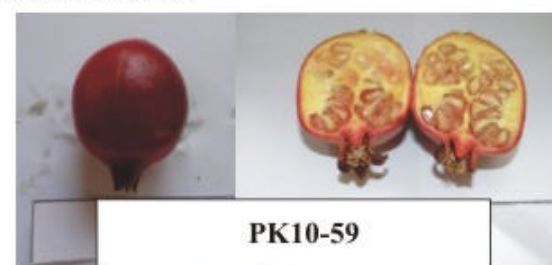
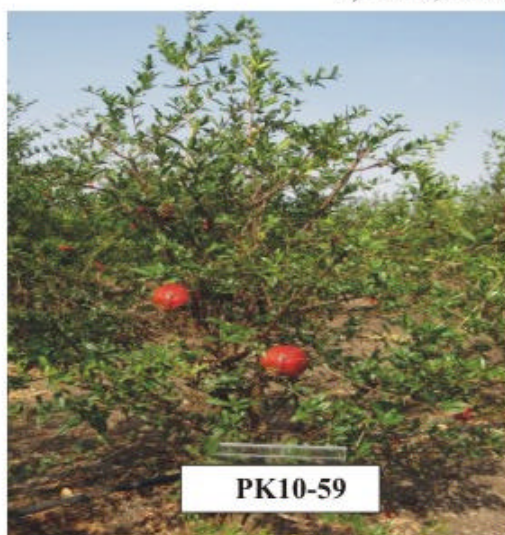
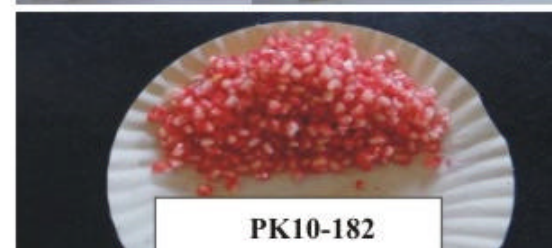
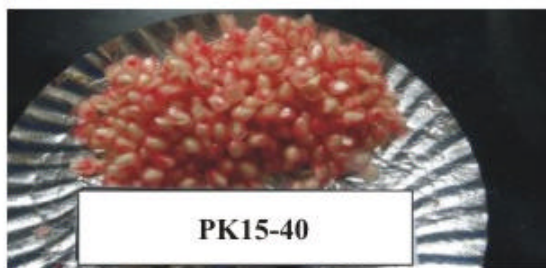
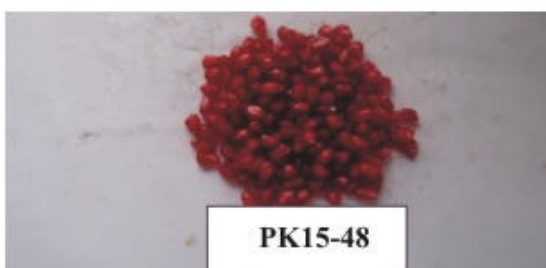
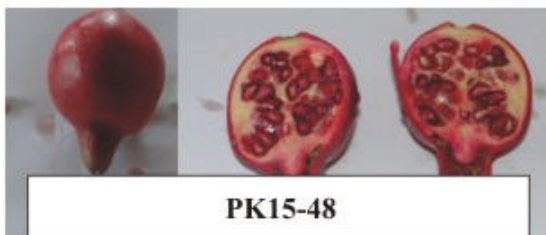
Plate 15 contd....**d) Plants, fruits and arils of PK10-37****e) Plants, fruits and arils of PK10-59****f) Plants, fruits and arils of PK10-182**

Plate 15 contd....



g) Plants, fruits and arils of PK15-40

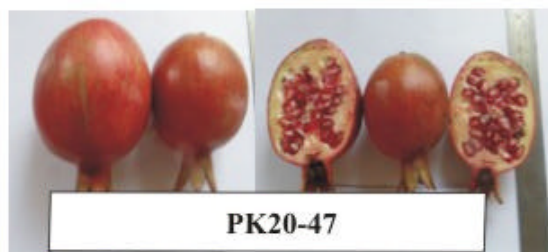


h) Plants, fruits and arils of PK15-48

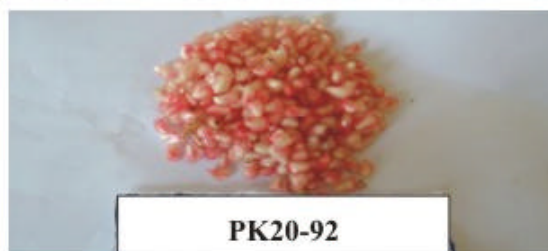
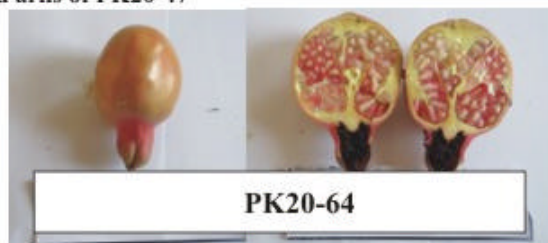


i) Plants, fruits and arils of PK15-78

Plate 15 contd....



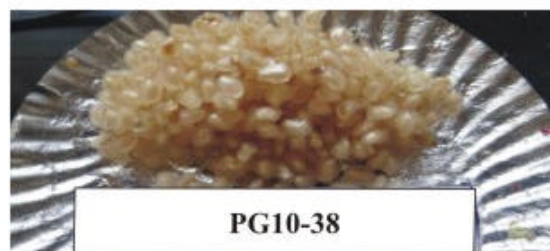
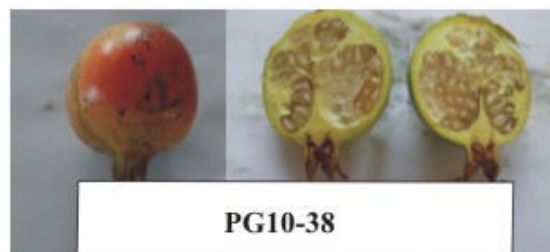
j) Plants, fruits and arils of PK20-47



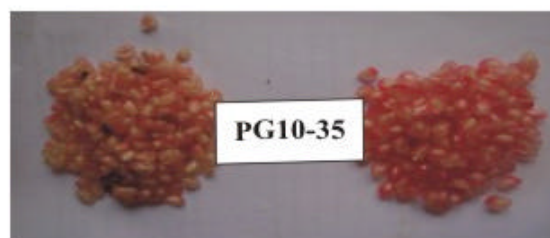
k) Plants, fruits and arils of PK20-92



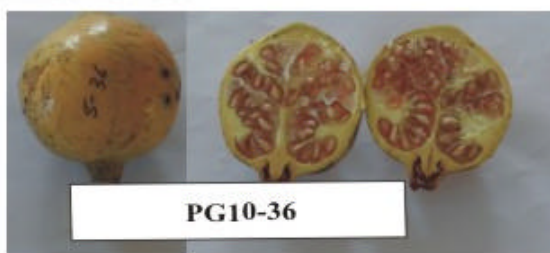
l) Plants, fruits and arils of PK20-92

Plate 15 contd....

m) Plants, fruits and arils of PG-10-38

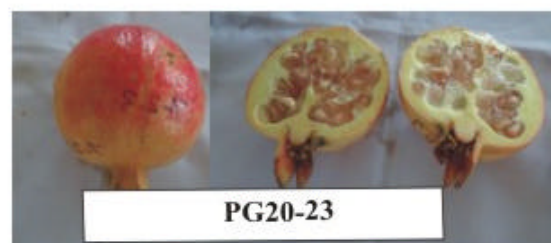


m) Plants, fruits and arils of PG-10-35

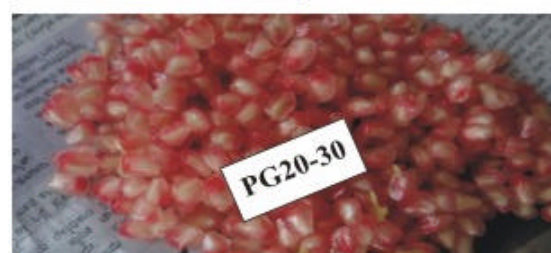
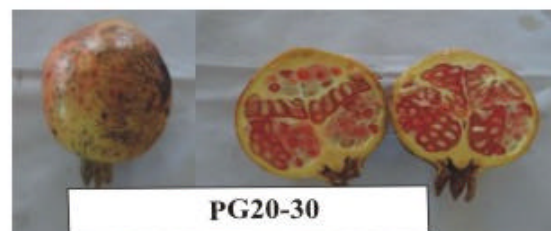


n) Plants, fruits and arils of PG10-36

Plate 15 contd....



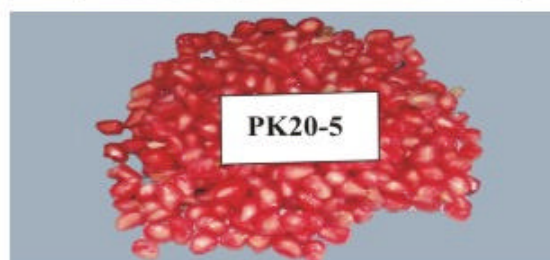
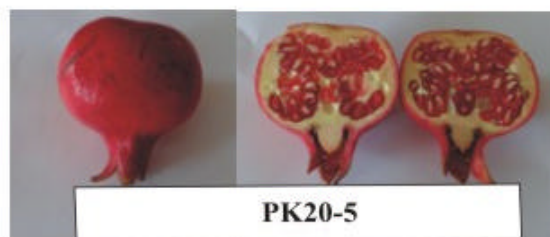
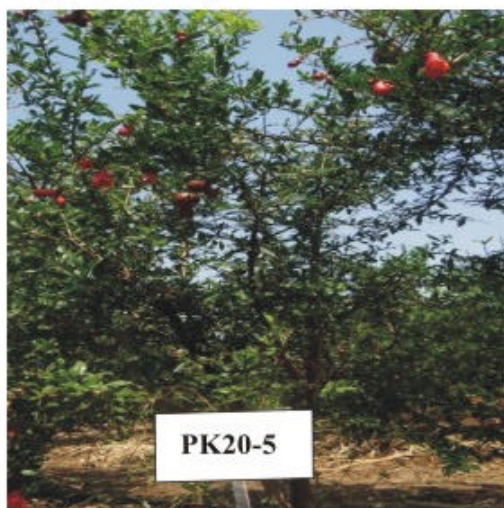
o) Plants, fruits and arils of PG20-23



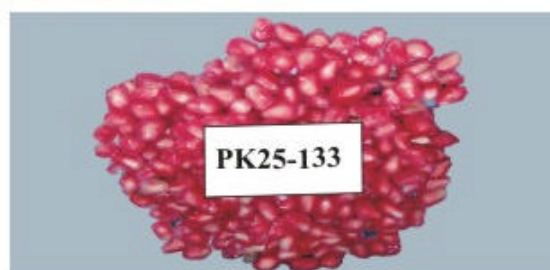
p) Plants, fruits and arils of PG20-30



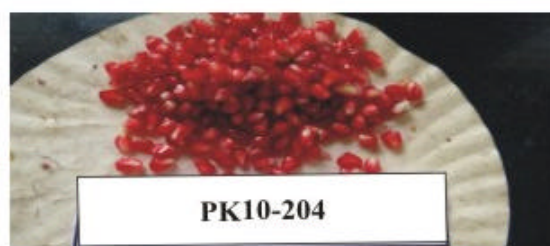
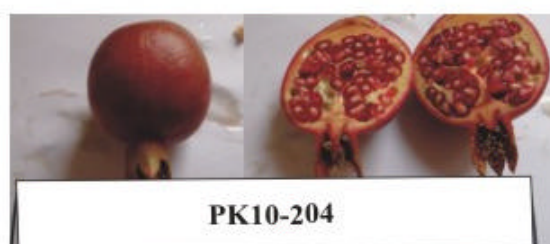
q) Plants, fruits and arils of PG25-37

Plate 15 contd....

r) Plants, fruits and arils of PK-20-5



s) Plants, fruits and arils of PK25-133



t) Plants, fruits and arils of PK10-204

All the γ irradiated seed derived progenies and germplasm of pomegranate were assessed based on nine selected quality parameters and disease severity on tree, only 20 genotypes were presented (Table 29 and Plate 16), which are superior to check varieties Bhagwa (68.95 %), Ganesh (57.63 %), Ruby (52.38 %), Patna-5 (35.34 %) and Nana (9.47 %). Among which only nine genotypes (PK20-38, PK10-90, PK10-204, PG25-48, PK20-5, PK10-201, PK10-37, PK25-133 and PK15-12) were found moderately susceptible compare to moderately susceptible check varieties such as Patna-5 and Nana. Out of nine moderately susceptible genotypes eight were showing high index score over highly susceptible checks (Bhagwa, Ganesh and Ruby). Generally, the superiority of the genotypes was due to less disease severity over checks and can be used further for selection.

4.2.5 Correlation studies in pomegranate genotypes for plant growth, fruit quality and its component traits

The Pearson correlation coefficients was estimated among 37 characters of 475 genotypes from pooled data of two seasons to find out the association of plant growth, fruit quality and its component traits (Table 30). Disease severity is dependent on different independent characters, therefore it is essential to know the relationship between the characters. Correlation analysis is generally used to find out mutual association between the level of resistance or susceptibility and its component traits. The estimate of correlation provides an effective way of predicting response of selection and isolating desirable individuals from the population. To study the level of correlation coefficients computed in all possible combinations among the characters is presented in Table 30. Correlation studies indicated that the bacterial blight disease on fruit, stem and leaves were positively correlated with disease severity of 0.97, 0.48 and 0.29, respectively. The disease severity showed significant negative correlation with acidity ($r = -0.18$) and days to maturity ($r = -0.18$), while non significant negative correlation with total phenols ($r = -0.023$), fruit calyx length ($r = 0.09$), rind proportion ($r = -0.04$) and pH of juice ($r = -0.004$).

The characters plant height ($r = 0.29$), plant spread (0.29), leaf thickness (0.13), leaf length (0.14), flower diameter (0.10), flower length (0.23), fruit diameter (0.28), fruit weight (0.26), total aril weight (0.28), total number of arils (0.19), volume of juice

Table 29: Performance of 20 superior genotypes of pomegranate for disease severity and other fruit quality traits during 2014-15 and 2015-16

S.No.	Genotypes	DS	Fruit weight	Aril (%)	Juice (%)	100 Aril weight	TSS	Acidity	Maturity index	Rind colour	Aril colour	Total selection index scores	Reaction to bacterial blight			
													Stem	Leaves	Fruits	DS on Tree
1	PK20-38		1	1	3	1	2	4	3	3	4	22	MS	MS	MS	10.08
2	PK10-90		1	1	1	1	1	5	3	3	4	20	MS	MS	MS	14.64
3	PK10-204		1	1	1	1	3	5	3	3	2	20	MS	MS	MS	14.83
4	PG25-48		1	1	1	1	1	4	3	3	1	16	MS	MS	MS	25.27
5	PK20-5		1	1	1	2	3	5	3	3	4	23	MS	MS	HS	27.04
6	PK10-201		1	1	1	1	3	5	4	3	4	23	MS	MS	HS	27.07
7	PK10-37		1	1	1	1	4	4	3	3	4	22	MS	MS	HS	28.24
8	PK25-133		1	1	1	1	1	5	3	3	4	20	MS	MS	HS	29.12
9	PK15-48		1	1	1	2	5	5	4	3	4	26	HS	HS	HS	30.16
10	PK15-12		1	1	2	1	1	5	3	3	4	21	HS	MS	HS	30.22
11	PK15-42		1	1	1	2	4	5	4	3	2	23	HS	HS	HS	31.12
12	PK20-50		1	1	1	1	4	5	4	2	2	21	HS	HS	HS	31.94
13	PK10-136		1	1	1	1	2	5	3	3	5	22	HS	HS	HS	32.16
14	PK10-59		1	1	2	2	5	5	4	3	4	27	HS	HS	HS	32.43
15	PK10-38		1	1	1	1	4	4	3	3	2	20	HS	HS	HS	33.04
16	PK10-124		1	1	1	2	5	5	4	3	4	26	HS	HS	HS	33.12
17	PK15-41		1	1	1	3	5	5	5	3	4	28	HS	HS	HS	33.35
18	Yarcaud 8		1	1	1	1	3	4	3	3	2	19	HS	HS	HS	33.44
19	PK10-120		1	1	1	1	4	5	4	3	4	24	HS	HS	HS	33.52
20	PK10-15		1	1	1	1	3	5	4	3	2	21	HS	HS	HS	33.76

Contd.....

Table 29 contd....

S.No.	Genotypes	Fruit weight	Aril (%)	Juice (%)	100 Aril weight	TSS	Acidity	Maturity index	Rind colour	Aril colour	Total selection index scores	Reaction to bacterial blight			
												Stem	Leaves	Fruits	DS on Tree
	Checks														
1	Ganesh	1	1	2	2	3	4	3	3	2	21	HS	HS	HS	57.63
2	Bhagwa	1	1	2	1	3	5	4	4	4	25	HS	HS	HS	68.95
3	Ruby	1	1	1	1	1	5	4	3	3	20	HS	HS	HS	52.38
4	Nana	1	1	1	1	2	1	1	3	3	14	MS	MS	MS	9.47
5	Patna-5	1	1	1	1	3	4	3	1	1	16	HS	MS	HS	35.34
														C.D. @ 5 %	9.84
														C.V.	9.58



a) PK20-38



b) PK10-59



c) PK10-90



d) PK10-204



e) Bhagwa

Plate 16. Field view of bacterial blight severity on leaves

Table 30: Pooled analysis of correlation coefficients in pomegranate genotypes

Variables	Days to maturity	Plant height (cm)	Plant spread (cm)	Leaf thickness (mm)	Leaf length (cm)	Leaf breadth (cm)	Flower length (cm)	Flower breadth (cm)	Pastille length (cm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Calyx length (cm)	Calyx diameter (cm)	Total aril weight (g)	Total No. of arils	% of arils per fruit	% of juice per fruit	Volume of juice	Rind thickness (mm)	100 aril weight (g)	Rind weight (g)	Single aril weight (g)	Rind percentage (%)	Total soluble solids (0B)	pH of juice	Acidity (%)	Maturity index	Phenolic content (mg/1000 ml gallic acid)	Anthocyanin (mg/100 ml)	Seed length (cm)	Seed weight (cm)	100 seed dry weight (g)	Bacterial blight on fruit (PDI)	Bacterial blight on stem (PDI)	Bacterial blight on leaves (PDI)	Disease severity (%)
Days to maturity	1.00	-0.10	-0.06	-0.09	-0.10	-0.06	0.02	-0.01	0.05	-0.23	-0.28	-0.32	-0.08	-0.13	-0.27	-0.12	0.01	0.03	-0.16	-0.19	-0.26	-0.27	-0.26	0.01	-0.10	0.25	-0.04	-0.01	-0.02	0.08	0.03	0.02	-0.15	-0.16	-0.07	0.05	-0.15
Plant height (cm)	-0.10	1.00	0.57	0.07	0.36	0.25	0.08	0.12	-0.07	0.32	0.37	0.31	-0.05	0.08	0.32	0.17	0.07	0.11	0.19	0.23	0.34	0.23	0.30	-0.05	0.16	0.05	-0.17	0.17	-0.11	-0.06	0.04	0.01	0.15	0.21	0.38	0.24	0.29
Plant spread (cm)	-0.06	0.57	1.00	0.11	0.32	0.25	0.11	0.08	-0.11	0.16	0.23	0.19	-0.05	0.09	0.23	0.15	0.11	0.03	0.06	0.12	0.17	0.14	0.15	-0.05	0.11	0.03	-0.23	0.12	-0.07	0.01	0.08	0.04	0.12	0.21	0.36	0.23	0.29
Leaf thickness (mm)	-0.09	0.07	0.11	1.00	0.30	0.22	-0.11	0.16	-0.05	0.03	0.06	0.06	-0.14	-0.08	0.08	0.00	0.06	0.08	0.07	0.10	0.11	0.02	0.11	-0.09	0.03	-0.04	-0.10	0.09	0.03	-0.01	0.11	0.11	0.06	0.13	0.09	0.07	0.13
Leaf length (cm)	-0.10	0.36	0.32	0.30	1.00	0.49	0.08	0.10	-0.09	0.24	0.25	0.24	-0.03	0.08	0.23	0.16	-0.01	0.00	0.12	0.15	0.19	0.23	0.18	0.06	0.09	0.09	-0.32	0.16	-0.02	0.03	0.10	0.00	0.07	0.11	0.22	0.07	0.14
Leaf breadth (cm)	-0.06	0.25	0.25	0.22	0.49	1.00	0.12	0.09	-0.05	0.12	0.14	0.14	0.00	0.11	0.14	0.11	0.00	0.05	0.08	0.06	0.08	0.11	0.08	0.00	0.06	0.08	-0.23	0.12	-0.02	0.00	0.00	-0.05	0.00	0.05	0.11	0.06	0.07
Flower length (cm)	0.02	0.08	0.11	-0.11	0.08	0.12	1.00	0.08	0.06	0.02	0.02	0.00	0.08	0.09	-0.03	0.08	-0.03	-0.03	-0.02	0.07	-0.03	0.01	-0.03	0.02	-0.01	0.06	-0.15	0.02	0.01	-0.03	-0.07	-0.06	-0.10	0.05	-0.03	0.09	0.05
Flower breadth (cm)	-0.01	0.12	0.08	0.16	0.10	0.09	0.08	1.00	0.23	-0.03	-0.03	-0.04	-0.01	-0.02	-0.02	-0.04	0.05	0.09	0.03	0.00	0.05	-0.06	0.04	-0.06	0.05	-0.06	-0.19	0.09	-0.02	-0.05	0.08	-0.01	0.04	0.08	0.07	0.07	0.10
Pastille length (cm)	0.05	-0.07	-0.11	-0.05	-0.09	-0.05	0.06	0.23	1.00	-0.03	-0.05	-0.05	-0.01	0.03	-0.05	0.02	-0.03	0.03	-0.01	-0.04	-0.09	-0.02	-0.09	0.05	-0.09	0.05	-0.14	-0.03	-0.05	0.02	-0.11	-0.13	-0.06	0.02	-0.05	0.06	0.01
Fruit length (cm)	-0.23	0.32	0.16	0.03	0.24	0.12	0.02	-0.03	-0.03	1.00	0.89	0.83	-0.13	0.09	0.82	0.50	0.05	0.09	0.40	0.29	0.55	0.69	0.55	0.04	0.20	-0.04	-0.18	0.19	-0.07	-0.10	0.02	-0.03	0.20	0.20	0.23	0.05	0.23
Fruit diameter (cm)	-0.28	0.37	0.23	0.06	0.25	0.14	0.02	-0.03	-0.05	0.89	1.00	0.90	-0.13	0.13	0.87	0.52	0.06	0.09	0.45	0.29	0.60	0.76	0.60	0.02	0.18	-0.03	-0.17	0.19	-0.08	-0.10	0.02	-0.02	0.24	0.25	0.23	0.03	0.28
Fruit weight (g)	-0.32	0.31	0.19	0.06	0.24	0.14	0.00	-0.04	-0.05	0.83	0.90	1.00	-0.13	0.09	0.88	0.54	-0.04	0.03	0.40	0.30	0.58	0.89	0.58	0.07	0.18	-0.04	-0.14	0.17	-0.10	-0.10	0.04	-0.01	0.21	0.24	0.20	0.00	0.26
Calyx length (cm)	-0.08	-0.05	-0.05	-0.14	-0.03	0.00	0.08	-0.01	-0.01	-0.13	-0.13	-0.13	1.00	0.50	-0.19	-0.13	-0.19	-0.02	-0.07	0.05	-0.14	-0.05	-0.13	0.16	-0.13	0.06	-0.04	-0.19	0.14	0.09	-0.03	0.09	0.01	-0.09	0.00	-0.01	-0.09
Calyx diameter (cm)	-0.13	0.08	0.09	-0.08	0.08	0.11	0.09	-0.02	0.03	0.09	0.13	0.09	0.50	1.00	0.06	0.10	-0.11	0.04	0.02	0.07	-0.05	0.11	-0.05	0.14	-0.15	-0.03	-0.16	-0.08	0.01	0.05	-0.08	0.04	0.00	-0.02	0.10	0.01	0.01
Total aril weight (g)	-0.27	0.32	0.23	0.08	0.23	0.14	-0.03	-0.02	-0.05	0.82	0.87	0.88	-0.19	0.06	1.00	0.65	0.36	0.07	0.43	0.22	0.56	0.66	0.55	-0.17	0.18	-0.07	-0.15	0.19	-0.08	-0.08	0.01	-0.04	0.18	0.25	0.20	0.01	0.28
Total No. of arils	-0.12	0.17	0.15	0.00	0.16	0.11	0.08	-0.04	0.02	0.50	0.52	0.54	-0.13	0.10	0.65	1.00	0.33	-0.09	0.30	0.07	-0.08	0.39	-0.10	-0.16	-0.18	-0.11	-0.06	-0.12	-0.10	-0.07	0.03	-0.09	-0.10	0.18	0.13	-0.02	0.20
% of arils per fruit	0.01	0.07	0.11	0.06	-0.01	0.00	-0.03	0.05	-0.03	0.05	0.06	-0.04	-0.19	-0.11	0.36	0.33	1.00	-0.02	0.14	-0.16	0.06	-0.29	0.06	-0.63	0.03	-0.09	0.06	0.02	0.01	-0.02	-0.02	-0.07	-0.04	0.07	0.04	-0.02	0.09
% of juice per fruit	0.03	0.11	0.03	0.08	0.00	0.05	-0.03	0.09	0.03	0.09	0.09	0.03	-0.02	0.04	0.07	-0.09	-0.02	1.00	0.42	0.10	0.13	0.02	0.13	0.04	0.16	-0.08	0.01	0.14	0.04	0.00	-0.05	0.01	0.03	0.06	0.04	0.10	0.07

Contd.....

Table 30 contd.....

Variables	Days to maturity	Plant height (cm)	Plant spread (cm)	Leaf thickness (mm)	Leaf length (cm)	Leaf breadth (cm)	Flower length (cm)	Flower breadth (cm)	Pastille length (cm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Calyx length (cm)	Calyx diameter (cm)	Total aril weight (g)	Total No. of arils	% of arils per fruit	% of juice per fruit	Volume of juice	Rind thickness (mm)	100 aril weight (g)	Rind weight (g)	Single aril weight (g)	Rind percentage (%)	Total soluble solids (OB)	pH of juice	Acidity (%)	Maturity index	Phenolic content (mg/1000 ml gallic acid)	Anthocynin (mg/100 ml)	Seed length (cm)	Seed weight (cm)	100 seed dry weight (g)	Bacterial blight on fruit (PDI)	Bacterial blight on stem (PDI)	Bacterial blight on leaves (PDI)	Disease severity (%)
Volume of juice	-0.16	0.19	0.06	0.07	0.12	0.08	-0.02	0.03	-0.01	0.40	0.45	0.40	-0.07	0.02	0.43	0.30	0.14	0.42	1.00	0.21	0.33	0.26	0.33	-0.16	0.18	-0.14	-0.07	0.13	0.04	-0.06	-0.05	-0.09	0.09	0.13	0.07	0.15	0.15
Rind thickness (mm)	-0.19	0.23	0.12	0.10	0.15	0.06	0.07	0.00	-0.04	0.29	0.29	0.30	0.05	0.07	0.22	0.07	-0.16	0.10	0.21	1.00	0.26	0.31	0.26	0.16	0.13	-0.05	-0.15	0.08	0.06	-0.02	-0.07	-0.03	0.15	0.03	0.22	0.11	0.08
100 aril weight (g)	-0.26	0.34	0.17	0.11	0.19	0.08	-0.03	0.05	-0.09	0.55	0.60	0.58	-0.14	-0.05	0.56	-0.08	0.06	0.13	0.33	0.26	1.00	0.48	0.99	-0.02	0.52	0.13	-0.15	0.44	-0.03	-0.09	-0.02	0.03	0.38	0.16	0.16	0.04	0.18
Rind weight (g)	-0.27	0.23	0.14	0.02	0.23	0.11	0.01	-0.06	-0.02	0.69	0.76	0.89	-0.05	0.11	0.66	0.39	-0.29	0.02	0.26	0.31	0.48	1.00	0.48	0.46	0.16	0.04	-0.11	0.13	-0.09	-0.07	0.06	-0.02	0.20	0.19	0.19	-0.03	0.21
Single aril weight (g)	-0.26	0.30	0.15	0.11	0.18	0.08	-0.03	0.04	-0.09	0.55	0.60	0.58	-0.13	-0.05	0.55	-0.10	0.06	0.13	0.33	0.26	0.99	0.48	1.00	-0.02	0.51	0.11	-0.15	0.44	-0.01	-0.09	-0.03	0.03	0.36	0.16	0.15	0.04	0.18
Rind percentage (%)	0.01	-0.05	-0.05	-0.09	0.06	0.00	0.02	-0.06	0.05	0.04	0.02	0.07	0.16	0.14	-0.17	-0.16	-0.63	0.04	-0.16	0.16	-0.02	0.46	-0.02	1.00	0.02	0.13	-0.08	0.04	-0.02	0.06	0.04	-0.05	0.06	-0.04	0.02	-0.03	-0.04
Total soluble solids (OB)	-0.10	0.16	0.11	0.03	0.09	0.06	-0.01	0.05	-0.09	0.20	0.18	0.18	-0.13	-0.15	0.18	-0.18	0.03	0.16	0.18	0.13	0.52	0.16	0.51	0.02	1.00	0.04	-0.03	0.56	0.07	-0.04	0.01	-0.09	0.34	0.02	0.05	0.01	0.03
pH of juice	0.25	0.05	0.03	-0.04	0.09	0.08	0.06	-0.06	0.05	-0.04	-0.03	-0.04	0.06	-0.03	-0.07	-0.11	-0.09	-0.08	-0.14	-0.05	0.13	0.04	0.11	0.13	0.04	1.00	-0.15	0.07	0.02	0.07	-0.01	0.03	-0.05	-0.01	0.09	-0.06	0.00
Acidity (%)	-0.04	-0.17	-0.23	-0.10	-0.32	-0.23	-0.15	-0.19	-0.14	-0.18	-0.17	-0.14	-0.04	-0.16	-0.15	-0.06	0.06	0.01	-0.07	-0.15	-0.15	-0.11	-0.15	-0.08	-0.03	-0.15	1.00	-0.51	0.05	0.00	0.01	0.03	-0.05	-0.14	-0.21	-0.20	-0.18
Maturity index	-0.01	0.17	0.12	0.09	0.16	0.12	0.02	0.09	-0.03	0.19	0.19	0.17	-0.19	-0.08	0.19	-0.12	0.02	0.14	0.13	0.08	0.44	0.13	0.44	0.04	0.56	0.07	-0.51	1.00	-0.07	-0.05	0.05	-0.07	0.22	0.11	0.12	0.09	0.13
Phenolic content (mg/1000 ml gallic acid)	-0.02	-0.11	-0.07	0.03	-0.02	-0.02	0.01	-0.02	-0.05	-0.07	-0.08	-0.10	0.14	0.01	-0.08	-0.10	0.01	0.04	0.04	0.06	-0.03	-0.09	-0.01	-0.02	0.07	0.02	0.05	-0.07	1.00	0.01	-0.06	-0.03	0.01	-0.01	-0.04	0.02	-0.02
Anthocynin (mg/100 ml)	0.08	-0.06	0.01	-0.01	0.03	0.00	-0.03	-0.05	0.02	-0.10	-0.10	-0.10	0.09	0.05	-0.08	-0.07	-0.02	0.00	-0.06	-0.02	-0.09	-0.07	-0.09	0.06	-0.04	0.07	0.00	-0.05	0.01	1.00	0.09	-0.01	0.01	0.00	0.00	0.03	0.00
Seed length (cm)	0.03	0.04	0.08	0.11	0.10	0.00	-0.07	0.08	-0.11	0.02	0.02	0.04	-0.03	-0.08	0.01	0.03	-0.02	-0.05	-0.05	-0.07	-0.02	0.06	-0.03	0.04	0.01	-0.01	0.01	0.05	-0.06	0.09	1.00	0.12	0.11	0.04	0.04	0.04	0.04
Seed weight (cm)	0.02	0.01	0.04	0.11	0.00	-0.05	-0.06	-0.01	-0.13	-0.03	-0.02	-0.01	0.09	0.04	-0.04	-0.09	-0.07	0.01	-0.09	-0.03	0.03	-0.02	0.03	-0.05	-0.09	0.03	0.03	-0.07	-0.03	-0.01	0.12	1.00	0.06	0.07	-0.02	0.02	0.05
100 seed dry weight (g)	-0.15	0.15	0.12	0.06	0.07	0.00	-0.10	0.04	-0.06	0.20	0.24	0.21	0.01	0.00	0.18	-0.10	-0.04	0.03	0.09	0.15	0.38	0.20	0.36	0.06	0.34	-0.05	-0.05	0.22	0.01	0.01	0.11	0.06	1.00	0.06	0.12	0.04	0.07
Bacterial blight on fruit (PDI)	-0.16	0.21	0.21	0.13	0.11	0.05	0.05	0.08	0.02	0.20	0.25	0.24	-0.09	-0.02	0.25	0.18	0.07	0.06	0.13	0.03	0.16	0.19	0.16	-0.04	0.02	-0.01	-0.14	0.11	-0.01	0.00	0.04	0.07	0.06	1.00	0.29	0.20	0.97
Bacterial blight on stem (PDI)	-0.07	0.38	0.36	0.09	0.22	0.11	-0.03	0.07	-0.05	0.23	0.23	0.20	0.00	0.10	0.20	0.13	0.04	0.04	0.07	0.22	0.16	0.19	0.15	0.02	0.05	0.09	-0.21	0.12	-0.04	0.00	0.04	-0.02	0.12	0.29	1.00	0.21	0.48
Bacterial blight on leaves (PDI)	0.05	0.24	0.23	0.07	0.07	0.06	0.09	0.07	0.06	0.05	0.03	0.00	-0.01	0.01	0.01	-0.02	-0.02	0.10	0.15	0.11	0.04	-0.03	0.04	-0.03	0.01	-0.06	-0.20	0.09	0.02	0.03	0.04	0.02	0.04	0.20	0.21	1.00	0.29
Disease severity (%)	-0.15	0.29	0.29	0.13	0.14	0.07	0.05	0.10	0.01	0.23	0.28	0.26	-0.09	0.01	0.28	0.20	0.09	0.07	0.15	0.08	0.18	0.21	0.18	-0.04	0.03	0.00	-0.18	0.13	-0.02	0.00	0.04	0.05	0.07	0.97	0.48	0.29	1.00

Figures with bold letter indicate 5 % level of significance

(0.15) hundred aril weight (0.18), rind weight (0.21) and single aril weight (0.17) also contributed significant positive association to the disease severity.

TSS has significant negative correlation with total number of arils (0.18), fruit calyx width (-0.14), days to maturity (-0.10) fruit calyx length (-0.12) and style length (-0.10) and positive significant with plant height (0.16), plant spread (0.11), fruit length (0.20) fruit diameter (0.18), fruit weight (0.18), total aril weight (0.18), per cent of juice per fruit (0.16), volume of juice (0.18), rind thickness (0.12), hundred aril weight (0.52), rind weight (0.15) and single aril weight (0.51). Similarly acidity had maximum negative significant correlation with much of the traits studied.

The characters, hundred aril weight showed highly significant positive correlation with characters, viz., fruit diameter (0.60), fruit weight (0.58), fruit length (0.551, plant height (0.34), plant spread (0.17), leaf thickness (0.16) and leaf length (0.19). It showed highly significant negative correlation with characters such as days to maturity (-0.26) and fruit calyx length (-0.14).

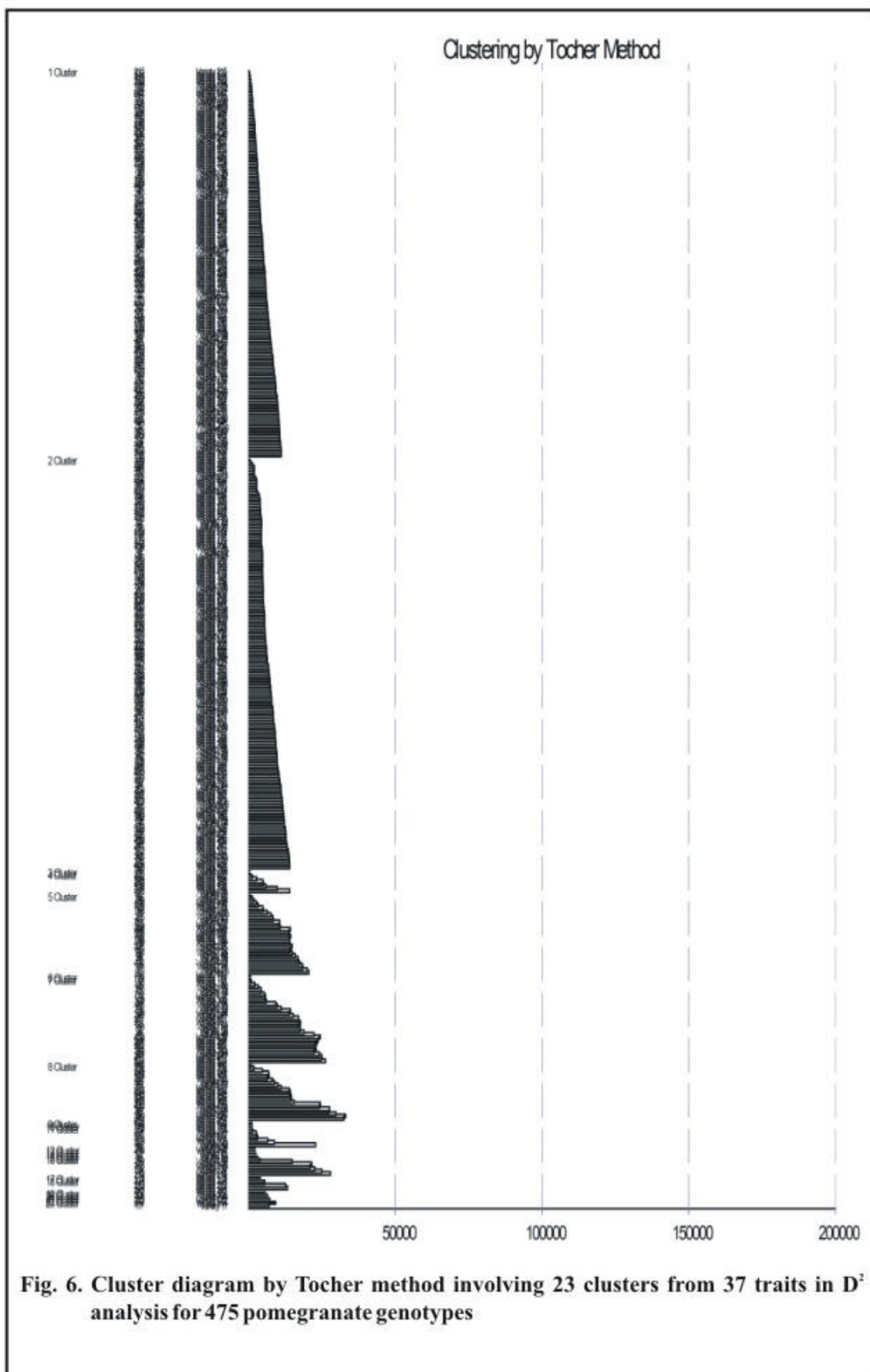
The characters fruit weight showed highly positive significant correlation with characters viz., fruit length (0.83) and fruit diameter (0.89), plant height (0.30), plant spread (0.19), leaf length (0.24) and leaf breadth (0.14).

4.2.6 Genetic diversity studies in pomegranate genotypes for plant growth, fruit quality and bacterial blight disease resistance traits (Mahalanobis's generalized distance D^2)

D^2 values were obtained for 475 genotypes for plant growth, fruit quality and bacterial blight disease resistance traits evaluated in two seasons are presented in Fig. 6.

4.2.6.1 Clustering of D^2 values

The 475 genotypes were grouped into 23 clusters based on Mahalanobis's generalized distance (D^2) values for plant growth, fruit quality and bacterial blight disease resistance traits. Among the 23 clusters, Cluster II was the largest with 172 genotypes followed by cluster I (162), VII (36), V (34), VIII (24), IV (9) and XII (9).



Clusters XIV, XVIII and XXIII included 8, 5 and 3 genotypes respectively and remaining cluster are solitary. The pattern of distribution of genotypes into various clusters is presented in Fig. 6.

4.2.6.2 Contribution of different characters towards divergence

Contribution of individual characters towards genetic divergence is presented in Table 31. Phenolic content ranks first with contribution of 61.55 per cent to the total divergence followed by anthocyanin content (17.51 %), number of arils per fruit (16.84 %), fruit weight (2.12 %), plant height (1.22 %), days to maturity (0.43 %), rind weight (0.22 %) and plant spread (0.06). The lowest contribution was from Bacterial blight disease resistance on fruit (0.03 %).

4.2.6.3 Cluster mean of different groups

The characters mean worked out for the genotypes falling in these 23 clusters is presented in Table 32. The cluster XXIII was found to be consisting of genotypes of high phenolic content (1897.02 mg), calyx length (2.21 cm). Similarly cluster XXII representing with highest number of arils per fruit (584.62), leaf breadth (1.69 cm) and pistil length. Cluster XXI was characterized with maximum juice percentage per fruit (53.54 %). Maximum rind thickness (4.27 mm), acidity (4.22 %) and anthocyanin content (548.60 mg) was found in cluster XIX and XVIII respectively. Cluster XVII has maximum values for fruit weight (202.61 g), rind weight (144.40 g) and fruit diameter (6.19 cm). Cluster XV represents the high bacterial blight disease severity on tree (50.36 %) and total aril weight (83.41 g). Similarly XIV cluster represents the longest fruit length (6.06 cm) and highest single aril weight (0.24 g), seed width (0.29 cm), dry seed weight (2.41 g), bacterial blight disease on fruit (54.77 %), bacterial blight disease on stem (60.30 %), bacterial blight disease on leaves (42.91 %) and bacterial blight disease severity on tree (54.76 %). Cluster XIII represented the highest rind thickness (4.39 mm), rind percentage (51.58 %), maturity index (42.62 %), leaf breadth (1.69 cm) and flower length (3.90 cm). Further the cluster XII characterized for leaf thickness (0.54 mm), pistil length (1.29 cm) and seed width (0.22 cm). Cluster X was found to be consisting of genotypes of long duration (183.14 days), 100 aril weight (26.76 g), TSS (17.16 °B), seed length (0.69 cm), seed width (0.29 cm) and low seed weight (1.2 g). Similarly cluster IX and VI were found to be consisting genotypes of highest plant height

Table 31: Relative per cent contribution of different characters to the total divergence in pomegranate genotypes

Sl. No.	Characters	No. of times ranked first	Per cent contribution
1.	Days to maturity of fruit	488	0.43
2.	Plant height	1378	1.22
3.	Plant spread	64	0.06
4.	Leaf thickness	-	-
5.	Leaf length	-	-
6.	Leaf breadth	-	-
7.	Flower length	-	-
8.	Flower diameter	-	-
9.	Pistil length	-	-
10.	Fruit height	-	-
11.	Fruit diameter	-	-
12.	Fresh fruit weight	2390	2.12
13.	Calyx length	-	-
14.	Calyx diameter	-	-
15.	Total aril weight		
16.	Number of arils per fruit	18963	16.84
17.	100 aril weight	-	-
18.	Single aril weight	-	-
19.	Aril percentage per fruit	-	-
20.	Juice percentage per fruit	-	-
21.	Volume of juice	1	-
22.	Total soluble solids	-	-
23.	pH of juice	-	-
24.	Acidity	-	-
25.	Maturity Index	-	-
26.	Phenolic content	69290	61.55
27.	Anthocyanins	19710	17.51
28.	Rind thickness	-	-
29.	Rind weight	248	0.22
30.	Rind percentage	-	-
31.	Seed length	-	-
32.	Seed width	-	-
33.	100 dry seed weight	-	-
34.	Bacterial blight on stem	-	-
35.	Bacterial blight on leaves	39	0.03
36.	Bacterial blight on fruit	3	-
37.	Disease severity on tree	-	-

Table 32: Pooled mean performance of different clusters

Traits	Clusters																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	18	20	21	22	23
Days to maturity of fruit	139.72	139.41	153.00	139.53	138.79	160.79	137.43	133.25	149.74	183.14	141.91	108.44	137.23	141.06	114.20	136.63	109.72	149.17	129.27	162.83	96.09	149.66	146.52
Plant height (cm)	157.24	157.64	161.86	158.74	155.18	138.61	163.90	164.61	202.61	119.45	149.33	138.02	190.27	161.86	154.09	136.87	157.00	179.02	63.02	153.87	139.06	162.71	153.77
Plant spread (cm)	123.55	122.17	125.36	123.85	122.49	121.48	121.52	125.04	134.89	101.38	116.96	123.57	114.71	124.19	118.87	116.69	133.85	187.96	54.29	132.85	121.03	120.45	120.48
Leaf thickness (mm)	0.50	0.51	0.52	0.51	0.51	0.50	0.51	0.51	0.49	0.53	0.50	0.54	0.49	0.52	0.52	0.51	0.50	0.50	0.47	0.49	0.53	0.46	0.50
Leaf length (cm)	4.83	4.83	4.59	4.89	4.92	5.07	4.86	5.00	5.20	4.80	4.82	5.08	4.92	4.61	4.54	4.71	5.33	4.88	2.88	4.63	5.44	4.96	4.77
Leaf breadth (cm)	1.63	1.64	1.44	1.62	1.63	1.68	1.63	1.68	1.84	1.62	1.61	1.61	1.69	1.51	1.63	1.56	1.73	1.64	0.82	1.50	1.69	1.69	1.67
Flower length (cm)	3.62	3.61	3.37	3.61	3.59	3.69	3.57	3.74	3.38	3.31	3.59	3.46	3.90	3.67	3.52	3.67	3.51	3.66	2.85	3.75	3.61	3.69	3.53
Flower breadth (cm)	1.46	1.49	1.40	1.46	1.47	1.53	1.46	1.46	1.51	1.38	1.48	1.56	1.49	1.31	1.49	1.44	1.36	1.46	0.90	1.50	1.56	1.47	1.45
Pistil length (cm)	1.27	1.25	1.21	1.27	1.26	1.24	1.26	1.23	1.24	1.28	1.27	1.29	1.21	1.26	1.31	1.26	1.30	1.26	0.85	1.28	1.22	1.33	1.23
Fruit length (cm)	5.57	5.64	5.50	5.62	5.64	4.80	5.80	5.87	5.41	4.85	5.54	5.41	5.14	6.06	5.79	5.31	6.03	5.40	4.35	4.98	5.75	5.40	5.19
Fruit diameter (cm)	5.52	5.59	5.23	5.55	5.58	4.73	5.83	5.95	4.97	4.69	5.51	5.56	4.74	5.98	5.40	5.17	6.19	5.36	3.91	4.50	5.86	4.98	4.92
Fruit weight (g)	103.18	106.58	90.62	99.09	105.06	60.62	137.27	133.77	79.84	62.04	97.49	84.54	57.30	120.78	167.41	91.26	223.72	86.08	23.19	51.04	129.65	71.08	65.28
Calyx length (cm)	1.74	1.73	1.85	1.81	1.70	1.56	1.78	1.75	1.77	1.20	1.77	1.85	1.80	1.43	1.60	1.81	2.02	1.85	1.04	1.80	1.71	1.93	2.21
Calyx diameter (cm)	1.63	1.62	1.87	1.63	1.64	1.33	1.66	1.69	1.84	1.39	1.68	1.76	1.74	1.75	1.52	1.61	1.72	1.70	0.86	1.64	1.77	1.81	1.70
Total aril weight (g)	53.87	55.31	42.45	53.37	55.38	39.94	66.78	66.19	37.37	40.38	52.31	38.79	22.14	33.07	83.41	48.58	73.85	47.70	20.13	26.25	71.80	35.71	39.15
Number of arils per fruit	288.01	295.15	174.93	242.34	293.51	211.04	358.11	353.63	144.81	144.05	274.98	523.89	130.76	115.53	523.23	259.89	406.81	260.06	165.27	120.95	358.30	584.62	252.87
Weight of 100 arils (g)	18.63	19.01	23.96	21.13	18.60	16.41	19.10	19.65	21.73	26.76	18.59	16.05	17.65	25.00	18.60	17.39	18.37	18.17	10.83	17.02	21.81	15.97	13.87
Single aril Weight (g)	0.19	0.19	0.23	0.21	0.19	0.17	0.19	0.20	0.21	0.26	0.19	0.16	0.17	0.24	0.19	0.18	0.18	0.18	0.12	0.17	0.21	0.16	0.14
Aril percentage per fruit (%)	45.89	45.93	42.10	45.15	45.82	48.84	45.90	45.86	42.32	46.91	45.80	43.90	39.18	34.32	43.71	46.67	42.20	46.18	49.73	41.09	47.40	41.81	45.92
Juice percentage per fruit (%)	41.64	42.24	40.18	43.72	42.22	38.44	41.58	42.78	43.90	39.15	42.89	27.33	46.46	45.21	36.68	41.31	38.33	42.38	44.36	42.65	53.54	45.26	39.76

Contd.....

Table 32 contd.....

Traits	Clusters																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	18	20	21	22	23
Volume of juice	26.56	27.64	23.10	28.28	27.74	34.54	27.57	31.33	20.96	28.10	28.61	28.80	31.46	28.77	27.91	27.00	33.08	25.66	19.06	19.54	30.82	27.76	23.13
Total soluble solids (°B)	14.08	14.20	15.15	14.83	14.02	15.49	13.98	14.08	15.08	17.16	14.35	13.31	14.79	14.64	14.17	14.33	15.15	14.95	13.81	15.81	13.70	13.63	13.89
pH of juice	3.30	3.30	3.33	3.47	3.33	3.23	3.33	3.29	3.18	3.83	3.27	3.40	3.30	3.29	3.29	3.32	3.22	3.34	2.86	3.52	3.27	3.25	3.39
Acidity (%)	0.48	0.48	0.54	0.50	0.47	0.51	0.48	0.48	0.57	0.46	0.49	0.52	0.37	0.47	0.52	0.52	0.58	0.64	4.22	0.47	0.40	0.67	0.56
Maturity Index (TSS/Acidity)	30.41	30.57	29.01	30.63	30.40	31.02	29.93	29.95	29.97	36.58	30.14	27.05	42.62	31.24	28.36	29.30	27.79	27.31	11.90	33.19	32.42	23.78	26.46
Phenolic content (mg/1000 ml gallic acid)	88.50	313.97	51.73	1107.19	297.27	186.68	82.11	459.07	209.50	81.34	874.28	249.49	90.92	251.67	211.69	1523.32	231.68	102.35	360.83	389.75	604.01	380.65	1897.02
Anthocyanins (mg/100 ml)	44.11	44.85	130.31	69.51	216.82	2.30	143.83	30.93	16.88	6.83	64.31	42.96	12.01	4.25	13.20	124.83	16.54	548.60	2.20	166.49	262.48	62.90	68.40
Rind Thickness (mm)	3.19	3.30	2.97	3.50	3.48	3.10	3.44	3.37	3.75	3.01	3.48	2.97	4.39	4.15	2.97	3.39	4.15	3.00	1.63	4.27	3.47	3.15	2.82
Rind weight (g)	51.64	51.97	48.24	48.99	52.52	23.98	70.14	67.80	42.96	25.16	47.71	41.73	43.95	51.18	79.74	46.13	144.40	42.45	10.06	34.18	69.04	38.11	32.04
Rind percentage (%)	44.81	44.47	46.79	45.09	45.35	40.80	44.90	44.95	45.15	41.99	44.57	43.93	51.55	43.73	44.61	43.86	47.70	44.18	40.60	50.24	46.56	45.46	44.72
Seed length (cm)	0.68	0.68	0.69	0.69	0.68	0.67	0.68	0.68	0.67	0.69	0.65	0.69	0.67	0.66	0.69	0.68	0.67	0.69	0.69	0.62	0.68	0.63	0.67
Seed width (cm)	0.27	0.27	0.29	0.27	0.27	0.23	0.27	0.28	0.29	0.29	0.25	0.22	0.22	0.29	0.27	0.27	0.27	0.27	0.29	0.28	0.26	0.29	0.27
100 dry seed weight (g)	1.80	1.83	2.08	1.96	1.94	1.98	1.84	1.85	1.72	1.20	1.89	2.10	1.54	2.41	1.61	1.80	1.56	1.78	1.68	1.32	1.99	1.64	1.50
Bacterial blight on stem (PDI)	51.32	52.08	55.40	52.43	53.82	50.82	51.03	54.02	48.60	36.60	49.99	50.82	57.79	60.30	48.59	46.12	55.40	55.47	15.31	55.38	55.40	50.82	53.29
Bacterial blight on leaves (PDI)	41.52	41.62	42.34	41.68	41.73	41.01	41.53	41.74	42.32	39.38	42.21	40.15	43.90	42.91	40.12	41.49	39.92	42.74	33.33	44.72	40.34	42.62	42.30
Bacterial blight on fruit (PDI)	48.46	49.16	40.81	41.43	49.93	50.51	47.53	50.66	44.11	39.02	42.64	47.87	48.79	64.77	52.29	49.16	39.74	56.04	25.63	29.08	52.80	50.33	56.86
Disease severity on tree (%)	48.25	48.83	43.89	43.61	49.69	49.35	47.59	50.11	45.07	38.41	44.14	47.64	50.25	54.76	50.36	47.83	42.58	54.58	23.67	36.00	52.08	49.72	54.02

(202.61 cm), leaf length (5.07 cm) and volume of juice (34.54 ml), respectively. Cluster IV representing the genotypes having higher pH of the juice (3.47) and seed length (0.69 cm). The least mean value for phenolic content (51.73 mg/l) was observed in cluster III. The analysis of variations concluded that cluster XIX appeared to be the most promising genotypes with least bacterial blight disease incidence on leaves, fruit and stem. The genotypes of cluster X were considered for better performance in most of the fruit quality traits (aril weight and TSS) with moderate bacterial blight disease incidence in the genotypes.

4.2.7 Variability analysis

Variability analysis among the different pomegranate genotypes was worked out by Principal Component Analysis mentioned in material and methods (PCA) using genstat version 13.1. PCA is a method that reduces data dimensionality by performing covariance analysis between factors. The PCA analysis is a covariance analysis between different factors where as covariance is always measured between two factors. PCA is a method of data reduction that transforms the original values into a limited number of uncorrelated new variables. PCA will find eigenvectors and eigen values relevant to the data using a covariance matrix. The technique is useful device for representing a set of variables by a much smaller set of composite variables that account for much of the variance among the set of original variables. It allows visualization of the differences among the individuals, identification of possible groups and relationships among individuals and variables.

As such, it is suitable for data sets in multiple dimensions identifying the patterns in the data and expressing the data in such a ways to highlight their similarities and differences. It has been used to establish the relationship among genotypes (cultivars and to study correlations between morphological, fruit physical and chemical traits within set of genotypes).

4.2.7.1 Variability in pomegranate genotypes based on morphological characters

The results pertaining to variability studies based on leaf, flower and fruit characters are presented in Table 33. The principal component analysis showed that more than 50 per cent of the variability observed for characters under study in different

Table 33: Eigen values and `proportion of total variability for the characters of pomegranate genotypes as explained by Principal Components

Sl. No.	Principal components	Eigen value	Variability (%)	Cumulative %
1	PC1	7.113	19.225	19.225
2	PC2	2.635	7.123	26.348
3	PC3	2.539	6.862	33.209
4	PC4	2.341	6.327	39.536
5	PC5	1.724	4.660	44.196
6	PC6	1.589	4.295	48.492
7	PC7	1.543	4.171	52.662
8	PC8	1.357	3.668	56.331
9	PC9	1.225	3.310	59.641
10	PC10	1.163	3.144	62.784
11	PC11	1.149	3.106	65.890
12	PC12	1.058	2.860	68.751
13	PC13	1.005	2.716	71.467
14	PC14	0.962	2.600	74.067
15	PC15	0.942	2.545	76.612
16	PC16	0.889	2.402	79.014
17	PC17	0.767	2.074	81.088
18	PC18	0.721	1.947	83.035
19	PC19	0.682	1.843	84.878
20	PC20	0.624	1.686	86.564
21	PC21	0.608	1.643	88.206
22	PC22	0.545	1.472	89.678
23	PC23	0.538	1.453	91.131
24	PC24	0.520	1.405	92.536
25	PC25	0.481	1.301	93.837
26	PC26	0.425	1.149	94.986
27	PC27	0.405	1.094	96.080
28	PC28	0.389	1.052	97.132
29	PC29	0.336	0.909	98.041
30	PC30	0.237	0.640	98.681
31	PC31	0.188	0.508	99.190
32	PC32	0.141	0.382	99.572
33	PC33	0.081	0.220	99.792
34	PC34	0.057	0.153	99.945
35	PC35	0.009	0.025	99.970
36	PC36	0.007	0.018	99.988
37	PC37	0.005	0.012	100.000

pomegranate genotypes was explained by the first seven components, which accounted for 52.66 per cent of the total variability observed with 19.22 per cent (Eigen value, 7.11), 7.12 per cent (Eigen value, 2.63) 6.86 per cent (Eigen value 2.54), 6.33 per cent (Eigen value 2.34), 4.66 per cent (1.72), 4.29 per cent (Eigen value 1.56) and 4.17 per cent (Eigen value 1.54) for., PC1, PC2, PC3, PC4, PC5, PC5, PC6 and PC7 respectively (Table 34 and Fig. 7 & 8). This study defined values above 0.20 as significant for important parameters.

The first component PC1 corresponds to the genotypes with plant height, plant spread, leaf length, total number of arils per fruit, rind thickness, fruit length, fruit diameter, fruit weight, total aril weight, volume of juice, 100 aril weight, rind weight, single aril weight, bacterial blight on fruit, stem and disease severity (Fig. 8) includes the genotypes viz., PK20-90, PG10-39, PG15-6 and PG25-9 with maximum values for this traits. Likewise PC2 values were also characterized by plant height, plant spread, leaf length, leaf breadth, fruit diameter, bacterial blight disease (stem, leaves and fruit) and disease severity on tree, genotypes corresponding to these characters with higher values were found in PK20-43, PK20-71 and PK10-204 (Table 34 and Fig 9). The component PC3 showed characters with higher values for total number of arils per fruit, per cent of arils per fruit and disease severity on tree and genotypes characterized with in this group were PK25-135, PG25-56 and PK25-11 corresponding to these traits. The characters fruit calyx length, fruit calyx width per cent of aril per fruit, rind weight and rind proportion with maximum values found in PC4 and genotypes characterized within this group were PK15-49, PK10-199 and Tobesta. The component PC5 showed characters with higher values were bacterial blight disease on fruit and disease severity on tree and grouped the genotypes like PK25-96, PK25-62 and PK10-204 corresponding to these traits.

The highest negative values for PC1 indicate the genotypes with minimum days to maturity, lower fruit calyx length, Acidity and anthocyanin content and the genotypes includes were nana, PK20-38, PK10-153, PK15-79 and PK20-25. The genotypes PK10-20, PK20-90 and PG10-35 which had the lowest PC2 value stands out especially due to the low value for acidity (Fig. 9 and 10). The highest negative value of PC3 was

Table 34: Proportion of variability for the principle components and Eigen values of different characters evaluated in the pomegranate genotypes

Sl No	Trait	PC1 (19.22 %)	PC2 (7.12 %)	PC3 (6.86 %)	PC4 (6.32)	PC5 (4.66)	PC6 (4.29)
1	Days to maturity of fruit	-0.348	0.146	-0.049	-0.063	-0.156	-0.181
2	Plant height (cm)	0.520	0.407	0.025	0.039	-0.124	-0.009
3	Plant spread (cm)	0.381	0.514	0.093	0.052	-0.140	-0.107
4	Leaf thickness (mm)	0.167	0.293	-0.033	-0.154	-0.101	-0.126
5	Leaf length (cm)	0.393	0.381	-0.011	0.181	-0.438	-0.184
6	Leaf breadth (cm)	0.248	0.348	0.012	0.143	-0.508	-0.051
7	Flower length (cm)	0.029	0.170	0.067	0.197	-0.220	0.196
8	Flower breadth (cm)	0.055	0.330	-0.053	-0.090	-0.110	0.202
9	Pistil length (cm)	-0.075	0.046	0.058	0.079	-0.027	0.264
10	Fruit length (cm)	0.839	-0.271	0.136	0.070	-0.048	-0.009
11	Fruit diameter (cm)	0.895	-0.260	0.154	0.072	-0.028	-0.023
12	Fruit weight (g)	0.880	-0.323	0.144	0.138	0.008	-0.098
13	Calyx length (cm)	-0.170	0.018	-0.038	0.533	0.012	0.352
14	Calyx diameter (cm)	0.076	0.029	0.136	0.539	-0.118	0.361
15	Total aril weight (g)	0.859	-0.249	0.292	-0.142	-0.098	-0.028
16	Number of arils per fruit	0.437	-0.168	0.729	0.017	-0.192	-0.049
17	100 aril weight (g)	0.741	-0.117	-0.472	-0.207	0.042	0.001
18	Single aril weight (g)	0.734	-0.128	-0.474	-0.204	0.049	0.017
19	Aril percentage per fruit (%)	0.093	0.073	0.398	-0.680	-0.216	0.080
20	Juice percentage per fruit (%)	0.154	0.071	-0.161	-0.113	0.086	0.512
21	Volume of juice	0.498	-0.115	0.091	-0.202	-0.024	0.477
22	Total soluble solids (°B)	0.361	-0.007	-0.610	-0.306	-0.012	0.066
23	pH of juice	-0.010	0.137	-0.237	0.165	-0.140	-0.303
24	Acidity (%)	-0.294	-0.437	0.141	-0.199	0.296	-0.076
25	Maturity Index (TSS/Acidity)	0.379	0.226	-0.527	-0.219	-0.132	-0.002
26	Phenolic content (mg/1000ml gallic acid)	-0.087	-0.011	-0.094	-0.007	0.073	0.296
27	Anthocyanins (mg/100ml)	-0.108	0.101	-0.019	0.118	0.037	-0.071
28	Rind thickness (mm)	0.384	-0.007	-0.147	0.254	0.026	0.233
29	Rind weight (g)	0.749	-0.330	-0.006	0.410	0.095	-0.175
30	Rind percentage (%)	0.019	-0.105	-0.334	0.707	0.173	-0.144
31	Seed length (cm)	0.042	0.113	-0.006	-0.009	0.068	-0.420
32	Seed width (cm)	-0.024	0.074	-0.028	0.044	0.204	-0.212
33	100 dry seed weight (g)	0.328	-0.022	-0.388	-0.067	0.165	-0.044
34	Bacterial blight on stem (PDI)	0.381	0.452	0.099	0.125	0.187	-0.052
35	Bacterial blight on leaves (PDI)	0.145	0.456	0.026	0.011	0.177	0.225
36	Bacterial blight on fruit (PDI)	0.390	0.449	0.266	-0.047	0.602	-0.038
37	Disease severity on tree (%)	0.443	0.527	0.274	-0.024	0.589	-0.022

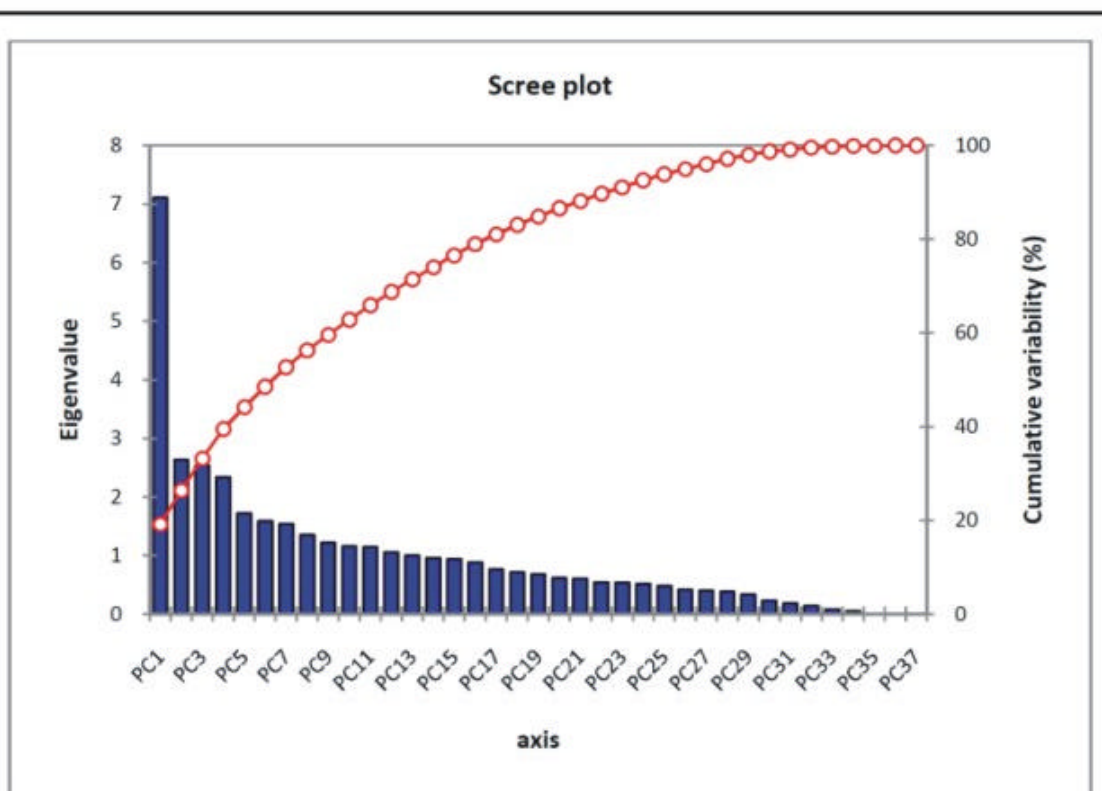


Fig. 7. Principal components screen plot

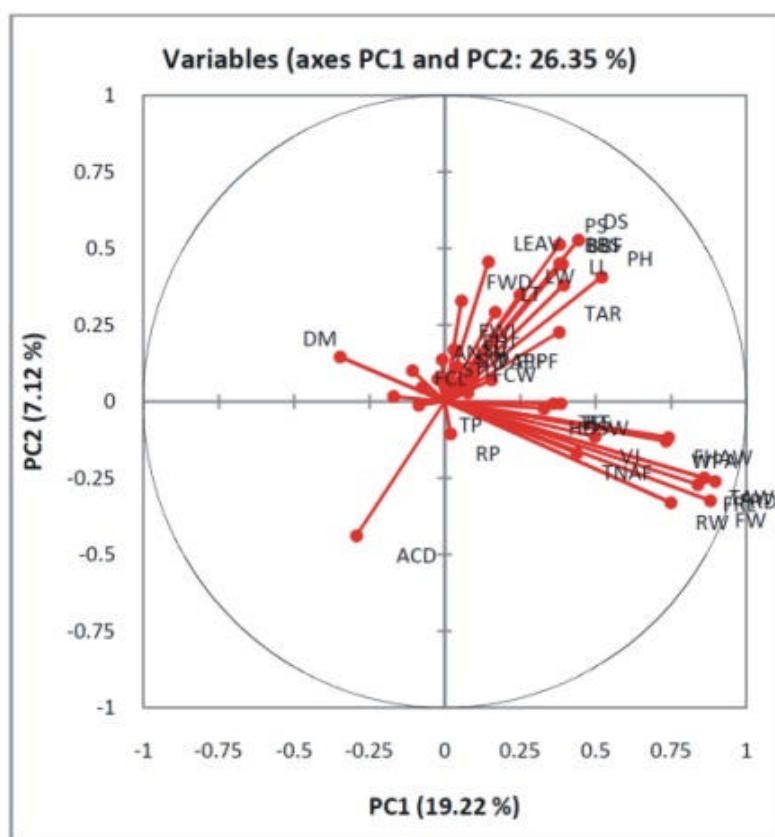


Fig. 8. Plot of principle components PC1-PC2 for the traits

Fig. 9. Plot of the accessions and morphological characters on the first and second components in pomegranate germplasms

Fig. 10: Plot of the accessions on the first and second components in pomegranate germplasms

obtained for character TSS, maturity index and 100 aril weight and single aril weight and the genotypes clubbed with the least value are PK15-41, PK10-59, PK20-10 and PG20-144. Likewise per cent of aril per fruit and TSS were found to have maximum negative value for component PCA with the genotypes viz., nana, PK15-41, PG20-34 and PK15-41.

The PCA analysis found to reduce the data of 37 traits in six most significant variables or components which showed maximum and stable variability among all other characters under study. The 20 most significant variables observed were plant height (0.520), plant spread (0.381), leaf length (0.393), leaf breadth (0.248), fruit length (0.839), fruit diameter (0.895), fruit weight (0.880), total number arils per fruit (0.437), total aril weight (0.859), 100 aril weight (0.741), single aril weight (0.734), volume of juice (0.498), TSS (0.361), maturity index (0.379) rind thickness (0.384), rind weight (0.749), 100 dry seed weight (0.328) disease severity on tree (0.443), bacterial blight disease on stem (0.381) and bacterial blight disease on fruit (0.390). The genotypes corresponding to these variables with maximum values would be taken into consideration for selection of diverse genotypes for future breeding programmes and also it is sufficient to take genotypes from these groups for genotyping with SSR markers.

4.3 Molecular characterization

Molecular markers assess variations at the DNA level in the nucleotide sequence of different genotypes in a population. This variation being purely genetic so is not influenced by the environment and the stage of development of the plant or the animal, unless there is any mutation. Molecular markers are numerous and therefore a large genome can be easily assayed for existence of any variation, such genetic markers are easy to score. DNA based markers allow direct comparison of the genetic material of two individual plants and have been used quite extensively in recent years. Use of molecular markers therefore provides an objective assessment of genetic diversity in a plant species and enables unequivocal identification of elite genotypes, besides acting as a database for the linkage mapping, identification of different quantitative trait loci, association mapping *etc.*

4.3.1 Genotyping and data analysis

A total of 150 SSR markers were examined for amplification of which 125 have been amplified in 188 genotypes of pomegranate. The amplicons were analyzed through capillary electrophoresis and scored for different allelic pattern. The details of the genotyping and analysis are presented in Table 35.

4.3.2 Heterozygosity

Heterozygosity is the proportion of heterozygous individual in the population. The highest was observed for PGCT098 (0.995) with a mean heterozygosity of 0.118, heterozygosity ranged from 0-99.46 per cent.

4.3.3 Allelic diversity

Allelic diversity was analyzed by using Power marker software. Highest allelic diversity 0.76 was found for the primer PGCT008 SSR primers and 45 SSR primers showed monomorphic. The average allelic diversity for the polymorphic SSR markers was 0.19.

4.3.4 Allele frequency

The allele frequency represents the incidence of a gene variant in the population. Allelic frequency was analyzed by using Power marker software. The average allelic frequency for the polymorphic SSR markers was 0.85. Lowest allele frequency of 0.26 was observed in PGCT008 and highest of 1.00 in majority of the screened primers.

4.3.5 Polymorphic Information Content (PIC)

To assess the quality of marker genotype data PIC is calculated. PIC content of each locus was analyzed to determine the extent of polymorphism explained by these markers using Powermarker. The PIC of markers exhibited a significant variation

Table 35: List of SSR markers showing polymorphism using 188 g-ray irradiated seed derived progenies and germplasm of pomegranate

Marker	Major Allele Frquency	Allele No.	Availability	Gene Diversity	Heterozygosity	PIC
PGCT009	0.9814	2.0000	1.0000	0.0365	0.0053	0.0359
PGCT030	0.7380	2.0000	0.9947	0.3867	0.0321	0.3120
EPS07	0.9677	2.0000	0.9894	0.0624	0.0323	0.0605
ABRII-MP07	0.9355	2.0000	0.9894	0.1207	0.0430	0.1134
PGCT002	1.0000	1.0000	0.9628	0.0000	0.0000	0.0000
PGCT090	0.6595	2.0000	0.9840	0.4491	0.3135	0.3483
EPS04	0.7802	2.0000	0.9681	0.3430	0.0769	0.2841
ABRII-MP12	0.9699	3.0000	0.9734	0.0586	0.0055	0.0574
PGCT025	0.8575	2.0000	0.9894	0.2443	0.0914	0.2145
PGCT057	0.6568	2.0000	0.9840	0.4509	0.3514	0.3492
EPS03	0.6984	3.0000	0.9787	0.4245	0.0272	0.3389
ABRII-MP28	0.8056	2.0000	0.9574	0.3133	0.0111	0.2642
POM_AAC12	1.0000	1.0000	0.9734	0.0000	0.0000	0.0000
EPS08	0.4098	7.0000	0.9734	0.6596	0.3607	0.5980
ABRII-MP30	0.9891	2.0000	0.9734	0.0216	0.0219	0.0214
PGCT097	0.7000	2.0000	0.9574	0.4200	0.0000	0.3318
PGCT062	1.0000	1.0000	0.9468	0.0000	0.0000	0.0000
pg17	1.0000	1.0000	0.9255	0.0000	0.0000	0.0000
ABRII-MP33-	1.0000	1.0000	0.9415	0.0000	0.0000	0.0000
PGCT070	0.4918	5.0000	0.9681	0.6678	0.1044	0.6193
ABRII-MP42	0.7088	6.0000	0.9681	0.4379	0.4890	0.3778
ABRII-MP39	0.9889	4.0000	0.9574	0.0221	0.0056	0.0220
PG13	0.9890	4.0000	0.9681	0.0218	0.0055	0.0217
POM_AGC11	0.9832	5.0000	0.9521	0.0332	0.0056	0.0330
ABRII-MP51	0.6438	5.0000	0.7766	0.4681	0.0068	0.3708
EPS10	0.8877	5.0000	0.9947	0.2016	0.1765	0.1853
EPS05	0.6695	6.0000	0.9415	0.4568	0.3503	0.3716
PGCT043	0.6452	4.0000	0.9894	0.4616	0.3602	0.3599
POM_AAC2	0.3984	6.0000	0.9947	0.7227	0.9786	0.6763
PGCT058	0.9836	3.0000	0.9734	0.0324	0.0000	0.0321
PGCT022	0.8962	2.0000	0.9734	0.1861	0.1639	0.1688
ABRII-MP04	0.9423	2.0000	0.9681	0.1087	0.0055	0.1028
pg22	0.7017	2.0000	0.9628	0.4187	0.0110	0.3310
PGCT024	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000

Contd.....

Table 35 contd.....

Marker	Major Allele Frquency	Allele No.	Availability	Gene Diversity	Heterozygosity	PIC
PGCT031A	1.0000	1.0000	0.9096	0.0000	0.0000	0.0000
POM_AAC1	0.6523	3.0000	0.9255	0.4613	0.5115	0.3650
PGCT014	0.8687	2.0000	0.9521	0.2281	0.1061	0.2021
PGCT003	1.0000	1.0000	0.9415	0.0000	0.0000	0.0000
ABRII-MP26	1.0000	1.0000	0.9202	0.0000	0.0000	0.0000
EPS06	0.9942	2.0000	0.9149	0.0116	0.0000	0.0115
POM_AAC7	0.9916	3.0000	0.9468	0.0167	0.0056	0.0167
pg23	0.9944	2.0000	0.9468	0.0112	0.0000	0.0111
ABRII-MP46	0.9942	2.0000	0.9202	0.0115	0.0000	0.0114
pg24	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000
pg7	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000
pg16	1.0000	1.0000	0.9574	0.0000	0.0000	0.0000
POM046	1.0000	1.0000	0.9840	0.0000	0.0000	0.0000
PG20	0.9830	2.0000	0.9362	0.0335	0.0000	0.0329
PGCT039	1.0000	1.0000	0.9947	0.0000	0.0000	0.0000
PGCT016	0.6330	5.0000	1.0000	0.4934	0.3989	0.4084
PGCT087	0.7473	3.0000	1.0000	0.3852	0.0053	0.3223
PGCT110	0.6765	2.0000	0.9947	0.4377	0.3155	0.3419
PGCT008	0.2596	9.0000	0.9734	0.7647	0.4809	0.7234
pg21	0.7039	2.0000	0.9521	0.4168	0.3017	0.3300
POM_AAC13	0.3936	4.0000	1.0000	0.6528	0.3883	0.5794
EPS01	1.0000	1.0000	0.9894	0.0000	0.0000	0.0000
PGCT102	0.9785	2.0000	0.9894	0.0421	0.0323	0.0412
EPS09	1.0000	1.0000	0.9894	0.0000	0.0000	0.0000
PGCT069	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000
pg19	1.0000	1.0000	0.9787	0.0000	0.0000	0.0000
PGCT082	1.0000	1.0000	0.9894	0.0000	0.0000	0.0000
PGCT038b	1.0000	1.0000	0.9840	0.0000	0.0000	0.0000
PGCT076	1.0000	1.0000	0.9894	0.0000	0.0000	0.0000
PGCT 035B	1.0000	1.0000	0.9947	0.0000	0.0000	0.0000
PGCT065	0.6862	2.0000	1.0000	0.4307	0.2979	0.3379
PGCT111	0.5430	2.0000	0.9894	0.4963	0.4516	0.3731
PGCT010	1.0000	1.0000	0.9947	0.0000	0.0000	0.0000
PGCT112	0.6845	2.0000	0.9947	0.4319	0.0214	0.3386
PGCT 028	1.0000	1.0000	0.9840	0.0000	0.0000	0.0000
PGCT061	0.5857	2.0000	0.9309	0.4853	0.3829	0.3675
PGCT085	0.5820	2.0000	0.9734	0.4866	0.3552	0.3682

Contd.....

Table 35 contd.....

Marker	Major Allele Frequency	Allele No.	Availability	Gene Diversity	Heterozygosity	PIC
PGCT047	0.6898	2.000	0.9947	0.4279	0.0321	0.3364
PGCT105	0.8497	2.000	0.9202	0.2554	0.1503	0.2228
PGCT086b	1.0000	1.000	0.9840	0.0000	0.0000	0.0000
PGCT067	0.6143	2.000	0.9309	0.4739	0.3486	0.3616
PGCT080	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
PGCT093A	1.0000	1.000	0.9787	0.0000	0.0000	0.0000
PGCT020	1.0000	1.000	0.9840	0.0000	0.0000	0.0000
PGCT034	1.0000	1.000	0.9894	0.0000	0.0000	0.0000
PGCT089b	0.6723	2.000	0.9415	0.4406	0.4633	0.3435
PGCT005	0.9758	2.000	0.9894	0.0472	0.0269	0.0461
PGCT109	0.9757	2.000	0.9840	0.0475	0.0162	0.0463
PGCT013	1.0000	1.000	0.9894	0.0000	0.0000	0.0000
PG10	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
PGCT083	1.0000	1.000	0.9947	0.0000	0.0000	0.0000
pg15	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
PGCT015	0.7038	2.000	0.9787	0.4169	0.0163	0.3300
PG5	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
PGCT104	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
PGCT088	0.3763	4.000	0.9894	0.6589	0.3441	0.5856
pg18b	1.0000	1.000	0.9894	0.0000	0.0000	0.0000
pg2	0.9785	2.000	0.9894	0.0421	0.0108	0.0412
PGCT099	0.9945	2.000	0.9734	0.0109	0.0109	0.0108
pg8	0.6919	2.000	0.9149	0.4264	0.0233	0.3355
pg10b	1.0000	1.000	0.9840	0.0000	0.0000	0.0000
pg3	1.0000	1.000	0.9894	0.0000	0.0000	0.0000
pg12	1.0000	1.000	0.9894	0.0000	0.0000	0.0000
PG6	0.6413	2.000	0.9787	0.4601	0.3804	0.3542
pg14b	0.5874	2.000	0.9734	0.4847	0.3552	0.3672
pg9	1.0000	1.000	0.9947	0.0000	0.0000	0.0000
pg25	1.0000	1.000	0.9947	0.0000	0.0000	0.0000
pg14a	0.5838	2.000	0.9840	0.4860	0.3676	0.3679
pg14c	0.5820	2.000	0.9734	0.4866	0.3661	0.3682
PGCT037A	0.9947	2.000	1.0000	0.0106	0.0106	0.0105
PGCT021	0.9669	3.000	0.9628	0.0646	0.0110	0.0634
PGCT094	0.6902	2.000	0.9787	0.4276	0.3696	0.3362
PGCT098	0.3649	4.000	0.9840	0.6982	0.9946	0.6427
ABRII-MP34	1.0000	1.000	0.9894	0.0000	0.0000	0.0000

Contd.....

Table 35 contd.....

Marker	Major Allele Frequency	Allele No.	Availability	Gene Diversity	Heterozygosity	PIC
PGCT001	0.9543	4.000	0.9894	0.0882	0.0161	0.0861
PGCT051	0.9947	2.000	0.9947	0.0106	0.0000	0.0106
PGCT066b	0.6766	2.000	0.9787	0.4376	0.3533	0.3419
PGCT032	0.5882	2.000	0.9947	0.4844	0.3636	0.3671
PGCT060	0.5189	3.000	0.9840	0.5070	0.3946	0.3864
PGCT004A	0.6405	2.000	0.9840	0.4605	0.3838	0.3545
pg18	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
pg11	1.0000	1.000	0.9947	0.0000	0.0000	0.0000
PGCT026	0.9838	3.000	0.9840	0.0320	0.0216	0.0316
PGCT075b	0.9451	4.000	0.9681	0.1055	0.0330	0.1030
PGCT004B	0.9892	2.000	0.9894	0.0213	0.0215	0.0210
PGCT093B	0.6995	2.000	1.0000	0.4204	0.0160	0.3320
PGCT050	0.5860	3.000	0.9894	0.4896	0.3441	0.3749
PGCT096b	1.0000	1.000	0.9947	0.0000	0.0000	0.0000
PGCT007	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
PGCT068	0.6648	2.000	0.9681	0.4457	0.3626	0.3464
PGCT046	1.0000	1.000	1.0000	0.0000	0.0000	0.0000
Mean	0.8497	2.144	0.9752	0.1952	0.1176	0.1609

ranging from 0.00 -0.72. The highest PIC (0.72) for the marker PGCT008. The average PIC for the polymorphic markers was 0.16.

4.3.6 Diversity studies with SSR marker

4.3.6.1 Cluster analysis with SSR

A dendrogram of the 188 Pomegranate accessions analyzed with the SSR marker system was drawn based on the DICE matrix and Neighbour joining using DARWIN (Dissimilarity Analysis and Representation for Windows) software programme (Fig. 11 and 12).

Based on the results, the Pomegranate accessions fell into three groups and the dendrogram scale varied from 0 to 0.1. Group A contained 128 accessions and these accessions were closely related to each other. Group B included 59 accessions and also these accessions were very similar. Group C contained one accessions and showed the most genetic diversity. When all groups are considered, the most distant accessions were PK15-41 in group A and PK10-5 in group B. Dissimilarity between these accessions was about 24 per cent.

In this study many pomegranate genotypes were γ -ray irradiated seed derived progenies of Bhagwa and Ganesh genotypes and germplasm however, genetic diversity was limited.

4.4 Marker trait association (MTA) / association mapping studies in pomegranate

Among 475 γ -ray irradiated seed derived progenies and germplasm of pomegranate evaluated for plant growth, fruit quality traits and bacterial blight disease resistance was subjected to D^2 analysis and 23 clusters formed. From each cluster, genotypes have been selected to represent the entire clustering pattern for constituting 188 association mapping panel for genotyping with SSR markers. The genotypic data based on SSR markers for the 188 different genotypes of the pomegranate was run for structural analysis using STRUCTURE 2.3.3 to know the population structure of the population.

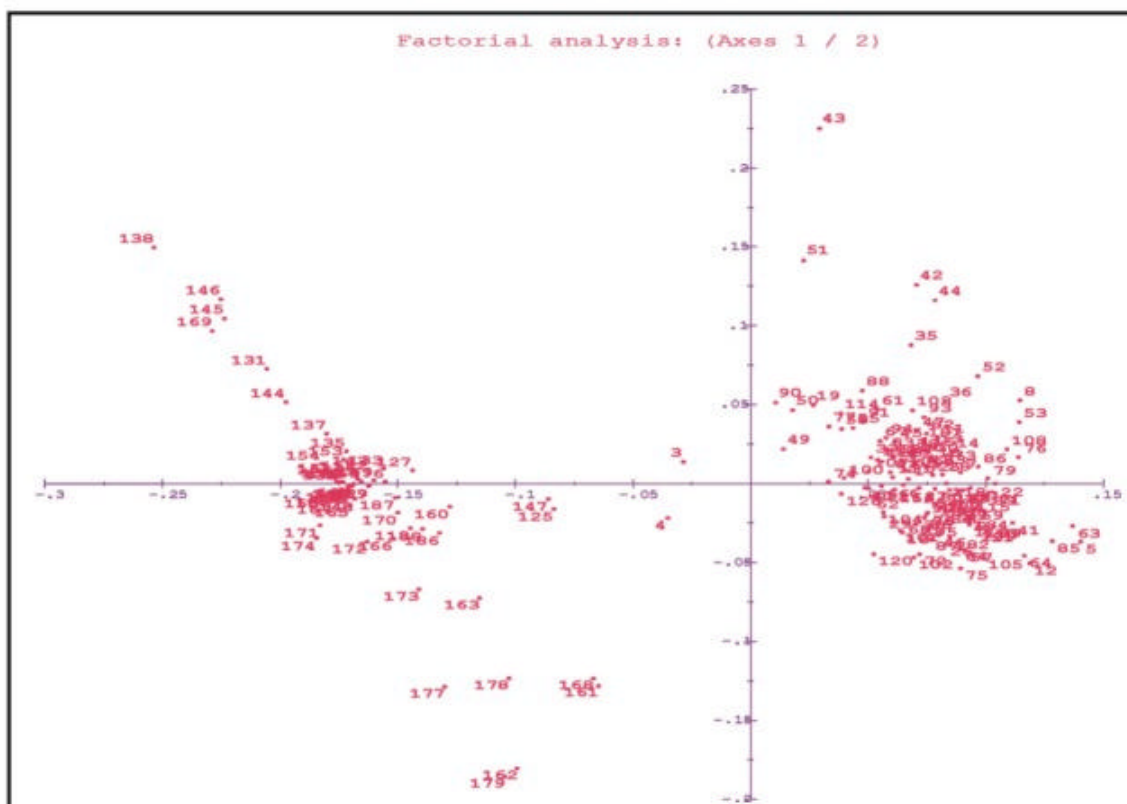
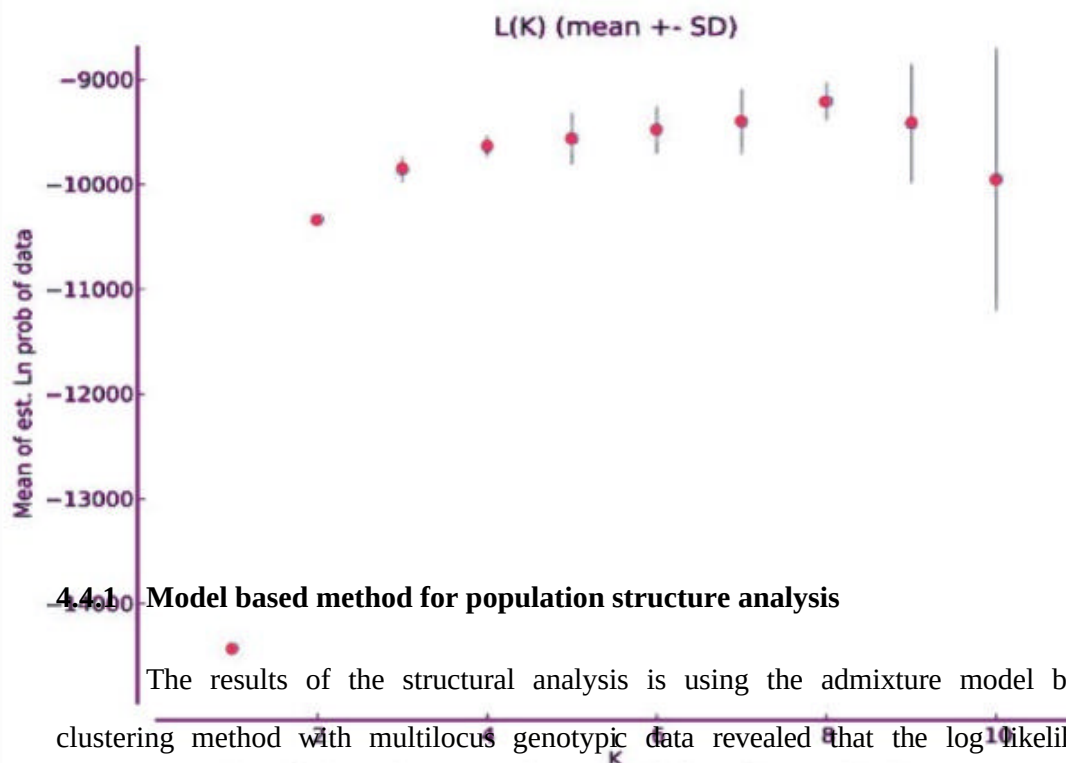


Fig. 12. Molecular diversity estimation in pomegranate genotypes on PCA plot



4.4.1 Model based method for population structure analysis

The results of the structural analysis is using the admixture model based clustering method with multilocus genotypic data revealed that the log likelihood steadily improved until $K=8$, with maximum natural log probability (-9207.36 ± 172.78),

which is proportional to posterior probability (Fig. 13). Estimated log probability ($LnP(D)$) represents the extent of genetic diversity hidden in the population. The $LnP(D)$ values increased with K from 1-10 but did not show a clear true K. To arrive at the real K value and ascertain about the actual number of subpopulations, an *adhoc* measure ΔK (Evanno, *et al.*, 2005) was used to detect the number of sub-groups using web based programme STRUCTURE HARVESTER. The rate of change of the mean likelihood distribution with K was plotted (Fig. 14). Fig. 15 revealed that there was peak at K = 2 (corresponding to number of sub-population) was inferred to be two based on likelihood value ($\Delta K = 5126.09$), which suggested two different groups belonging to Bhagwa and Ganesh, with each genotype having = 90 per cent affiliation probability in its respective group. Each individual is represented by a vertical line broken into K coloured segments, with lengths proportional to each of the K inferred clusters. The numbers (1-188) corresponded to the codes of the different genotypes, as given in Table 36. Further, the statistical functions for the K from Structure Analysis for clustering are presented in Table 36.

The structural analysis from SSR data for the different genotypes is presented in Fig. 16. Two major groups were formed here also. Group I contained 124 genotypes and group II contained 41 genotypes. Their sum constitutes 87.76 per cent of the genotypes in these two groups. The rest 12.24 per cent are admixtures amongst the two groups.

4.4.2 Marker trait association (MTA)

SSR markers were used for estimation of LD-based association mapping of phenotypic traits using TASSEL 3.0 (Bradbury *et al.*, 2007) to identify genomic regions that determine the variability in specific traits. Association analysis was performed on a panel (188) of pomegranate genotypes using 125 SSR markers. A General Linear Model

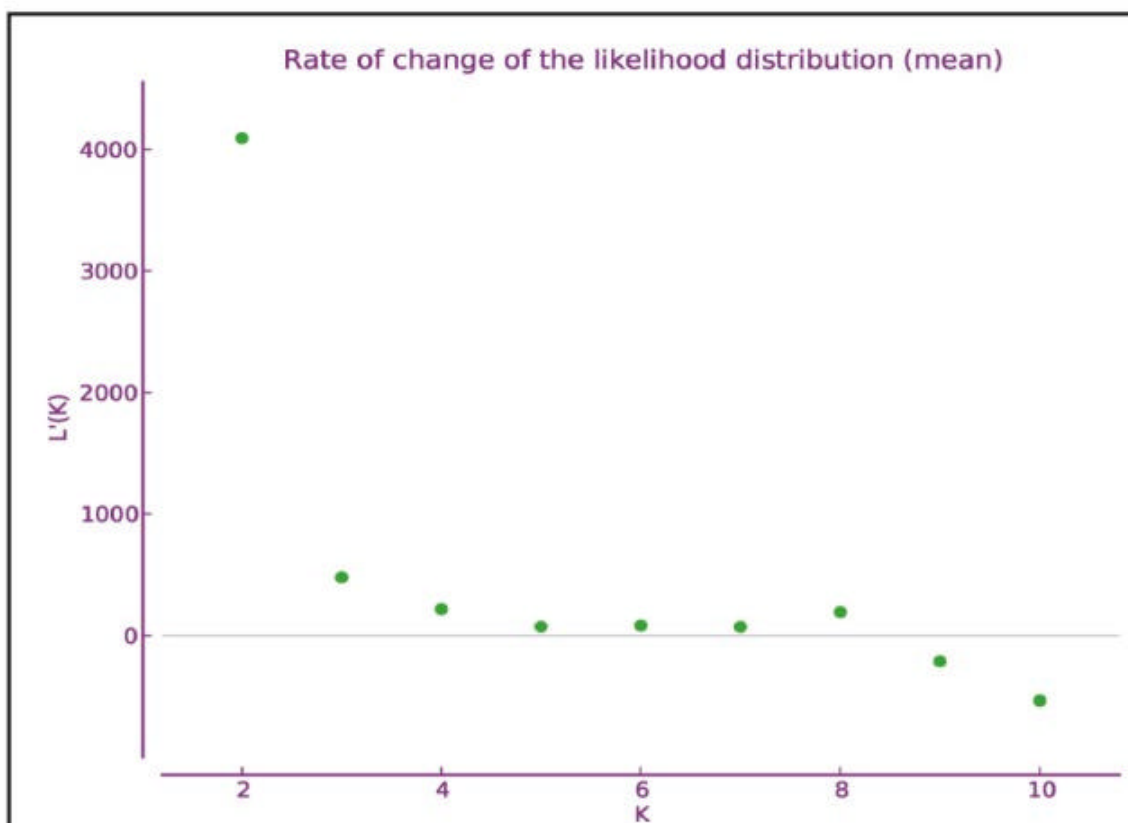


Fig. 14. Rate of change of likelihood distribution

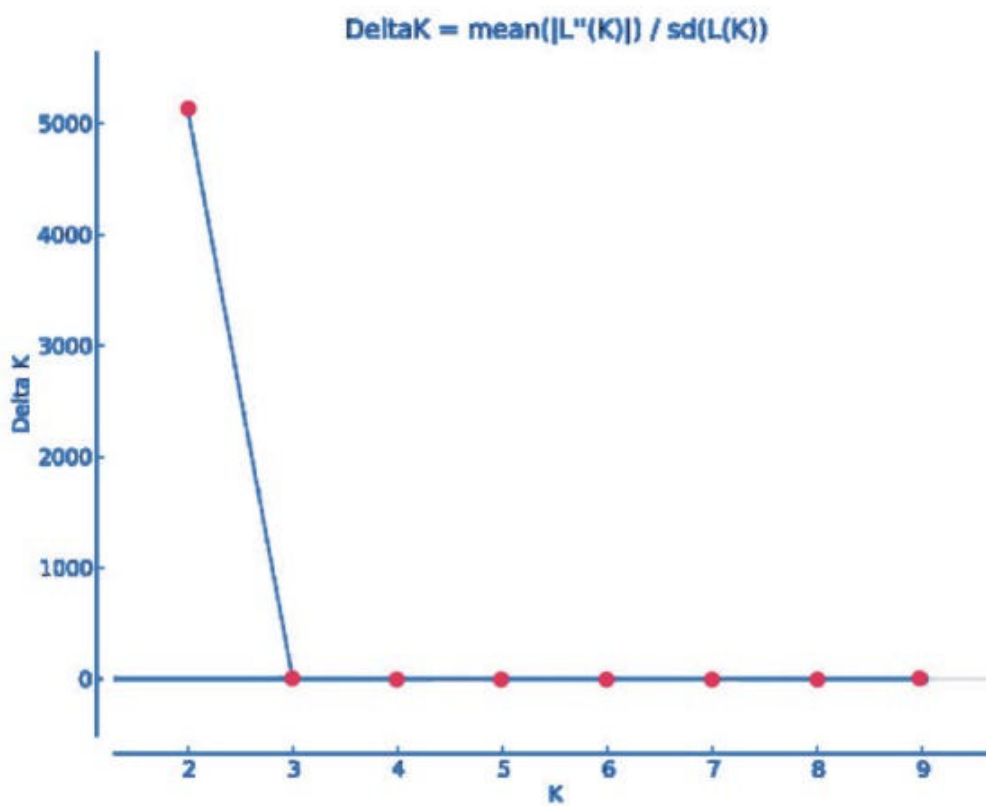


Fig. 15: Magnitude of ΔK as a function of K in the population

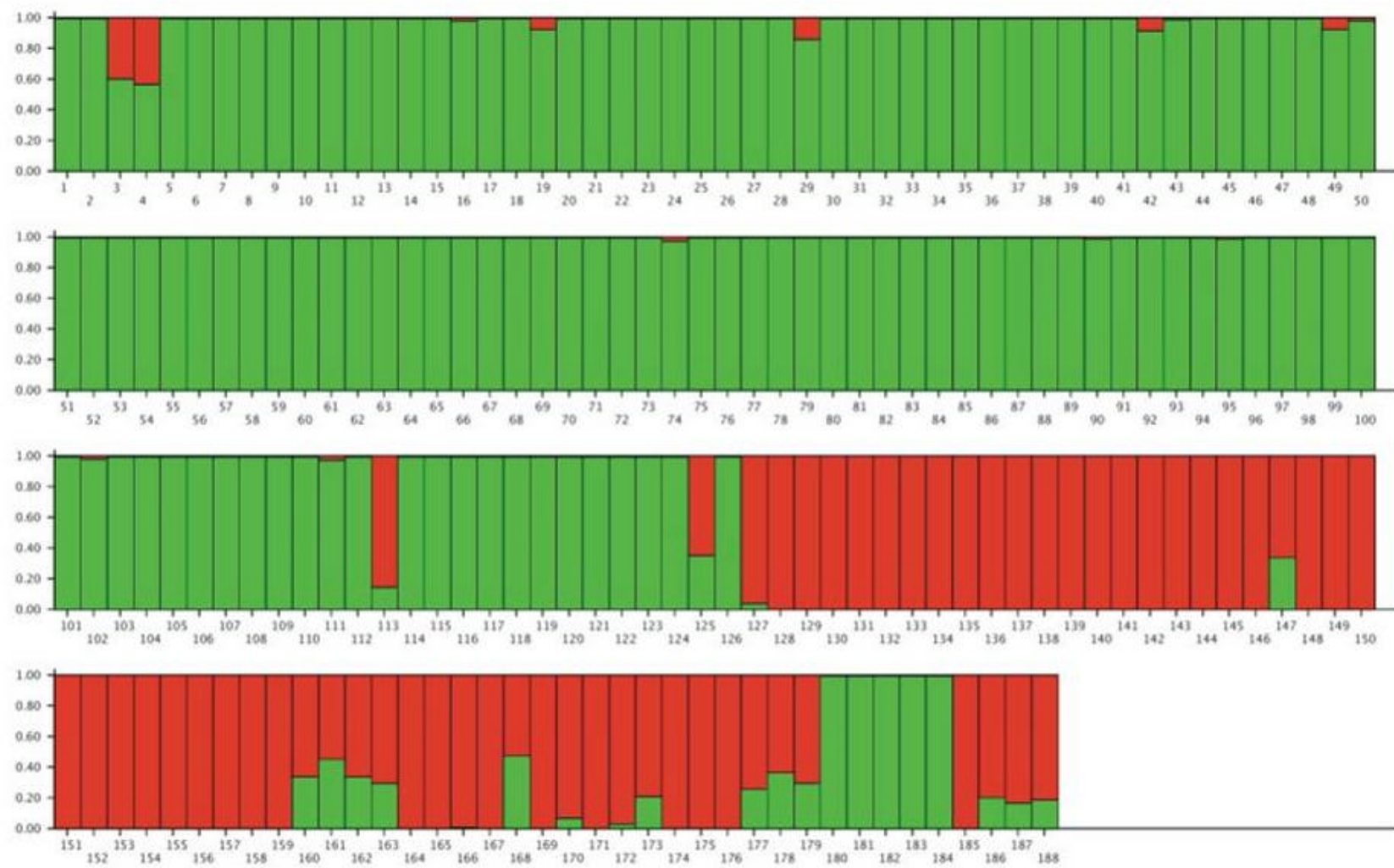


Fig. 16: Estimated genotypic structure for the 188 genotypes of pomegranate using SSR markers

Table 36: Mean and standard deviation of the logarithm of the probability of data for different K tested

K	Replication	Mean LnP (K)	Stdev LnP (K)	Ln' (K)	 Ln'' (K) 	Delta K
1	5	14428.000000	0.158114	—	—	—
2	5	-10334.720000	0.704982	4093.280000	3613.800000	5126.086368
3	5	-9855.240000	122.627048	479.480000	260.600000	2.125143
4	5	-9636.360000	95.494492	218.880000	143.260000	1.500191
5	5	-9560.740000	243.278447	75.620000	8.580000	0.035268
6	5	-9476.540000	220.311445	84.200000	10.120000	0.045935
7	5	-9402.460000	305.298523	74.080000	121.020000	0.396399
8	5	-9207.360000	172.781851	195.100000	406.160000	2.350710
9	5	-9418.420000	560.348795	-211.060000	322.420000	0.575392
10	5	-9951.900000	1245.798812	-533.480000	—	—

(GLM) and single marker analysis (SMA) was employed to discover genomic region with the different phenotypic traits.

4.4.2.1 General linear model (GLM)

Association between the individual markers and the different traits studied for the genotypes using TASSEL 3. The general linear model (GLM) based on the chosen Q matrix produced by the software STRUCTURE 2.2 (Appendix III).

The different marker trait associations along with their probability value (at 5 % level of significance) and their respective contribution (R^2 value) for SSR markers is presented in Table 37. In all 208 significant markers trait associations were identified for 32 traits. Out of 125 markers 43 markers showed significant association with the trait. Among all MTA, 25 associations were specifically found for the trait-acidity. Markers linked to acidity high phenotypic variance ($R^2 > 0.90$). Out of 43 markers 9 markers viz., PGCT001, PGCT051, ABRIIMP39, PG13, POM_AGC11, ABRIIMP51, EPS10, PH05 and PG20 were significantly associated with more than ten traits.

The plant height was associated with EPS-07 ($R^2 = 0.083$), ABRII-MP-51 ($R^2 = 0.11$), PGCT051 ($R^2 = 0.056$), PGCT014 ($R^2 = 0.070$), PGCT001 ($R^2 = 0.076$) and EPS04 (0.066), with their respective contribution.

The EPS-10 is common marker showing association with disease resistance characters such as bacterial blight on leaves, fruit, stem and disease severity on tree with contribution of 10.84, 10.40, 12.66 and 12.71 per cent respectively, where as the marker PG-20 also associated with disease resistance characters except in bacterial blight on fruit.

The markers such as PG-14a, PG-14b and PG-14c is the only marker showing the common association with the most of fruit characters such as fruit length, fruit diameter, fruit weight, total aril weight, rind thickness, hundred aril weight and single aril weight. However, this has to be confirmed by using more number of markers and genotypes through multi-locational trial in different agro-climatic zones.

Table 37: Association of marker loci with phenotypic traits of plant growth, fruit quality traits and bacterial blight disease resistance using GLM (Q) model

Sl. No.	Characters	SSR Marker/primer	P value (<0.05)	R ² value
1.	Plant height (cm)	EPS07	7.30E-05	0.083
		ABRII-MP51	2.37E-04	0.102
		PGCT051	3.30E-04	0.056
		PGCT014	4.69E-04	0.070
		PGCT001	5.14E-04	0.076
		EPS04	6.57E-04	0.066
2.	Plant spread (cm)	ABRII-MP51	6.67E-06	0.160
		PGCT051	1.55E-05	0.091
		PGCT026	1.95E-05	0.119
		EPS05	9.37E-05	0.132
		PG13	1.03E-04	0.092
		ABRII-MP39	1.12E-04	0.093
		PGCT043	1.20E-04	0.100
		PGCT001	1.29E-04	0.100
		POM_AGC11	2.16E-04	0.100
		EPS10	3.27E-04	0.102
		PGCT021	5.53E-04	0.089
3.	Leaf thickness (mm)	PGCT008	7.70E-08	0.2636
		PGCT022	4.92E-04	0.0772
		EPS10	7.34E-04	0.0940
4.	Leaf length (cm)	POM_AGC11	3.06E-11	0.231
		PGCT051	1.66E-10	0.179
		ABRII-MP51	3.02E-10	0.247
		PG13	4.15E-10	0.192
		ABRII-MP39	4.18E-10	0.194
		PGCT043	1.57E-09	0.193
		PGCT001	1.70E-09	0.194
		PGCT026	2.09E-09	0.193
		EPS05	3.53E-09	0.218
		EPS10	1.60E-08	0.187
		PGCT058	1.97E-08	0.163
		ABRII-MP42	7.92E-08	0.198
		PGCT087	1.93E-07	0.153
		PGCT009	1.54E-05	0.102
		PGCT021	2.36E-05	0.114
		PG20	5.80E-05	0.080
		EPS07	7.58E-04	0.069

Contd.....

Table 37 contd.....

Sl. No.	Characters	SSR Marker/primer	P value (<0.05)	R ² value
5.	Leaf breadth (cm)	PGCT051	4.84E-08	0.138
		PG13	2.32E-07	0.145
		POM_AGC11	3.31E-07	0.159
		EPS05	3.76E-07	0.180
		ABRII-MP39	4.19E-07	0.142
		PGCT026	7.49E-07	0.147
		PGCT001	7.77E-07	0.147
		PGCT043	9.81E-07	0.144
		ABRII-MP51	1.25E-06	0.175
		EPS10	1.44E-06	0.152
		ABRII-MP42	1.86E-05	0.152
		PGCT058	4.84E-05	0.098
		PGCT021	2.10E-04	0.097
		PGCT087	2.12E-04	0.094
		PGCT009	5.04E-04	0.073
		EPS07	5.68E-04	0.073
6.	Flower length (cm)	PGCT051	5.22E-04	0.062
7.	Flower breadth (cm)	PGCT051	2.54E-04	0.066
		PG13	8.35E-04	0.071
8.	Pistil length (cm)	ABRII-MP51	4.65E-04	0.118
9.	Fruit length (cm)	PG20	4.74E-05	0.077
		PGCT051	2.08E-04	0.061
		PGCT050	2.76E-04	0.084
		ABRII-MP39	3.64E-04	0.072
		PGCT001	3.99E-04	0.081
		PG13	4.06E-04	0.071
		pg14a	4.32E-04	0.070
		pg14c	4.48E-04	0.071
		EPS10	6.95E-04	0.085
		ABRII-MP51	7.27E-04	0.097
		EPS04	8.27E-04	0.064
10.	Fruit diameter (cm)	pg14c	1.61E-04	0.075
		pg14a	1.61E-04	0.073
		PGCT050	2.92E-04	0.078
		pg14b	5.43E-04	0.064
		PGCT001	6.19E-04	0.071
		EPS05	7.93E-04	0.092

Contd.....

Table 37 contd.....

Sl. No.	Characters	SSR Marker/primer	P value (<0.05)	R ² value
11.	Fruit weight (g)	pg14b	1.61E-05	0.105
		PGCT050	1.78E-05	0.115
		pg14a	2.56E-05	0.099
		pg14c	2.72E-05	0.100
		ABRII-MP39	2.79E-04	0.078
		PG13	3.39E-04	0.077
		POM_AAC7	3.68E-04	0.078
		POM_AGC11	4.06E-04	0.089
		EPS05	5.52E-04	0.108
12.	Calyx length (cm)	PG20	6.65E-05	0.073
		ABRII-MP07	7.49E-04	0.063
13.	Calyx diameter (cm)	M110	9.53E-06	0.099
		EPS08	1.66E-05	0.181
		PGCT043	2.49E-05	0.121
		PGCT032	3.54E-05	0.104
		PGCT001	4.25E-05	0.116
		ABRII-MP39	4.61E-05	0.105
		POM_AGC11	4.61E-05	0.120
		PG13	5.84E-05	0.101
		PGCT026	1.07E-04	0.107
		EPS10	1.17E-04	0.117
		ABRII-MP51	1.78E-04	0.128
		EPS05	2.70E-04	0.126
		pg14c	3.12E-04	0.131
		pg14a	3.37E-04	0.071
		ABRII-MP42	6.94E-04	0.076
		PG20	7.44E-04	0.074
14.	Total aril weight (g)	pg14c	2.74E-04	0.069
		pg14a	2.76E-04	0.067
		pg14b	5.04E-04	0.063
		PGCT050	8.15E-04	0.068
15.	100 aril weight (g)	pg14a	7.68E-05	0.094
		pg14c	8.40E-05	0.095
		pg14b	1.19E-04	0.091
16.	Single aril weight (g)	pg14a	8.20E-05	0.093
		pg14c	9.02E-05	0.094
		pg14b	1.31E-04	0.090

Contd.....

Table 37 contd.....

Sl. No.	Characters	SSR Marker/primer	P value (<0.05)	R ² value
17.	pH of juice	PGCT001	3.05E-04	0.098
		PG20	7.16E-04	0.064
18.	Acidity (%)	PGCT051	1.44E-130	0.957
		PGCT043	8.87E-127	0.957
		PGCT001	1.01E-126	0.957
		PG13	8.62E-126	0.957
		PGCT026	1.63E-125	0.957
		ABRII-MP39	2.69E-125	0.958
		EPS10	3.31E-125	0.957
		POM_AGC11	3.45E-123	0.959
		EPS05	4.53E-122	0.962
		ABRII-MP42	6.48E-121	0.959
		ABRII-MP51	6.32E-109	0.968
		PGCT058	1.86E-27	0.495
		PGCT021	6.89E-24	0.465
		EPS07	8.33E-17	0.333
		PGCT087	1.76E-16	0.340
		PG20	6.08E-16	0.314
		PGCT009	6.54E-16	0.315
		PGCT093B	8.86E-11	0.222
		ABRII-MP12	4.13E-09	0.209
		PGCT075b	3.74E-08	0.215
		pg22	3.85E-07	0.153
		ABRII-MP07	2.87E-06	0.130
		PGCT098b	4.80E-04	0.105
		PGCT105	7.71E-04	0.081
		pg8	9.46E-04	0.079
19.	Maturity Index (TSS/Acidity)	PGCT051	1.93E-08	0.152
		ABRII-MP39	1.45E-07	0.159
		PG13	1.73E-07	0.155
		PGCT043	1.95E-07	0.164
		POM_AGC11	3.44E-07	0.166
		ABRII-MP51	3.46E-07	0.200
		PGCT001	5.28E-07	0.157
		PGCT026	5.89E-07	0.155
		EPS05	7.25E-07	0.185
		EPS10	1.55E-06	0.158
		ABRII-MP42	8.75E-06	0.167
		PGCT058	7.10E-05	0.098
		PGCT087	8.78E-05	0.106
		PGCT105	3.20E-04	0.088
		PGCT093B	3.99E-04	0.079
		EPS07	8.87E-04	0.072

Contd.....

Table 37 contd.....

Sl. No.	Characters	SSR Marker/primer	P value (<0.05)	R ² value
20.	Phenolic content (mg/1000ml gallic acid)	EPS08	2.44E-04	0.150
21.	Anthocyanins (mg/100ml)	PGCT004B	1.64E-04	0.071
22.	100 dry seed weight (g)	ABRII-MP04	6.44E-05	0.095
23.	Rind thickness (mm)	PG20	1.20E-05	0.104
		PGCT050	7.13E-05	0.112
		ABRII-MP07	1.53E-04	0.091
		pg14c	2.61E-04	0.087
		pg14b	2.91E-04	0.086
		pg14a	3.14E-04	0.085
		ABRII-MP39	4.54E-04	0.083
		PG13	5.57E-04	0.080
		PGCT051	5.79E-04	0.062
		EPS05	6.80E-04	0.116
		EPS10	9.15E-04	0.097
24.	Rind weight (g)	POM_AAC7	2.87E-05	0.109
		PGCT050	2.96E-05	0.117
		EPS05	1.19E-04	0.132
		ABRII-MP39	1.21E-04	0.092
		POM_AGC11	1.39E-04	0.105
		PG13	1.56E-04	0.090
		pg23	3.57E-04	0.068
		EPS06	3.88E-04	0.069
		ABRII-MP46	3.98E-04	0.068
		pg14c	4.04E-04	0.080
		pg14b	4.72E-04	0.079
		pg14a	4.96E-04	0.077
25.	Bacterial blight on stem (PDI)	PGCT051	4.24E-05	0.087
		PG20	4.78E-05	0.091
		ABRII-MP51	4.82E-05	0.147
		EPS10	7.77E-05	0.124
		ABRII-MP39	1.77E-04	0.093
		PG13	2.37E-04	0.089
		POM_AGC11	3.90E-04	0.099
		PGCT001	4.30E-04	0.094
		PGCT026	6.40E-04	0.091
		PGCT 043	8.57E-04	0.087

Contd.....

Table 37 contd.....

Sl. No.	Characters	SSR Marker/primer	P value (<0.05)	R ² value
26.	Bacterial blight on leaves (PDI)	POM_AGC11	1.89E-05	0.131
		EPS05	1.01E-04	0.139
		PGCT051	1.15E-04	0.078
		PGCT043	1.80E-04	0.104
		PGCT058	2.14E-04	0.090
		EPS10	3.32E-04	0.108
		ABRII-MP42	4.93E-04	0.128
		PG20	5.16E-04	0.067
		PG13	5.64E-04	0.081
		ABRII-MP39	6.21E-04	0.080
27.	Bacterial blight on fruit (PDI)	EPS10	4.51E-04	0.104
28.	Disease severity on tree (%)	EPS10	4.96E-05	0.127
		PGCT058	4.75E-04	0.080
		PG20	9.46E-04	0.061

4.4.2.2 Single marker analysis (SMA)

Single marker analysis was performed using 188 genotypes to determine the strength of association between linked markers and Trait by calculating *F* statistic and simple linear regression coefficient (Haley and Knott, 1992) using WinQTL Cartographer version 2.5 (Wang *et al.*, 2007). In total, 125 SSR markers were considered for SMA as mentioned in materials and method.

About 343 significant ($P < 0.001$) marker-trait associations in SMA, significant associations were found for plant growth, fruit quality and bacterial blight disease resistance component traits. Highest number of markers (32) explaining more than 5 per cent phenotypic variation were found associated with fruit diameter followed by 29 markers for fruit length and total aril weight, 24 markers for fruit weight and 23 markers for calyx length. Similarly about 50 markers having more than 5 per cent contribution showed significant association with leaf length, leaf breadth and leaf thickness. Highest markers with respect to bacterial blight disease, six were found associated with bacterial blight on stem and two markers each associated with bacterial blight on leaves, fruit and disease severity (Table 38).

4.4.3 Comparison of General linear model (GLM) and Single marker analysis (SMA) for marker trait association in pomegranate

About 208 significant ($P < 0.001$) marker-trait associations in GLM and 343 significant associations were found in the SMA model for the plant growth, fruit quality, bacterial blight disease resistance and its component traits. About 25 associations in GLM and 16 significant associations were found in the SMA model for the acidity (Table 37 and 38). Highest number of markers (20) explaining more than five per cent phenotypic variation were found associated with calyx diameter followed by 17 markers for leaf length, 16 for leaf breadth and maturity index and 12 markers for rind weight, 11 markers for plant spread, fruit length and 10 markers for bacterial blight on leaves and stem and only three markers for disease severity on tree. Similarly in SMA about 32 markers having more than five per cent contribution showed significant association with fruit diameter followed by 29 and 24 markers for fruit length, total aril weight and fruit weight, respectively. Only six markers were found associated with bacterial blight on stem and followed by two marker each associated with bacterial blight on leaves, stem and disease severity (Table 39).

Table 38: Association of Marker loci with phenotypic traits of plant growth, fruit quality traits and bacterial blight disease resistance using SMA model

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
1.	Plant height (cm)	PGCT030	0.000000011	0.1591
		EPS04	0.000044942	0.0856
		PGCT097	0.000005958	0.1293
		PGCT070	0.000005811	0.1233
		Pg-22	0.000000004	0.159
		PGCT112	0.000000569	0.1325
		PGCT085	0.000055950	0.0923
		PGCT047	0.000000107	0.1402
		PGCT105	0.000011231	0.0805
		PGCT015	0.000000193	0.1385
		PGCT088	0.000077553	0.0845
		Pg-8	0.000000047	0.1359
		PGCT098	0.000000002	0.1776
		PGCT060	0.000000006	0.1668
		PGCT004	0.000000884	0.1184
		PGCT093	0.000000000	0.2147
		PGCT068	0.000019104	0.0897
2.	Plant spread (cm)	PGCT043	0.000039454	0.0586
		PG-22	0.000051023	0.0882
		PGCT112	0.000325656	0.0661
		PGCT047	0.000349771	0.0652
		PGCT015	0.000918892	0.0528
		PGCT094	0.002718111	0.0526
		PGCT004	0.000874823	0.0543
		PGCT093	0.000024004	0.0917
3.	Leaf thickness (mm)	PGCT030	0.001006305	0.0566
		PGCT057	0.000047441	0.0861
		PGCT097	0.000103692	0.0797
		PGCT070	0.000696858	0.0649
		PGCT014	0.000611016	0.0649
		PGCT008	0.000000490	0.0747
		PGCT065	0.000574740	0.1325
		PGCT112	0.000121485	0.0619
		PGCT047	0.000321493	0.0798
		PGCT034	0.000027659	0.0646
		PGCT015	0.000552288	0.067
		PGCT096	0.000049667	0.0653
		PGCT068	0.000952908	0.0849
4.	Leaf length (cm)	PGCT009	0.000485511	0.0635
		PGCT030	0.000022809	0.0876
		PGCT097	0.000341190	0.0719
		PGCT070	0.001294063	0.0641
		PG13	0.023212015	0.1193

Contd....

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
5.	Leaf breadth (cm)	POM_AGC11	0.000001205	0.1193
		ABRII-MP51	0.000001205	0.1193
		EPS10	0.000056219	0.0838
		PGCT043	0.000000013	0.1198
		Pg22	0.000001217	0.1213
		PGCT112	0.000620085	0.0622
		PGCT047	0.000067061	0.0821
		PGCT015	0.000291889	0.0712
		Pg8	0.000004460	0.0987
		PGCT098	0.000000230	0.1279
		PGCT051	0.000000173	0.156
		PGCT060	0.000153146	0.0719
		PGCT004	0.000042832	0.0793
		PGCT093	0.000009742	0.1001
		PGCT030	0.000254584	0.0679
		POM_AGC11	0.000322686	0.0673
		ABRII-MP51	0.000322686	0.0673
		EPS10	0.000322686	0.0673
		PGCT043	0.000004161	0.0738
		Pg22	0.000187098	0.0742
		PGCT047	0.000607611	0.0625
		PGCT015	0.002018789	0.0546
		Pg8	0.000384724	0.062
		pg14b	0.000621209	0.0654
		pg14a	0.000618358	0.0663
		pg14c	0.000616606	0.0625
		PGCT098	0.000014655	0.0937
		PGCT051	0.000008594	0.1149
		PGCT060	0.001246414	0.0523
		PGCT004	0.000050998	0.0816
		PGCT093	0.000060510	0.0831
		PGCT050	0.000178258	0.0751
6.	Flower length (cm)	PGCT051	0.003398343	0.0533
7.	Flower breadth (cm)	pg22	0.000625701	0.0647
		pg16	0.007818254	0.0565
		PGCT008	0.000157210	0.0783
		Pg8	0.000077965	0.0761
		PGCT098	0.000144681	0.0745
		PGCT093	0.000208603	0.0715
		PGCT030	0.000000315	0.1285
		EPS04	0.000041551	0.083
		PGCT057	0.001069971	0.0608
		ABRII-MP28	0.000595387	0.0579
8.	Fruit length (cm)	EPS08	0.000035032	0.0977
		PGCT097	0.000000028	0.1535

Contd....

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
		PGCT070	0.000006565	0.1214
		Pg22	0.000000119	0.1409
		PGCT014	0.001282325	0.0539
		PGCT087	0.000008463	0.1014
		PGCT008	0.000014225	0.0924
		PGCT112	0.000001551	0.1162
		PGCT085	0.000007127	0.102
		PGCT047	0.000000533	0.1272
		PGCT105	0.000036227	0.0931
		PGCT015	0.000000120	0.1468
		PGCT088	0.000012284	0.0962
		Pg8	0.000000993	0.1067
		pg14b	0.000013691	0.1048
		pg14a	0.000013596	0.1035
		pg14c	0.000013633	0.0943
		PGCT098	0.000000001	0.1725
		PGCT066	0.000295841	0.0677
		PGCT032	0.000005951	0.1066
		PGCT060	0.000005404	0.1077
		PGCT004	0.000000998	0.1186
		PGCT093	0.000000054	0.1473
		PGCT050	0.000006520	0.1057
		PGCT068	0.000863869	0.0575
9.	Fruit diameter (cm)	PGCT030	0.000000005	0.1653
		EPS04	0.001135108	0.0531
		PGCT057	0.000398086	0.07
		ABRII-MP28	0.000444519	0.0605
		EPS08	0.000000152	0.1576
		PGCT097	0.000000000	0.2032
		PGCT070	0.000000858	0.1458
		Pg22	0.000000000	0.1879
		PGCT014	0.000150202	0.0716
		PGCT087	0.000002548	0.1125
		PGCT008	0.000010173	0.1018
		POM_AAC13	0.001327131	0.054
		PGCT111	0.001320613	0.0506
		PGCT112	0.000000004	0.1718
		PGCT085	0.000001373	0.121
		PGCT047	0.000000000	0.1907
		PGCT105	0.000006859	0.104
		PGCT015	0.000000000	0.2003
		PGCT088	0.000006434	0.1054
		Pg8	0.000000001	0.1667
		Pg6	0.001763845	0.0543
		pg14b	0.000228756	0.0753
		pg14a	0.000228527	0.072
		pg14c	0.000230156	0.0642

Contd....

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
		PGCT098	0.000000000	0.2219
		PGCT066	0.000136758	0.07
		PGCT032	0.000020811	0.095
		PGCT060	0.000000339	0.132
		PGCT004	0.000000003	0.1723
		PGCT093	0.000000000	0.1948
		PGCT050	0.000084182	0.0819
		PGCT068	0.000016540	0.0921
10.	Fruit weight (g)	PGCT030	0.000021542	0.0897
		EPS08	0.000011277	0.1166
		PGCT097	0.000000834	0.122
		PGCT070	0.000002128	0.1261
		POM_AAC11	0.001070575	0.0561
		ABRII-MP51	0.001070575	0.0561
		Pg22	0.000003173	0.0972
		PGCT087	0.000000890	0.1221
		PGCT111	0.000549206	0.061
		PGCT112	0.000144177	0.075
		PGCT085	0.000516801	0.0605
		PGCT047	0.000031121	0.0886
		PGCT105	0.000770802	0.066
		PGCT15	0.000026862	0.0942
		Pg8	0.000070993	0.0694
		pg14b	0.000768688	0.0679
		pg14a	0.000765655	0.0631
		pg14c	0.000767424	0.0572
		PGCT08	0.00000143	0.1107
		PGCT032	0.000009301	0.1042
		PGCT060	0.000014318	0.0981
		PGCT004	0.000089511	0.08
		PGCT093	0.000034846	0.0882
		PGCT050	0.000239291	0.073
11.	Calyx length (cm)	PGCT030	0.000000187	0.1368
		EPS07	0.000186798	0.077
		ABRII-MP07	0.000000103	0.1438
		PGCT057	0.000000009	0.1683
		EPS08	0.000006666	0.1128
		PGCT097	0.000001973	0.1186
		PGCT070	0.004503563	0.0544
		ABRII-MP04	0.001054989	0.0641
		pg22	0.000000040	0.1523
		PGCT014	0.000031980	0.0779
		PG20	0.000096289	0.1084
		PGCT112	0.000000021	0.1552
		PGCT047	0.000000000	0.1965
		PGCT105	0.000001674	0.1101
		PGCT015	0.000000005	0.1523

Contd....

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
		PGCT088	0.002260076	0.0546
		pg8	0.000010462	0.1024
		PGCT098	0.000028105	0.1005
		PGCT066	0.000351830	0.0617
		PGCT032	0.000001390	0.1182
		PGCT075	0.001239311	0.0575
		PGCT093	0.000000002	0.1744
		PGCT068	0.000220645	0.0672
12.	Calyx diameter (cm)	EPS07	0.000133460	0.0806
		POM_AGC11	0.000342623	0.0668
		ABRII-MP51	0.000342623	0.0668
		PGCT043	0.000020908	0.0676
		PG20	0.000079544	0.1018
		PGCT051	0.000135992	0.0913
		PGCT032	0.000003415	0.1107
13.	Total aril weight (g)	PGCT030	0.000000000	0.196
		PGCT057	0.000234639	0.0752
		EPS08	0.000000295	0.1496
		PGCT097	0.000000000	0.2266
		PGCT070	0.000005358	0.1308
		Pg22	0.000000000	0.2038
		PGCT014	0.001482471	0.0505
		PGCT087	0.000003637	0.1092
		PGCT008	0.000112132	0.0795
		POM_AAC13	0.000345011	0.0667
		PGCT111	0.000682969	0.06
		PGCT112	0.000000009	0.1665
		PGCT085	0.000016206	0.0974
		PGCT047	0.000000000	0.2153
		PGCT105	0.000000695	0.1259
		PGCT15	0.000000000	0.2162
		PGCT088	0.000019017	0.0958
		Pg8	0.000000013	0.1441
		pg14b	0.000038204	0.0914
		pg14a	0.000038160	0.0891
		pg14c	0.000038406	0.0808
		PGCT098	0.000000000	0.2347
		PGCT066	0.000036900	0.0802
		PGCT032	0.000021380	0.0945
		PGCT060	0.000004154	0.1099
		PGCT004	0.000000304	0.1302
		PGCT093	0.000000000	0.2204
		PGCT050	0.000018486	0.0987
		PGCT068	0.000004593	0.1037
14.	Total number of	PGCT030	0.000000011	0.1617

Contd....

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
	arils/fruit	PGCT057 EPS08 PGCT097 PGCT070 Pg22 PGCT087 PGCT008 PGCT111 PGCT112 PGCT085 PGCT047 PGCT105 PGCT15 PGCT088 Pg8 PGCT098 PGCT066 PGCT032 PGCT060 PGCT004 PGCT093 PGCT068	0.000138698 0.000003585 0.000000000 0.000067997 0.000000000 0.000011690 0.000009540 0.000698385 0.000000002 0.000017450 0.000000000 0.000029077 0.000000001 0.000020373 0.000000021 0.000000001 0.000040630 0.000175116 0.000571138 0.000010430 0.000000000 0.000052604	0.0804 0.1167 0.1969 0.1086 0.2116 0.0984 0.1042 0.059 0.174 0.0922 0.2009 0.0909 0.1869 0.0936 0.1467 0.1781 0.0833 0.0749 0.0636 0.1007 0.1929 0.0849
15.	100 aril weight (g)	PGCT105 pg14b pg14a pg14c PGCT098 PGCT060 PGCT093 PGCT050	0.000522764 0.000108842 0.0001087 0.00010877757 0.000179467 0.000089936 0.000781793 0.000113921	0.0597 0.0809 0.0797 0.0751 0.0727 0.0797 0.059 0.0806
16.	Single aril weight (g)	PGCT098 PGCT060	0.001282363 0.002035853	0.0509 0.0514
17.	Aril percentage per fruit (%)	PGCT086 PGCT104 PGCT096	0.000143713 0.000122784 0.000129362	0.0705 0.0644 0.0759
18.	Percentage of juice/fruit	PGCT093	0.000249098	0.0835
19.	Volume of juice	PG6 pg14b PGCT050	0.001492705 0.001709052 0.000920312	0.0539 0.0563 0.0567
20.	TSS	PG22 PGCT112 PGCT047 PGCT015 PG08 PGCT098	0.000984322 0.000764288 0.000289898 0.000092316 0.001144450 0.000650340	0.0574 0.0595 0.0666 0.0683 0.0519 0.0704

Contd....

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
		PGCT004	0.000617771	0.063
		PGCT093	0.000596509	0.0616
21.	pH of juice	PGCT090	0.001085731	0.0549
		PGCT016	0.012940195	0.0549
22.	Acidity (%)	PGCT009	0.000000000	0.2306
		EPS07	0.000012072	0.0988
		ABRII-MP07	0.001580515	0.052
		ABRIi-MP12	0.000019695	0.1445
		ABRII-MP42	0.000920333	0.0635
		ABRII-MP39	0.000106637	0.0819
		PG13	0.000000375	0.4489
		POM_AGC11	0.000000000	0.4489
		ABRII-MP51	0.000000000	0.4489
		EPS10	0.000000000	0.4735
		PGCT043	0.000000000	0.6254
		PG20	0.000646990	0.1546
		PGCT001	0.000034886	0.1012
		PGCT051	0.000000000	0.7665
		PGCT026	0.000992188	0.0815
		PGCT075	0.000625257	0.0769
23.	Maturity Index (TSS/Acidity)	PG13	0.062668546	0.0509
		POM_AGC11	0.001856829	0.0509
		ABRII-MP51	0.001856829	0.0509
		EPS10	0.000547080	0.0624
		PGCT043	0.000000105	0.1038
		PGCT051	0.000002755	0.1302
		PGCT093	0.000698599	0.0601
24.	Phenolic content (mg/1000ml gallic acid)	PGCT035	0.000015128	0.096
		PGCT068	0.001775247	0.052
25.	Anthocyanins (mg/100ml)	PGCT097	0.003032500	0.0506
		PGCT008	0.000460177	0.0707
		PGCT047	0.001007205	0.0538
		PGCT015	0.003213317	0.0531
		PGCT004	0.000158435	0.0748
		PGCT068	0.000059028	0.0902
26.	Rind thickness (mm)	ABRII-MP07	0.000488054	0.063
		POM_AGC11	0.000143323	0.075
		ABRII-MP51	0.000143323	0.075
		PGCT043	0.000207190	0.0665
		PG20	0.000742059	0.091
		Pg21	0.002759246	0.0528
		PGCT051	0.002036565	0.0588
27.	Rind weight (g)	EPS08	0.000380142	0.0815
		PGCT097	0.001141017	0.0563
		PGCT070	0.000009525	0.1044

Contd....

Sl. No.	Characters	SSR Marker/primer	P value (<0.01)	R ² value
		POM_AGC11	0.000373713	0.066
		ABRII-MP51	0.000373713	0.066
		PGCT087	0.000037574	0.0875
		PGCT032	0.000308629	0.07
		PGCT060	0.000052060	0.0827
28.	100 dry seed weight (g)	EPS08	0.017586393	0.0517
		PGCT097	0.004064110	0.0556
		ABRII-MP04	0.000019541	0.1032
		PG22	0.000376134	0.0685
		PGCT112	0.000069163	0.0832
		PGCT047	0.000214379	0.0717
		PGCT015	0.000753833	0.0556
		PG8	0.000058252	0.0795
		PGCT001	0.000779600	0.0514
29.	Bacterial blight on leaves (PDI)	PGCT043	0.000086831	0.0698
		PGCT051	0.000127151	0.0831
30.	Bacterial blight on fruit (PDI)	PG6	0.001034124	0.0701
		PGCT096	0.001073871	0.056
31.	Bacterial blight on stem (PDI)	EPS04	0.001604048	0.0579
		PG13	0.086626237	0.0538
		POM_AGC11	0.001364801	0.0538
		ABRII-MP51	0.001364801	0.0538
		PGCT043	0.000073958	0.0535
		PGCT051	0.000217234	0.0815
32.	Disease severity on tree (%)	PG6	0.000599198	0.0741
		EPS10	0.001283844	0.057

Table 39: Common SSR marker loci identified in both GLM and SMA for Marker trait association in pomegranate

Sl. No.	Characters	SSR Marker/primer	GLM		SMA	
			P value (<0.05)	R ² value	P value (<0.01)	R ² value
1.	Plant height (cm)	EPS04	6.57E-04	0.0657294	0.000044942	0.086
2.	Plant spread (cm)	PGCT043	1.20E-04	0.09994814	0.000039454	0.059
3.	Leaf thickness (mm)	PGCT008	7.70E-08	0.26357876	0.000000490	0.132
4.	Leaf length (cm)	POM_AGC11	3.06E-11	0.23118578	0.000001205	0.119
		PGCT051	1.66E-10	0.17933271	0.000000173	0.156
		ABRII-MP51	3.02E-10	0.24684702	0.000001205	0.119
		PGCT043	1.57E-09	0.1929465	0.000000013	0.120
		EPS10	1.60E-08	0.18671753	0.000056219	0.084
		PGCT009	1.54E-05	0.10245607	0.000485511	0.063
		PG13	4.15E-10	0.19221746	0.023212015	0.119
5.	Leaf breadth (cm)	PGCT051	4.84E-08	0.1384158	0.0000086	0.115
		POM_AGC11	3.31E-07	0.15930682	0.000323	0.067
		PGCT043	9.81E-07	0.14430098	0.00000416	0.074
		ABRII-MP51	1.25E-06	0.17547077	0.0003227	0.067
		EPS10	1.44E-06	0.15225888	0.000323	0.067
6.	Flower length (cm)	PGCT051	5.22E-04	0.06285583	0.003398	0.053
7.	Fruit length (cm)	PGCT050	2.76E-04	0.08430737	0.0000065	0.105
		pg14a	4.32E-04	0.0695854	0.0000136	0.103
		pg14c	4.48E-04	0.07071825	0.0000136	0.094
		EPS04	8.27E-04	0.06449577	0.00004155	0.083
8.	Fruit diameter (cm)	pg14c	1.61E-04	0.07476254	0.00023	0.064
		pg14a	1.61E-04	0.07305239	0.00023	0.072
		PGCT050	2.92E-04	0.07827681	0.00008	0.082
		pg14b	5.43E-04	0.06405086	0.00023	0.075

Contd.....

Table 39 contd.....

Sl. No.	Characters	SSR Marker/primer	GLM		SMA	
			P value (<0.05)	R ² value	P value (<0.01)	R ² value
9.	Fruit weight (g)	pg14b	1.61E-05	0.10451237	0.00077	0.068
		PGCT050	1.78E-05	0.11450634	0.00024	0.073
		pg14a	2.56E-05	0.0990617	0.00076	0.063
		pg14c	2.72E-05	0.10046629	0.00077	0.057
10.	Calyx length (cm)	PG20	6.65E-05	0.07385824	0.00009	0.108
		ABRII-MP07	7.49E-04	0.06385476	0.00000	0.144
11.	Calyx diameter (cm)	PGCT051	9.53E-06	0.09911785	0.00013	0.091
		PGCT043	2.49E-05	0.12073825	0.00002	0.068
		PGCT032	3.54E-05	0.10359225	0.00000	0.110
		ABRII-MP51	1.78E-04	0.12840122	0.00013	0.091
		PG20	7.44E-04	0.07430398	0.00008	0.102
12.	Total aril weight (g)	pg14c	2.74E-04	0.0689448	0.00004	0.081
		pg14a	2.76E-04	0.0671714	0.00004	0.089
		pg14b	5.04E-04	0.06311757	0.00004	0.091
		PGCT050	8.15E-04	0.06807367	0.00002	0.099
13.	100 aril weight (g)	pg14a	7.68E-05	0.09423155	0.00011	0.080
		pg14c	8.40E-05	0.09498057	0.00011	0.075
		pg14b	1.19E-04	0.09105415	0.00011	0.081
14.	Acidity (%)	PGCT051	1.44E-130	0.95678887	0.00000	0.766
		PGCT043	8.87E-127	0.95708443	0.00000	0.625
		PGCT001	1.01E-126	0.95679357	0.00003	0.101
		PG13	8.62E-126	0.95736602	0.00000	0.449
		PGCT026	1.63E-125	0.9568532	0.00099	0.081
		ABRII-MP39	2.69E-125	0.95822444	0.00011	0.082
		EPS10	3.31E-125	0.95658592	0.00000	0.473
		POM_AGC11	3.45E-123	0.95854221	0.00000	0.449
		ABRII-MP42	6.48E-121	0.95915734	0.00092	0.063
		ABRII-MP51	6.32E-109	0.96802789	0.00000	0.449
		EPS 07	8.33E-17	0.33301905	0.00001	0.099
		PG20	6.08E-16	0.31400862	0.00064	0.155
		PGCT009	6.54E-16	0.31508809	0.00000	0.231
		ABRII-MP12	4.13E-09	0.2087172	0.00002	0.144
		PGCT075	3.74E-08	0.21548111	0.00062	0.077
		ABRII-MP07	2.87E-06	0.13033137	0.00158	0.052

Contd.....

Table 39 contd.....

Sl. No.	Characters	SSR Marker/primer	GLM		SMA	
			P value (<0.05)	R ² value	P value (<0.01)	R ² value
15.	Maturity Index (TSS/Acidity)	PGCT051	1.93E-08	0.15223939	0.00000	0.130
		PG13	1.73E-07	0.15461582	0.06267	0.051
		PGCT043	1.95E-07	0.16444544	0.00000	0.104
		POM_AGC11	3.44E-07	0.16600579	0.00186	0.051
		ABRII-MP51	3.46E-07	0.19983333	0.00186	0.051
		EPS10	1.55E-06	0.08839786	0.00055	0.062
		PGCT093	3.99E-04	0.07867132	0.00070	0.060
16.	Anthocyanins (mg/100ml)	PGCT004	1.64E-04	0.07114673	0.00016	0.075
17.	100 dry seed weight (g)	ABRII-MP04	6.44E-05	0.09570296	0.00406	0.103
18.	Rind thickness (mm)	PG20	1.20E-05	0.10434612	0.00074	0.091
		ABRII-MP07	1.53E-04	0.09113814	0.00049	0.063
		PGCT051	5.79E-04	0.06196688	0.00204	0.059
19.	Rind weight (g)	POM_AGC11	1.39E-04	0.10539587	0.00037	0.066
20.	Bacterial blight on stem (PDI)	PGCT051	4.24E-05	0.08713469	0.00022	0.081
		ABRII-MP51	4.82E-05	0.14738645	0.00136	0.054
		PG13	2.37E-04	0.08942465	0.08663	0.054
		POM_AGC11	3.90E-04	0.09915009	0.00136	0.0538
21.	Bacterial blight on leaves (PDI)	PGCT051	1.15E-04	0.07782316	0.00013	0.083
		PGCT043	1.80E-04	0.10372978	0.00009	0.070
22.	Disease severity on tree (%)	EPS10	4.96E-05	0.12717507	0.00128	0.057

5. DISCUSSION

Pomegranate (*Punica granatum* L.) is one of the most important fruit crops used for fresh consumption and processing. It is cultivated in Mediterranean region and all over the world. Due to increase in worldwide demand for superior nutritional and therapeutic properties of pomegranate, there is a need to initiate well planned breeding programmes to meet the increasing demands of local and international consumers, processors, growers and exporters. Pomegranate is genetically heterozygous and sufficient variations for several plant and fruits has been generated in nature, as well as by crossing and mutations (Mars, 2000). Cultivation of one particular cultivar for long period of time may lead to the genetic erosion and restricting the subsequent breeding programme.

Pomegranate a dollar earning fruit crop boon to the farmers, turned a big bane after the severe incidence of the bacterial blight and occurring in devastating form in several parts of Karnataka, Maharashtra, Andhra Pradesh and Tamil Nadu states of India, causing wide spread damage to the pomegranate orchards since 2000 and disease continued to occur in epidemic form and became difficult to manage effectively due to faster inoculum build up and its subsequent spread during favourable weather conditions. Many growers in the affected area were forced to uproot their crop after making only a couple of harvests.

Though in recent years, a lot of information has accumulated however, much emphasis needs to be given to the resistant germplasm, various facets of the pathogen and disease, survival of the pathogen, epidemiology, host range, pathogenic variation and effective management strategies. Therefore, it is necessary to document the available diversity and new variability (Sheidai *et al.*, 2008) in the γ -ray irradiated seed derived progenies and germplasm of the pomegranate, studying the strains variation on the genotypes/ germplasm and finally to develop marker trait association for plant growth, fruits quality traits and bacterial blight disease resistance.

The results of the experiments conducted are discussed on the following headings.

- 5.1 Morphological, pathogenic and molecular variability in *X. axonopodis* pv. *punicae*
- 5.2 Evaluation of γ -ray irradiated seed derived progenies and germplasm of pomegranate (*Punica granatum* L.) for plant growth, fruit quality traits and bacterial blight disease resistance
- 5.3 Correlation studies in γ -ray irradiated seed derived progenies and germplasm of pomegranate (*Punica granatum* L.) germplasm lines for plant growth, fruit quality traits and bacterial blight disease resistance
- 5.4 Genetic and molecular diversity studies in γ -ray irradiated seed derived progenies and germplasm of pomegranate (*Punica granatum* L.) germplasm lines for plant growth, fruit quality traits and bacterial blight disease resistance
- 5.5 Marker trait association (MTA) for plant growth, fruit quality, bacterial blight disease resistance and its component traits

5.1 Morphological, pathogenic and molecular variability in *X. axonopodis* pv. *punicae*

5.1.1 Symptomatology

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* appeared on all aerial parts of the plants such as leaf, stem and fruits. Initially on the lower surface of leaves, small water-soaked lesions were surrounded by diffused margins. Correspondingly on the upper leaf surface, small brown to black coloured spots were seen. Spots were angular to irregular in shape. As the disease progressed, the spots also grew, coalesced and extended up to midrib in a week time and occupied the major portion of leaf lamina. At later stages, the infected leaves turned yellow, chlorotic and shed off finally. The oozed out bacteria from the infected tissue under wet weather conditions formed a thin scale on the leaves. Similar kind of symptoms on leaf were described by Hingorani and Mehta (1952) as irregular spots varying 2 to 5 mm diameter

in size, initially light brown in colour surrounded by a water soaked margin. Later on, spots turned dark brown, coalesced and covered the larger areas of leaf lamina lead to the shedding of leaf at the final stage.

Stem infection was manifested in the form of long, narrow, brown to black coloured lesions on the main stem and branches. The lesions later on turned rough and cankerous. Stem girdling followed by breaking at the point of infection was noticed eventually.

Symptoms on fruits were very prominent with small pin head sized lesions surrounded by diffused water-soaked margin as a mark of bacterial infection. The lesions later on developed into brownish black coloured, medium to big sized spots. One too many such spots could be seen on the single fruits. In severe case of infection, the affected fruits split opens with L, Y or star shaped cracks on the pericarp. Infected fruits did not develop further nor dropped but dried did up and remain hanged in the plant itself. Kanwar (1976) reported the flower and fruit infection apart from leaf as studied in the present investigation. Similarly, other workers also described bacterial blight disease symptoms of pomegranate on leaves, stems and fruits (Kishun, 1993; Rangaswamy, 1962; Sohi *et al.*, 1964; Yenjerappa *et al.*, 2004; Manjula and Khan, 2003; Jalaraddi, 2006; Benagiand Kumar, 2011, Divya, 2013 and Katwal *et al.*, 2015).

5.1.2 Isolation and pathogenicity

Isolation of bacteria was made from the infected plant parts showing typical symptoms of bacterial blight disease collected from different parts of Karnataka by following standard serial dilution plating technique using nutrient glucose agar medium. Repeated isolation of all the isolates collected from different areas yielded well separated, circular, convex to slightly raised, light yellow to deep yellow, mucoid and small to medium sized bacterial colonies on nutrient agar (NA) medium after 72 hrs of incubation at $28 \pm 1^{\circ}\text{C}$. The well separated colonies which appeared as pale yellow to dark yellow after 72 hrs of incubation at $28 \pm 1^{\circ}\text{C}$ were picked and purified by single colony isolation technique on NA medium and suspended in sterile distilled water taken in sterile polypropylene tubes and stored at 4°C , which served as a stock culture for further studies. All the four isolates produced light yellow to yellowish brown colonies on NA medium. Pathogenicity was proved by inoculating bacterial cell suspension (4.5

$\times 10^6$ cfu/ml) to the pomegranate plants of Bhagwa and Ganesh cultivars. The characteristic symptoms of the disease appeared after 15-18 days of inoculation in the form of small, water-soaked, brown to black coloured lesions, which later on developed into angular to irregular shaped spots. Re-isolations were carried out from artificially inoculated plants yielded the bacterial colonies similar to the original culture.

Hingorani and Mehta (1952) isolated the bacterial pathogen from infected pomegranate leaves and proved the pathogenicity. Infection was readily seen by them on tender leaves artificially inoculated plants in seven to ten days of incubation. Isolation and pathogenicity studies were also carried out in a similar fashion by Kanwar (1976). He has observed the symptoms within four to seven days on injured portions and it took eight to twelve days to get the symptoms on uninjured parts.

The observations made pertaining to the isolation and pathogenicity in the present investigation were in conformity with the work of Manjula (2002), who obtained pure culture of the seven isolates of the pathogen from infected plant parts on nutrient agar medium by dilution plating technique and pathogenicity of all the isolates were proved by spray method of inoculation using 45 days old pomegranate cv. Jyothi.

5.1.3 Identification and variability of the pathogen

The identification of the pathogen involved in causation of bacterial blight disease of pomegranate was done by conducting studies on its morphological, cultural and molecular characteristics of pathogen as per the standard procedures.

5.1.3.1 Morphological characters

The colonies of all the isolates were circular with smooth margin pale white to yellow coloured, circular, convex to slightly raised, mucoid, small to medium in size and single and paired colonies were recorded on nutrient agar medium. The results obtained in the present study on morphological characters were in agreement with the reports of Hingorani and Singh (1959); Kanwar, 1976; Manjula, 2002; Jalaraddi, 2006; Kirankumar, 2007; Yenjerappa, 2009 and Wayde, 2015).

Kirankumar (2007) observed that the bacterium appeared as small rods, gram negative, non capsulated and non spore forming with monotrichous flagellation. The

bacterial colonies of all the isolates were yellow coloured, slightly mucoid, circular, single and small in size on (YDCA) medium. Yenjerappa (2009) also reported that the bacterium was rod shaped with rounded ends occurred singly or rarely in pairs, gram negative, capsulated, non-spore forming with single polar flagellum.

5.1.3.2 Cultural characters

The knowledge on extent of variability in a given pathogen has a wide practical utility, which can be made out from differences in virulence and nutrition (Venugopal, 1983). All the microorganisms possess traits that can be used to distinguish them and often artificial culture media can capitalize on these characters to facilitate selection (Schaad and Stall, 1988). Muras *et al.* (1983) studied the natural variability of morphological, cultural and biochemical properties of culture collection of phytopathogenic bacteria and stated that, among more than 1000 cultures of *Pseudomonas*, *Xanthomonas*, *Corynebacterium*, *Erwinia* and *Agrobacterium* spp. three per cent showed variability in colony morphology. *Xanthomonas* and *Pseudomonas* spp. were the most sensitive to change under storage. The extent of variability with regard to the growth characters of four isolates of *Xanthomonas axonopodis* pv. *punicae* collected from different locations viz., Vijayapur, Bagalkot, Koppal and Chitradurga were assessed. Schaad (1992) reported that, most pathovars of *Xanthomonas campestris* can be differentiated by growth and colony morphology on selective and semi-selective medium.

The isolates of *Xanthomonas axonopodis* pv. *punicae* differed in respect of size of colony as they developed medium to large, small to medium and small colonies. The isolates of *Xanthomonas axonopodis* pv. *punicae* also differed greatly in shape of colony as some of bacterial isolates produced circular, while others are circular to irregular in shape. The colour of the colony also differed greatly as the isolates were creamish yellow, light brown, light yellow in colour. Only two isolates produce fuscan (brown pigment) in the medium while remaining isolates couldn't produce any brown pigmentation. The number of days taken for the appearance of bacterial colonies has also differed to some extent that the isolates were appeared 4 to 5 days after streaking

on Nutrient Agar (NA) at $28\pm1^{\circ}\text{C}$. These results indicated the presence of variability with respect to colony morphology of different isolates of *X. axonopodis* pv. *punicae*. Similar observations were recorded by Raut (1990) and Manjula (2002).

The isolates showed variability with respect to colony diameter and growth rate. Among four isolates screened on solid media, *Xap* 3 isolate (Koppal) showed maximum growth (with 0.33 μm diameter) compared to all other isolates and least growth was recorded in *Xap* 2 (Bagalkot) isolate (0.24 μm diameter). This implies to the variation among the isolates of *X. axonopodis* pv. *punicae*. Present findings are comparable with the report by Hingorani and Singh (1959). They opined that, nutrient agar (NA) was the best media for the cultivation of pomegranate bacterium.

5.1.3.3 Pathogenic characters

All the isolates exhibits water soaked, circular to irregular, dark brown symptoms on leaves of Bhagwa and Ganesh variety in pot plants. There was significant difference within *Xanthomonas axonopodis* pv. *punicae* isolates with respect to their virulence and reaction with different varieties. Among the test isolates, *Xap* 1 was found to be virulent (PDI 41.32) across the varieties and was superior over other isolates viz., *Xap* 2, *Xap* 3 and *Xap* 4, which were statistically on par and were rated as moderately virulent.

Pomegranate varieties expressed variation in their susceptibility to *Xanthomonas axonopodis* pv. *punicae*. The variety Bhagwa recorded highest mean PDI followed by P-16 and Ganesh and rated as highly susceptible. Variety 'Patna 5' shown moderate susceptibility, Nana as moderately resistant. It was observed that the interaction between varieties and isolates was statistically non significant. There was considerable variation in virulence of isolates and susceptibility of varieties. Isolates *Xap* 1 (Vijayapur) and *Xap* 2 (Bagalkot) were found more virulent irrespective of varieties compared to other isolates. Varieties Bhagwa, P13 and Ganesh were found to be highly susceptible irrespective of isolates and recorded maximum mean PDI. Resistant varieties Patna-5 and Nana showed uniform resistant reaction against all the four isolates.

5.1.3.4 Molecular characterization of different isolates of *X. axonopodis* pv. *punicae* on the basis of 16s rRNA and *gyrB* gene analysis

The four isolates were characterized based on the 16s rRNA and *gyrB* gene analysis. Four isolates were used for the amplification of 753 bp of the 16s rRNA coding region and 491 bp of *gyrB* gene. The amplicon size of around 753 bp and 491 bp were noticed in all the four isolates using 16s rRNA and *gyrB* gene primer respectively; and confirms that, all these isolates belong to *Xap*.

A more conserved genetic characteristic, the 16s-23s intergenic spacer (IGS) of rDNA operon, has also been used to infer the phylogenetic relationship and identification of bacteria at different taxonomic levels (Gurtler and Stanisich, 1996). The 49 strains tested were amplified and produced a single band with each primer set and these sizes corresponded to the expected sizes of the targeted 16s rRNA and 23s rRNA genes respectively. For the ITS region, they produced a band of approximately 1600 bp and the resulting size of the ITS region ranged from 500 to 550 bp (Hirokaku and Hisatoshi, 1999). It was the expected size, since they have recently determined the sequence of this region among *Xanthomonas* strains.

Mondal *et al.* (2012) identified the *Xap* isolates through 16s rRNA gene based universal primers. The pathogenecity of the *Xap* isolates were confirmed following Koch's postulate. The 16s rRNA sequence of *Xap* isolates were deposited at NCBI Bank (Accession number of Delhi isolates are JN036620, JN036621, JN036622, JN036623, JN036624 and 3 Solhapur isolares are JN109171, JN109172 and Jn109173) and also attempted to understand the genetic variability among the *Xap* strains from diverse geographical origins (Delhi, Maharashtra and Andhra Pradesh) as well as to determine the phylogentic relationship between *Xap* and other phytopathogenic *Xanthomonas* using two important housekeeping genes, 16s rRNA and *gyrB*. Phylogeny based on 16s rRNA gene revealed that all 15 strains of *Xap* from Delhi, Maharashtra and Andhra Pradesh formed one cluster sharing 100 per cent identity among them, suggesting that geographical distance does not necessarily contribute to the variation in *Xap* strains with respect to the two important housekeeping gene loci, 16s rRNA and *gyrB*.

5.1.3.5 ERIC, BOX and Rep PCR generated genomic finger prints and their relationship with pathogenic variability of *X. axonopodis* pv. *punicae*

ERIC, BOX and Rep PCR have shown to be useful in diversity analysis of bacterial plant pathogens, including the genera of *Xanthomonas* and *Pseudomonas*. In this study genomic and pathogenic variability of four pomegranate isolates were collected from different locations of Karnataka viz., *Xap* 1 (Vijayapur), *Xap* 2 (Bagalkot), *Xap* 3 (Koppal) and *Xap* 4 (Chitradurga).

Amplification of ERIC, BOX and Rep region DNA from four isolates of pomegranate bacterial blight disease from four locations with three different primers yielded a total 28, 20 and 24 bands respectively (Table 20 and Plate 11, 12 and 13) with monomorphic banding pattern in all four isolates and amplicon size ranging from 300 to 2000 bp with BOX and ERIC primers whereas in case of Rep primers the range is 200 to 1000 bp only. From this it is confirmed that not much variation is existing in the collected isolates. Hence, isolate from Vijayapur was used for the further screening of the pomegranate genotypes against bacterial blight disease both *in vivo* and *in vitro*. The ERIC –PCR has been demonstrated to be an effective method in determining the genetic diversity among the population of many bacterial plant pathogenic genera, including *Xanthomonas* and *Pseudomonas* (Louws *et al.*, 1994 and Louws *et al.*, 1998).

Arshiya *et al.* (2014) isolated 56 strains of *Xanthomonas axonopodis* pv. *citri* (*Xac*) from different citrus growing areas of Marathwada region of Maharashtra state and 20 representative strains of *Xac* were selected for genetic diversity. Repetitive DNA polymerase chain reaction-based fingerprinting (rep-PCR) using repetitive extragenic palindromic (rep) and enterobacterial repetitive intergenic consensus (ERIC) and BOX primers were used to assess the genetic diversity. Cluster analysis done by combining the banding patterns of REP-PCR, ERIC-PCR and BOX-PCR clearly separated two diverse groups within species of *Xanthomonas axonopodis* pv *citri* based on the geographic region of isolation.

5.1.4 *In vivo* and *in vitro* screening of g-ray irradiated seed derived progenies and germplasm of pomegranate for bacterial blight disease resistance

Genetic variability as a result of induced mutation by various mutagens has contributed to modern plant breeding. Over the past five decades, it has played a major role all over the world in the development of superior plant varieties (Kharkwalet *et al.*, 2009) with desirable characteristics. Global impact of developed and released varieties in major crops all over the world has been reviewed by Ahloowalia *et al.* (2004). Physical mutagens, mostly ionizing radiations have been used widely for inducing hereditary aberrations and more than 70 per cent of mutant varieties were developed using physical mutagenesis (Mba, 2012 and Mba, 2013). The major advantage of using physical mutagenesis compared to chemical mutagenesis is the degree of accuracy and sufficient reproducibility, particularly for gamma rays, which have a uniform penetrating power in the tissue (Yamaguchi, 2003)

Mutation breeding via gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars (Nakagawa 2009). Mutation induction is the starting step in mutation breeding and its low frequency has been a severe limiting factor. Mutation breeding has its own advantages and limitations. The advantages include, creation of new gene alleles that do not exist in germplasm pools; induction of new gene alleles for a commercial variety so, new varieties carrying desired mutation alleles can be directly use as a commercial variety. The limited genetic changes of any single plant of a mutated population and the often recessive nature enable breeders to develop a new variety in a short breeding cycle. The disadvantage of mutation breeding is in its limited power in generating dominant alleles that might be desired; it is also less effective than cross breeding for a trait needs a combination of multiple alleles, such as tolerance to abiotic stresses. The low mutation frequency requires growing and screening of large population for selection of desired mutants at a reasonable confidence. There is no doubt that there are many more cultivars/varieties derived by means of mutation breeding all over the world. Against the backdrop of the still ongoing debates concerning the safety of genetically modified organisms, crop varieties and cultivars that have been derived by mutation breeding in the last 50 years, continue to play a key role in present-day agriculture.

A total of 475 γ -ray irradiated seed derived progenies of Bhagwa and Ganesh, and germplasm lines of pomegranate were screened *in vivo* and *in vitro* for bacterial blight disease incidence in *mrigbahar* season of 2014-15 and 2015- 16. The *mrigbahar* season was highly favourable for bacterial blight incidence and majority of the genotypes recorded moderate to high level of disease incidence as also reported by Ashish and Arora (2014) in germplasm of pomegranate. The mean disease incidence was as high as 43.39 per cent (*in vivo*) and 40.56 per cent (*in vitro*) indicating the severity of disease. The results indicated Nana was found to be resistant whereas, Patna-5 and PK20-64) were tolerant and 12 genotypes were moderately susceptible under *in vivo*. Similarly, under *in vitro* condition only Nana and PG20-80 genotypes were found to be resistant and tolerant respectively whereas 17 genotypes were moderately susceptible. However, significant differences were observed among the genotypes tested under *in vitro* and *in vivo*. Majority of the genotypes are susceptible to bacterial blight as also reported by Ashish and Arora (2014); Katwal (2015) and Priya *et al.* (2016)

5.2 Evaluation of γ -ray irradiated seed derived progenies and germplasm of pomegranate (*Punica granatum* L.) for plant growth, fruit quality traits and bacterial blight disease resistance

5.2.1 Genetic variability

Genetic variability is the gift of nature and its fruitful utilization in any crop species calls for systematic collection, evaluation and grouping, based on economic descriptors. Genetic diversity estimates among genotypes are helpful in selecting parental combinations for creating segregating populations. A critical estimate and study of genetic variability is pre-requisite for initiating appropriate breeding procedure for effective selection of superior genotypes. Plant breeders should know thoroughly the nature and expression of different characters and range of variability present in the population of crop before adopting different breeding methods. Assessment of variability in the population therefore becomes the basic prerequisite for any breeding programme. In the present investigation, γ -ray irradiated seed derived progenies and germplasm lines of pomegranate exhibited a wide range of variation for almost all the

characters under the study. Earlier workers (Prasad and Bankar, 2000; Sarkhosh *et al.*, 2009 and Singh *et al.*, 2010) found a wide range of variability for almost all the characters studied.

The analysis of variance showed that the differences among the genotypes were highly significant for all the characters under study except leaf thickness, flower length, flower breadth and number of arils per fruit. The range of variation was wide for plant growth characters like days to maturity, plant spread, flower length, flower breadth, fruit weight, total aril weight, total number of arils, 100 aril weight, aril percentage, juice percentage, TSS, acidity, maturity index, phenolic content, anthocyanin content, rind weight and bacterial blight disease incidence. Meena *et al.* (2003) recorded highly significant variance for characters such as fruit weight, weight of 100 arils, TSS and acidity. Earlier studies conducted by Prasad and Bankar (2000), Sarkhosh *et al.* (2009), Singh *et al.* (2010) found wide range of variability for the characters studied.

The number of days required for maturity varied with significant range from 74 days in PG10-50 to 209 days in PG25-38. Singh *et al.* (2004) observed the earliest fruit set and fruit maturity in Ganesh and G-137 respectively. Plant height and plant spread is the important trait in determining the plant architecture suggesting its importance in high density planting, mechanical and easy harvesting in particular growing conditions (Levin, 2006). The plant height ranged from 63.02 to 228.17 cm, while plant spread ranged from 54.29 to 161.32. Good amount of variation for these characters was also found by Balamohan *et al.* (2001); Sharma and Dhillon (1996); Mir *et al.* (2007a) and Mir *et al.* (2010)

The fruit length ranged from 4.35 to 6.62cm, while fruit diameter was from 0.9-3.04 cm. Proportion of fruit length and diameter determines the shape of fruit rendering it the attractiveness. Karale and Desai (1998) found non additive gene action for these characters viz., length and diameter of fruit. Thus, suggesting that improvement in these characters through selection will be relatively low. Good amount of variation for these characters was also found by Josan *et al.* (1979); Karale (1997); Karale and Desai (1998); Mars and Marrakchi (1999); Prasad and Bankar (2000); Padmavathamma and Hulmani (2007); Celik and Erasl (2009); Singh *et al.* (2010); Wani *et al.* (2012);

Chandra *et al.* (2013); Caliskan and Bayazit (2013); Faten and Messaoud (2014); Kumar and Rana (2014) and Khadivi-Khub *et al.* (2015).

The character fruit weight ranged from 23.19 to 235.86 g and total seed weight ranged from 99.4 to 301.8 g. Viswanath (1999) observed that fruit weight ranged from 141.5 to 543 g. Wide range of variation for these characters was also reported by Manohar *et al.* (1981); Bist *et al.* (1994); Karale, 1997; Pandey and Bist (1998); Jalikop and Kumar (1998); Nageswari *et al.* (1999); Prasad and Bankar (2000); Karale and Desai (2000); Singh and Meena (2003); Meena *et al.* (2003); Singh *et al.* (2004); Aulakh (2004); Dhillon and Kumar (2004); Sharma and Bist (2005); Samadia and Pareek (2006a); Jalikop *et al.* (2005); Samadia and Pareek (2006b); Padmavathamma and Hulimani (2007); Durgac *et al.* (2008); Islam *et al.* (2009); Rao and Subramanyam (2009); Singh *et al.* (2010); Chandra *et al.* (2013); Wani *et al.* (2012); Caliskan and Bayazit (2013); Faten and Messaoud (2014); Kumar and Rana (2014) and Khadivi-Khub *et al.* (2015).

The highest and lowest values for number of arils per fruit and 100 aril weight were observed in PK25-71 (585) and PK10-107 (115) and for PK20-90 (34.03g) and PK20-233 (8.17g) respectively. Jalikop and Kumar (1998) found highly significant differences for 100 aril weight, total aril weight, fruit weight, rind thickness, TSS and acidity. While total seed weight ranged from 99.4 to 301.8 g. Large amount of variation was observed for size of arils. Similar findings were also reported by Bist *et al.* (1994); Karale (1997); Sharma and Bist (2005) and Singh *et al.* (2010); Mundhe (2011); Chandra *et al.* (2013); Kumar and Rana (2014); Khadivi-Khub *et al.* (2015). This study reveals that there was maximum range discrimination in the characters fruit weight and number of arils per fruit.

Rind thickness is important character as it affects the keeping quality as well as the aril content of the fruit. Rind thickness ranged from 1.63 mm in Nana to 4.94 mm in PK10-216. Similar differences for rind thickness were also observed by Karale (1997); Jalikop and Kumar (1998); Karale and Desai (2000); Singh and Meena (2003); Islam *et al.* (2009); Singh *et al.* (2010); Wani *et al.* (2012); Chandra *et al.* (2013); Caliskan and Bayazit (2013); Faten and Messaoud (2014); Kumar and Rana (2014) and Khadivi-Khub *et al.* (2015).

Total soluble solids (TSS) ranged from 11.03 in PK10-133 to 17.16 in PK15-41. Choudhari and Shirsath (1976) noted that content of total soluble solids (TSS) was related to fruit colour and shape, red-coloured fruits and those with red markings and ridged appearance had the highest TSS content. However, fruits with the highest content of TSS (180 °Brix) had hard seeds. Singh (2004) recorded the highest total soluble solids (19.7 °Brix) in variety Kandhari. Wide range of variation for TSS was also reported by Malhotra *et al.* (1983a); Sharma and Sharma (1990); Karale (1997); Jalikop and Kumar (1998); Nageswari *et al.* (1999); Prasad and Bankar (2000); Singh and Meena (2003); Meena *et al.* (2003); Singh *et al.* (2004); Aulakh (2004); Singh (2004); Jalikop *et al.* (2005); Mirdehghan and Rahemi (2006); Padmavathamma and Hulemani (2007); Mir *et al.* (2007a); Singh *et al.* (2010); Wani *et al.* (2012); Chandra *et al.* (2013); Caliskan and Bayazit (2013); Faten and Messaoud (2014); Kumar and Rana (2014) and Khadivi-Khub *et al.* (2015).

The acidity ranged from 0.34 in (PK10-119, PK20-233) to 4.22 (Nana). Manohar *et al.* (1981); Malhotra *et al.* (1983a), Jalikop and Kumar (1998); Prasad Bankar (2000); Sharma and Dhillon (2002); Meena *et al.* (2003); Singh *et al.* (2004); Aulakh (2004); Singh (2004); Jalikop *et al.* (2005); Sharma and Bist (2005); Mirdehghan and Rahemi (2006); Padmavathamma and Hulemani (2007); Mir *et al.* (2007b); Singh *et al.* (2010); Wani *et al.* (2012); Chandra *et al.* (2013); Caliskan and Bayazit (2013); Faten and Messaoud (2014); Kumar and Rana (2014) and Khadivi-Khub *et al.* (2015) also found a wide range of variability for this character.

The range for maturity index was from 11.9 in Nana to 43.76 in PG20-96. Sharma *et al.* (2005) also recorded highest maturity index in G-137. Wide range of variability for this character was also recorded by Viswanath *et al.* (1999); Padmavathamma and Humani (2007); Durgac *et al.* (1999); Mansour *et al.* (2011); Mundhe (2011); Chandra *et al.* (2013); Caliskan and Bayazit (2013) and Raina (2013).

The pH values ranged from 2.86 in Nana to 3.83 in PK15-41, Similar results were observed by Mirdehghan and Rahemi (2006); Singh *et al.* (2004); Meena *et al.* (2004); Sharma and Bist (2005); Munde (2011); Caliskan and Bayazit (2013); Raina (2013); Faten and Messaoud (2014) and Khadivi-Khub *et al.* (2015)

The phenolic content ranged from 18.75 to 1938.59 mg, while anthocyanin from 0.17 to 679.97 mg. The phenolic compounds have many biological and functional activities for fruit quality and human health. They are highly varied in species and cultivars (Tomas Barberan and Espin, 2001). Whereas the anthocyanin is one of the most important quality factors of the pomegranate and the colour of pomegranate is attributed to these compounds (Zaouay *et al.*, 2012)

The seed length ranged from 0.52 to 0.76 cm, while fruit diameter was from 0.21-0.30 cm. Proportion of fruit length and diameter determines the shape. Good amount of variation for these characters was also found by Martinez *et al.* (2007).

The bacterial blight on leaves, stem and fruit is ranging from 33.33 to 46.38 PDI, 15.31 to 72.66 and 18.22 to 81.96, respectively; while disease severity on tree is ranging from 23.33 to 70.02. The lowest disease severity was observed in the PK20-38 (23.33 PDI) followed by PK10-90, PK10-201, PK10-204, PK15-12 and PK25-18.

The total variability in each of these characters could be partitioned in to three components viz., phenotypic, genotypic and environmental. This helps to determine heritable and non heritable portion of variation in respect of characters under study. The characters days to maturity, fruit weight, total aril weight, number of arils per fruit, 100 aril weight, volume of juice, acidity, phenolic content, anthocyanin, rind weight and bacterial blight on leaves showed maximum variability at phenotypic and genotypic levels.

The phenotypic and genotypic coefficient of variation measures the extent of variation present in the population for particular character. The phenotypic coefficient of variation (PCV) was high for phenolic content (105.09), Anthocyanin (125.59), acidity (38.04), fruit weight (33.31), rind weight (41.58), total aril weight (31.82), number of arils per fruit (26.70), 100 aril weight (22.32), bacterial blight on fruit (22.59), while it was low for seed length (4.41).

The genotypic coefficient of variation (GCV) aids in assessing genetic variability for various characters and enables to compare the magnitude of variation. The highest magnitude of genotypic coefficient of variation was noticed for the characters viz., phenolic content (102.17), anthocyanin (116.38), acidity (35.89), total aril weight (20.90), rind weight (22.96), days to maturity (19.45), fruit weight (19.36),

volume of juice (19.46) and bacterial blight on fruit (19.89), while it was low for pH of the juice. Manohar *et al.* (1981) reported that arils per fruit, rind weight, fruit weight and acidity showed high GCV. Bist *et al.* (1994) recorded maximum coefficient of variation for aril size followed by fruit weight. Meena *et al.* (2003) recorded significantly higher genotypic coefficient of variation for characters like fruit weight, seed content and weight of 100 arils, juice acidity and weight of 100 seeds.

Both phenotypic and genotypic coefficients of variation were high for the characters phenolic content, anthocyanin, days to maturity, acidity, maturity index, fruit weight, total aril weight, juice volume, number of arils per fruit, 100 aril weight, rind weight, rind thickness and bacterial blight disease on leaves indicating that phenotypic variability is a reliable measure of genotypic variability. Mundhe (2011) recorded higher GCV and PCV for acidity, TSS/acidity ratio (maturity index), rind thickness and weight of arils per fruit, number of arils per fruit, fruit volume and fruit weight. Faten and Messaoud (2014) recorded moderate GCV and PCV for rind weight, skin colour and higher values for acidity and juice colour.

5.2.2 Heritability, genetic advance and GAM

The magnitude of heritable variation is of utmost importance as it has relevance to selection response. Heritability refers to the extent of variability for a quantitative character that is transmitted to its progeny. However, the genetic gain from selection for a particular character is the function of its heritability, selection differential and phenotypic variance in the basal population (Burton and Devane, 1953). Characters with high heritability reveal that breeder can select a genotype on its own phenotypic performance. Characters having medium heritability indicate that these were influenced by environmental conditions and cannot be selected based on phenotypic values alone. The characters exhibited low to moderate heritability accompanied by low genetic gain indicate predominantly non additive gene action for these characters. Thus improvement in these characters through selection will be relatively low. The characters showing low heritability would need further selection pressure. Higher magnitude of non-additive gene action revealed the need to go for developing new hybrids using superior segregates from bi-parental progenies.

The characters exhibited high estimates of heritability were days to maturity (97.97), rind thickness (87.85), acidity (89.01), phenolic content (94.52), anthocyanins (85.86), seed width (87.18), bacterial blight disease on stem (86.06) and disease severity on tree (81.32), plant height (67.93), plant spread (70.03), juice percentage (62.54), volume of juice (72.54), rind percentage (68.21) and bacterial blight disease on fruit. The characters exhibited moderate estimates of heritability were pistil length (37.43), fruit height (52.88), fruit diameter (47.33), fruit weight (33.79) and calyx length (48.46). The low estimate of heritability was noticed in leaf breadth (22.07), flower length (10.89), flower diameter (17.30), number of arils per fruit (15.67), juice pH (29.56), maturity index (27.50) and seed length (14.13).

Karale and Desai (1998) reported that all the characters were not equally heritable (*viz.*, 32.80 % for TSS:acid ratio to 96.74 % for seed mellowness). High heritability (more than 80 %) was observed for the characters fruit weight, fruit diameter, fruit volume, number of seeds per fruit, aril weight, number of arils per fruit, TSS and acidity by Mir *et al.* (2010) and Mundhe (2011). Similarly Faten and Messaoud (2014) observed high heritability for the characters *viz.*, fruit weight, fruit height, fruit diameter, juice pH and least for rind thickness

The heritability estimates provide the information on the magnitude of inheritance of quantitative characters but it does not indicate the magnitude of genetic gain obtained by selection of best individual from the best population. So heritability along with genetic advance is more useful for selection than the heritability alone. In the present investigation, the highest genetic advance was observed for phenolic content (568.35) followed by anthocyanin (159.22), days to maturity (55.14), plant height (30.67), plant spread (24.54), fruit weight (24.96), number of arils per fruit (25.62), aril weight (15.71), rind weight (13.99), bacterial blight on fruit (17.54), bacterial blight on stem (15.4) and disease severity (13.36).

Manohar *et al.* (1981) reported that arils per fruit, rind weight, fruit weight and acidity showed high heritability and genetic advance. Meena *et al.* (2003) recorded high heritability, significantly higher genotypic coefficient of variation, heritability and genetic advance for characters like fruit weight and 100 aril weight. Thus, these traits

may be effectively used as selection criterion for screening potential genotypes in a breeding programme. Meena *et al.* (2004) also recorded high heritability in association with high genetic advance for maturity duration and number of fruits per plant, indicating additive gene effects and emphasizing the effectiveness of selection for these traits to improve economic yield in pomegranate. Samadia and Pareek (2006a) observed that characters viz., fruit weight, plant spread and plant height were observed to be highly heritable traits which also showed large magnitude of genetic advance. Similar results were also observed by Faten and Messaoud (2014). Thus, these characters should respond favourably to simple selection procedure in cultivar improvement attempts.

The overall segregation pattern observed in the study done by Jalikop *et al.* (2005) and Faten and Messaoud (2014) revealed that high variability existed for fruit yield contributing traits, offering opportunity for improvement through selection. Individual plant selection for yield, aril percentage, fruit weight and number of arils would be satisfactorily effective in pomegranate as these exhibited high heritability as well as high genetic advance.

The genetic advance expressed in percentage of mean (GAM) ranged from 0 for leaf thickness to 222.16 for anthocyanin content. In the present study, high heritability was associated with high genetic advance for majority of the characters. High heritability coupled with high genetic advances have also been reported by Navjot *et al.* (2009). Panse and Sukhatme (1997) emphasized that if character is governed by additive gene action, both heritability and genetic advances would be high. Some of the characters exhibited comparatively lower heritability accompanied by low genetic advance, indicating the dominance or epistatic effects are of considerable value for these characters and hence little improvement in these characters is possible through selection.

In general the characters days to maturity, plant spread, plant height, juice percentage, rind thickness, rind percentage, acidity, phenolic content, anthocyanin content, seed width, bacterial blight disease on leaves, stem and disease severity on tree had higher heritability as well as high genetic advance indicating an additive nature of genes and that there is chances of improvement in these characters through selection.

Fruit colour at maturity and aril colour are important characters, which are highly visible for characterization of genotypes. For both these characters high variability has been observed. For fruit colour, the highest frequency was for red followed by dull red and yellow. In general red colour arils are accepted by consumers and creamy white are not preferred. It is necessary to have red fruit and aril colour for the acceptance from consumers. The variation in fruit and aril was also observed by Chandra *et al.* (2013) and Sonawane (2015).

5.2.3 Identification of superior genotypes for fruit quality traits and bacterial blight disease resistance

The genotypes exhibiting high index score for fruit quality traits and bacterial blight disease resistance were different. Out of 20 numerically superior genotypes over the best check Bhagwa, only 11 genotypes (PK10-190, PK15-41, PG10-37, PK10-59, PK20-47, PG25-37, PK15-40, PK10-2, PK15-48, PK15-85 and PK20-64) showed superiority for selection index score (Chandra *et al.*, 2013). All the genotype had high disease severity (>50 %) except PK15-41 (33.35 %), PK10-59 (32.43 %) and PK15-48 (30.16). Therefore, these genotypes can be taken up for large scale trials.

Among the superior genotypes for disease severity majority of them were low selection index score for fruit quality traits as compared to check variety Bhagwa. The genotypes PK20-38, PK10-90 and PK10-204 were moderately susceptible to bacterial blight disease, while PK15-41 and PK10-59 were having high selection index score. These genotypes are to be tested over years and locations to confirm their superiority.

5.3 Correlation studies in *g*-ray irradiated seed derived progenies and germplasm of pomegranate (*Punica granatum* L.) germplasm lines for plant growth, fruit quality traits and bacterial blight disease resistance

Correlation between different characters of plant could arise because of linkage, pleiotropy or developmentally influenced functional relationships. Correlation studies provide information on the nature and extent of association between any two characters. From this, it would be possible to bring about genetic up gradation in one character by the selection of the other pair. The correlation coefficient, measures the relationship existing between pairs of characters.

For a rational approach towards the improvement of fruit yield, selection has to be made for the component of yield and resistance to various stresses. This is determined by the interaction of a number of characters among themselves together with environment and hence, has a highly variable expression. So the knowledge of association of various characters helps in determining relative importance of the yield components to be considered to improve yield. A critical review of literature of the correlation pattern in any crop species particularly in pomegranate indicates that the association analysis has been reported mostly on morphological traits. But with the recent interest on disease resistance, it is all the more desirable to elicit the information of their association with fruit quality traits in order to have more clear picture on the fruit quality and consumer preference. In the present study, a number of morphological, biochemical and disease reaction were investigated and their relationship with disease severity as well as among themselves was examined using Pearson correlation analysis. The character fruit weight was found to have significant positive correlation with plant height, plant spread, leaf length, leaf breadth, fruit length, fruit diameter, total aril weight, number of arils per fruit, volume of juice, rind thickness, 100 aril weight, rind weight, single aril weight, TSS, 100 dry seed weight, bacterial blight disease on stem, fruit and disease severity on tree, while significant positive correlation was revealed by leaf thickness, per cent of juice per fruit, rind proportion and seed length. Significant negative correlation with fruit weight was observed by days to maturity, acidity, phenolic content and anthocyanin content. This result is in harmony with earlier report by Mir *et al.* (2007a) and Faten and Messaoud (2014), where they found a significant positive correlation between fruit weight and fruit length, fruit diameter, rind weight, rind thickness and juice content.

Positive and significant association was observed between majority of the characters studied. Desai *et al.* (1992) found that most of the undesirable fruit characters had positive association amongst themselves and negative with desirable characters. Desai *et al.* (1994) noted that the desired characteristics of bigger fruits, more number of arils, juice, soluble solids and vitamin C content, large arils, coloured arils and soft seeds were favourably associated with each other.

Pandey and Bist (1998) observed that fruit weight was positively associated with fruit length and fruit diameter. Viswanath *et al.* (1999) found a positive significant association between total seed weight and fruit weight, while a negative association existed between total seed weight, acidity and maturity index. Karale and Desai (2000) observed TSS content in the juice was positively and significantly associated with acidity.

Samadia and Pareek (2006b) reported that fruit weight, diameter and length were positively and significantly correlated with fruit yield per plant. Fruit weight had highly significant and positive correlation with fruit diameter, fruit length and weight of 100 arils. Rind weight and thickness had significantly positive correlation with fruit weight, fruit length, fruit diameter, total aril weight, number of arils per fruit and 100 dry seed weight. These findings are in agreement with the results of Zamani *et al.* (2013) and Karimi and Mirdehghan (2013).

Acidity was found to be significantly negatively correlated with majority of the characters studied, which is in agreement with findings of Khadivi-Khub *et al.* (2015). Zamani *et al.* (2013) reported titratable acidity (TA) negatively correlated with TSS and pH and similar reports are available from the investigation of Zamani *et al.* (2007).

The disease severity on tree showed positive and significant correlation for the majority of the character studied except for days to maturity, calyx length, rind percentage, acidity and phenolic content. Acidity is significantly and negatively associated with the bacterial blight disease on leaves, stem and fruit indicating that genotypes having more acidity will show less incidence of the bacterial blight. Jalikop (2010) reported that the varieties nana and daru were resistant to bacterial blight disease as these are more acidic in nature.

Hence, selection of genotypes showing maximum values for the characters fruit length, fruit diameter, number arils per fruit, aril weight, rind weight and bacterial blight on fruit would be effective to obtain higher quality yields. Mir *et al.* (2007b) noted that the number of fruits per plant and fruit weight could form selection criteria for yield improvement in pomegranate.

5.4 Genetic and molecular diversity studies in γ -ray irradiated seed derived progenies and germplasm of pomegranate (*Punica granatum* L.) germplasm lines for plant growth, fruit quality traits and bacterial blight disease resistance

5.4.1 Genetic diversity studies for plant growth, fruit quality and bacterial blight disease and its component traits

Genetic relationship among genotypes can be measured by similarity or dissimilarity of any number of quantitative characters assuming that the difference between characters of genotypes reflects the divergence of genotypes. The amount of diversity available in the crop decides the success of any crop improvement programme with manifold objectives. Assemblage and assessment of divergence in the germplasm is essential to know the spectrum of diversity. In the present investigation, 475 genotypes of pomegranate were considered for assessment of genetic diversity by adopting Mahalanobis (1936) concept of generalized distance (D^2).

5.4.1.1 Clustering of D^2 values

Based on D^2 values, 475 γ -irradiated seed derived progenies and germplasm lines were grouped into 23 clusters indicating high amount of diversity among the germplasm lines in this study. Cluster II was the largest with 172 genotypes followed by cluster I (162), VII (36), V (34), VIII (24), IV (9) and XII (9). Clusters XIV, XVIII and XXIII included 8, 5 and 3 genotypes respectively and remaining cluster having one genotype each. Raina (2013) grouped 30 pomegranate genotypes into eight clusters based on plant morphological, yield and yield contributing characters. Mundhe (2011) reported four clusters from 20 genotypes using morphological and fruit quality traits and Caliskan and Bayazit (2013) grouped 76 pomegranate genotypes in to four clusters. These authors have divided few genotypes into many groups; this may be due to the presence of higher genetic diversity. Mansour *et al.* (2011) and Karimi and Mirdeghan (2013) reported three clusters from 21 and 8 genotypes respectively based on morphological and biochemical characters.

Zamani *et al.* (2013) reported two clusters from 50 pomegranate genotypes, the studied genotypes have shown morphological differences which could be attributed to several factors including differences in geographical origin, ecological adaptation to sites and nature. Cluster analysis revealed a considerable variability that may be due

mainly to recombination (resulting from out-crossing) combined with sexual propagation for long term and controlled spread of plant material (Mars, 1996). Pomegranate is known to be at least partially cross pollinated (Jalilop and Kumar, 1990).

5.4.1.2 Contribution of different traits towards divergence

Among the traits studied, phenolic content ranked first and contributed 61.55 per cent to the total divergence followed by anthocyanin content (17.51 %), number of arils per fruit (16.84 %), fruit weight (2.12 %). The lowest contribution was from plant spread (0.06 %). Presence of higher contribution to genetic diversity by phenolic content indicates higher genetic diversity existed among these germplasm lines. These lines have higher potential use in breeding for plant ideotype.

5.4.1.3 Cluster mean and range of different groups

It is possible to identify clusters having higher diversity for more number of characters and it also helps to identify clusters having less diversity for more number of characters based on analysis of cluster means. Utilization of high and low clusters in breeding are expected to yield desirable lines in advanced generation of selection.

In the present study, it was observed that only three germplasm lines grouped under cluster XXIII were superior for phenolic content and calyx length at desirable direction followed by germplasm lines under cluster XXII for three traits (number of arils per fruit, leaf breadth and pistil length). Genotypes grouped under cluster XXI, was characterized with juice percentage. Cluster XX was having only one genotype showed highest mean value for flower length, rind thickness and bacterial blight disease on leaves.

5.4.2 Principal Component Analysis (PCA)

In the characterization of genotypes, it is a paramount to evaluate the traits which show variation in the population in order to identify the traits of interests and variations therein. This becomes complex while dealing with a large number of traits at a time. In general, the yield attributing traits as well as traits with bearing on the quality

of produce exhibit polygenic or complex inheritance and are highly influenced by the environmental conditions. Under such circumstances, Principal Component Analysis (PCA) is one of the most efficient approaches to find out the variations exhibited by different traits.

Principal components analysis (PCA) is a method that reduces data dimensionality by performing covariance analysis between factors. As such, it is suitable for data sets in multiple dimensions to identify the patterns and expressing the data highlighting their similarities and differences (Winterova *et al.*, 2008). It has been used to establish the relationships among genotypes (cultivars) and to study the correlations between morphological, fruit physical and chemical traits within sets of genotypes (Badenes *et al.*, 1998; Gurrieri *et al.*, 2001; Azodanlou *et al.*, 2003; Ruiz and Egea, 2008). Further, PCA had been used to evaluate germplasm of different species viz., olive (Cantini *et al.*, 1999), pomegranate (Mars and Marrakchi, 1999; Badenes *et al.*, 2000 and Khadivi-Khub *et al.*, 2015).

In the present study a total of 37 traits were subjected to principal components analysis (PCA). The results indicated that the total variability is being explained by 37 principal components, where first seven Principal components with eigen values greater than one explained highest variation with 52.66 per cent of the total variability observed. Variations in most of the studied characters like plant height, plant spread, leaf length, number of arils per fruit, total aril weight, rind thickness, fruit length, fruit diameter, fruit weight, volume of juice, 100 aril weight, single aril weight, rind weight, bacterial blight on disease on fruit, stem and disease severity on tree was explained by principal component1 (PC1), which accounted for 19.22 per cent of the total variation.

The results obtained in the present studies are in accordance to those of Noormohammadi *et al.* (2012), who revealed that PCA ordination based on two first components (factors) confirmed cluster analysis using combined data when eigen value for first and second components were 20.88 per cent and 9.45 per cent respectively in Iranian pomegranate germplasm. Yilmaz *et al.* (2012) revealed that eigen values of the first 3 components were able to represent 32.67 per cent of total variance in PCA and further, eigen value of pomological PCA analysis represented 73 per cent of total

variance. Zamani *et al.* (2013) reported that eigen values of the first three components represented 61.64 per cent of total variability. Rakonjac *et al.* (2010) reported that morphological characterization of sour cherry genotypes with high PC1 scores could be good genitors for large fruit size and yield potential. On the other hand, if genotypes with higher PC2 scores are used as genitors, a later flowering time could be achieved. PC analysis helped to select a set of genotypes with better fruit quality performances (Mratinic *et al.*, 2011; Drogoudi *et al.*, 2005 and Durgac *et al.*, 2008). According to Zamani *et al.* (2007), fruit characteristics in pomegranate had the highest loading values for the first component analysis. The results obtained in this study along with the earlier reports indicate that fruit traits could be successfully used for the study of pomegranate genotypes as they exhibit highest variation.

5.4.3 Molecular diversity analysis using SSR markers

Assessment of genetic diversity is important in germplasm characterization and conservation. A wide genetic diversity is of great importance for the development of improved varieties. The results derived from analyses of genetic diversity could be used for designing effective breeding programs to broaden the genetic base of commercially grown pomegranate varieties.

In the present study, a set of 125 SSR primer pairs were employed to assess the genetic diversity among the 188 genotypes. *Punica granatum* accessions selected from the total plant material were representing all the 23 clusters obtained by Mahalanobis's D^2 analysis. The selection of such a 188 diverse subset of evaluated germplasm for SSR diversity analysis ensured the informative allelic data. The markers were selected from NCBI website (<http://www.ncbi.nlm.gov/genbank/>) and the published literature. Genetic diversity studies of pomegranate have been carried out by several groups in India, Turkey, Morocco, Iran, Tunisia, Spain, Italy and China who used various molecular marker tools (Yuan *et al.*, 2007; Ebrahimi *et al.*, 2010; Narzary *et al.*, 2010; Sarkhosh *et al.*, 2011; Soriano *et al.*, 2011; Ercisli *et al.*, 2011a; Ercisli *et al.*, 2011b; Ferrara *et al.*, 2011; Noormohammadi *et al.*, 2012; Hasnaoui *et al.*, 2012; Zhang *et al.*, 2012; Caliskan and Bayazit 2013; Zhao *et al.*, 2013 and Singh *et al.*, 2015).

The data of 125 SSR markers were used for identification and evaluation of genetic diversity in 188 pomegranate genotypes. A total of 268 alleles were detected by 125 primers in the 188 genotypes with an average of 2.14 alleles per primer. However, Jian *et al.* (2012) developed 18 markers that resulted in amplification in 42 pomegranate accessions, with an average of 2-5 alleles (mean = 2.80) per locus. The 9 SSR loci provided 22 alleles, with the allele numbers per locus ranging from 1 to 5 among 34 accessions of *Punica granatum* (Curro *et al.*, 2010). One hundred and twenty five SSR primers revealed clear and consistent amplification profile. Among these 125 markers, 78 SSR markers amplified 268 alleles across 188 genotypes. Only 47 markers showed monomorphic pattern, revealing single allele across all the 188 genotypes. (Koohi *et al.*, 2005; Koohi-Dehkordi *et al.*, 2006 and Curro *et al.*, 2010). Out of 78 polymorphic SSR loci, 50 (PGCT009, PGCT030, EPS07, ABRII-MP07, PGCT090, EPS04, PGCT025, PGCT057, ABRII-MP28, ABRII-MP30, PGCGT097, PGCT022, ABRII-MP04, pg22, PGCT014, EPS06, pg23, ABRII-MP46, pg20, PGCT110, pg21, PGCT102, PGCT065, PGCT111, PGCT112, PGCT061, PGCT085, PGCT047, PGCT105, PGCT067, PGCT089b, PGCT005, PGCT109, PGCT015, pg2, PGCT099, pg8, pg14a, pg14b, pg14c, POM_AAC14, PGCT037A, PGCT094, PGCT051, PGCT066b, PGCT032, PGCT004A, PGCT004B, PGCT093B and PGCT068) showed two alleles each, 10 primers (ABRII-MP12, EPS03, PPGCT058, POM_AAC1, POM_AAC7, PGCT021, PGCT060, PGCT026 and PGCT050) amplified three alleles each, 8 primers (ABRII-MP39, PG13, PGCT043, POM_AAC13, PGCT088, PGCT098b, PGCT001 and PGCT075b) amplified four alleles, 5 primers (PGCT070, POM_AGC11, ABRII-MP51, EPS10 and PGCT010) amplified five alleles, three markers (ABRII-MP42, EPS05 and POM_AAC2) amplified six alleles for pomegranate genotypes. High number of alleles 7 and 9 obtained in markers like EPS08 and PGCT008 respectively. The variation in the number of alleles produced by SSR markers demonstrates heterozygosity in different alleles at a given locus in which the heterozygosity could reflect greatly the state of genetic variability, suggests their suitability for screening large number of genotypes (Elfalleh *et al.*, 2008).

Per cent heterozygosity ranged from 0 to 99.46 per cent, highest for markers PGCT098b (99 %) and POM_AAC2 (98 %), showing high heterozygosity of this locus, could be due to its clonally propagated as well as cross pollinated nature. The highest

allelic diversity was shown by the primer PGCT008 among the primers showing polymorphic bands. The gene diversity in the present investigation is low with a mean gene diversity of 0.19. Among the SSR primers giving polymorphic, allele frequency ranged from 0.26 for the SSR primer PGCT008 to 1 for majority of the primers with an average of 0.85 across all the SSR primers. In a population, allele frequencies are a reflection of genetic diversity. In population genetics, allele frequencies are used to depict the amount of genetic diversity at the individual population or species level. Changes in allelic frequencies over time can indicate that genetic drift is occurring or that new mutations have been introduced in to the population.

Another measure of diversity among the population is the extent of polymorphic information content (PIC) value of markers. In the present study, the average PIC for all SSR primers was 0.16 suggesting existence of genetic diversity among pomegranate accessions. Soriano *et al.* (2011) reported the polymorphism information content (PIC) value across all loci range between 0.09 and 0.71, with an average of 0.37. Thus, the higher number of alleles amplified cannot be considered for higher PIC values (Zamani *et al.*, 2007). The PIC values of a primer vary with the crop and the set of the genotypes used. Lower PIC value may be the result of closely related genotypes and higher PIC values may be the result of diverse genotypes. Marker loci with an average number of alleles running at equal frequencies will have the highest PIC values (Senior *et al.*, 1998). In the present study, the probable reason for lower PIC value may be the closely related genotypes.

The cluster analysis was done using Squared Euclidean Distance (SED) for 188 pomegranate genotypes with SSR data. A dendrogram (Fig. 11) was constructed by UPGMA method. The genetic distance values in the distance matrix ranged from 0.012 to 0.519, suggesting a moderate genetic variation within pomegranate genotypes. The highest genetic distance of 0.519 observed between genotype PG20-9 and Nana genotype and least genetic distance of 0.09 was observed between genotype PK10-60 and genotype PK10-2. Three clusters were formed based on the molecular data. Using SSR markers, Jian *et al.* (2012) reported similarity coefficient values ranged from 0.706 to 0.975 with a mean of 0.842. Similarly, Durgac *et al.* (2008) described genetic similarity ranged from 0.83 to 1.0 among six pomegranate cultivar. Raina *et al.* (2013) reported similarity coefficient values ranged from 0.78 to 0.95.

The observed pattern of genetic variation was able to distinguish the pomegranate accessions based on different perspective. Cluster I was found to be constituted by Ganesh derived progenies and germplasm and cluster II indicated Bhagwa derived progenies and germplasm and distinct cluster III with only three genotypes (PK10-5, PK25-134 and PG20-72). Thus grouping of the genotypes in different clusters showed considerable variation in pomegranate germplasm and proved that SSR markers an excellent genetic marker system for pedigree analysis. Hence, SSR markers provide accurate genetic information for allelic profile of genotypes in pomegranate germplasm. Diverse genotypes placed in cluster irrespective of their origin with other genotypes showed that geographical diversity of the genotypes is not corroborating with the genetic diversity and pomegranate plants are independent of their geographical affiliations. The lack of correlation or correspondence between geographical origin of the genotypes and their genetic characters seems to be a features for some genotypes in pomegranate germplasm, Nazary *et al.* (2009). A significant result showed that the different pomegranate genotypes collected do not show high similarities amongst provinces, because these may be related to each other by descent. Within species genetic exchange rather than past relationship has been emphasized as the determinant of genetic diversity or genetic structure.

The present study found in concord with Mars and Marrakchi (1999) revealed that the geographical origin of the cultivars did not determine their clustering on the basis of morphological characters. Similarly, in the observations in Tunisian genotypes based on AFLP profiles (Jbir *et al.*, 2008) reported the clustering of the genotypes independent of their geographical origins and had further assumed a continuous distribution of diversity in the region. Moslemi *et al.* (2010) also not found significant differences between Iranian pomegranate genotypes from Markazi, Yazd and Kerman provinces by using AFLP markers. Similar results were reported by Yuan *et al.* (2007); Raina (2013) and Singh *et al.* (2015) using SSR markers on pomegranate cultivars. The high level of genetic diversity within group (population) and low level among them may be explained by clonal propagation of pomegranate and the extensive gene flow between localities due to material exchange.

5.5 Marker trait association (MTA)/Association mapping for plant growth, fruit quality traits and bacterial blight disease resistance

In any tree improvement program, longer juvenile period, generation interval and delayed expression of traits are always been a major constraint for fruit breeder. But due to advancement in modern biotechnological research, different approaches have helped breeders in overcoming the constraints. Some of them are recombinant technology, marker assisted selection, omic studies, viz., proteomics, metabolimics and the emerging field of genomics. Linkage mapping, QTL mapping and association mapping have been done in annual crops. However, in fruit crops, these methods are emerging for qualitative and quantitative improvement of fruits traits.

Conducting association genetic studies in a well defined ecological and evolutionary context is an emerging approach in which phenotypes and environmental components are dissected to allow correlative analysis between phenotypes and genotypes at a fine scale. It can be used more precisely to determine the exact genes controlling complex traits and therefore ultimately identify mutations responsible for phenotypic differences among individuals for quantitative traits. Each functional variant (*i.e.*, mutation) is referred to as a quantitative trait nucleotide (QTN). The fundamental difference between QTL mapping and association genetics is that the former relies on genetic linkage following a generation or two of crossing, whereas the later takes advantage of population-level linkage disequilibrium (LD) between genetic markers and QTNs following many generations in a large intermating population. In association genetics, chromosomes being mapped are more highly recombined than those of QTL mapping populations due to the accumulation of recombination events over evolutionary time. This enables greater resolution of genes controlling complex traits (White *et al.*, 2007). Absence of full-sib progenies is the greatest limitation in linkage mapping of important traits in fruit trees. In such case, linkage disequilibrium (LD) or association mapping of candidate genes is the most promising approach for identifying genes from natural populations.

There are two basic approaches to association genetics-genome scan and candidate gene. In the genome scan approach, genetic markers are throughout the genome so that the entire genome can be searched for QTNs. In the candidate gene approach, genetic markers are used only within individual genes that are thought to be involved in determining the phenotype. The genome scan approach is thus more exhaustive in its search but is also more expensive to conduct (White *et al.*, 2007).

Genetic association studies have emerged as a powerful tool for dissecting the genetic contribution to complex traits, to identify inter-individual genetic variants, which show the strongest association with the phenotype of interest, either because they are causal or, more likely, statistically correlated or in linkage disequilibrium (LD) with an unobserved causal variants (Tachmazidou *et al.*, 2007).

The association mapping approach has been developed for identifying genes controlling complex traits in perennial crops like fruits and trees (Neale and Savolainen, 2004; Devey *et al.*, 2004; Brown *et al.*, 2004; Quesada *et al.*, 2010; Dillon *et al.*, 2010; Eckert *et al.*, 2012; Xu *et al.*, 2014; Yang *et al.*, 2015 and Ipek *et al.*, 2015).

What make pomegranate a suitable for association genetics studies is the higher genetic variation present, low levels of domestication, out crossing nature leading to recombination and marked phenotypic differences. This is because the informational content of an association genetics population depends on the amount of population-level LD between genetic markers and QTNs. Further, artificially constructed populations, such as breeding populations, might be expected to have much higher LD. The greater the LD, the more likely associations between genetic markers and QTNs will be detected. This is advantageous for breeding applications. However, if the goal is to exactly pinpoint the QTN, somewhat less LD would be required (White *et al.*, 2007).

Marker trait association was carried out for different growth traits, fruit quality traits and bacterial blight disease resistance for the different genotypes. Molecular markers used for association studies were 125 SSRs and the results have been presented in Table 36. Population structure was analyzed using STRUCTURE 2.3.3 and marker trait association was carried out using TASSEL 3.0 software

The results from Structural analysis revealed that there was peak at the log likelihood, $K = 2$ (at $\Delta K = 5126.08$), which suggested two different clusters; with each genotype having = 80 per cent affiliation probability in its respective cluster. Based on the different marker systems used, the population was grouped into two main clusters, leaving some genotypes as admixture. The results revealed that most of the genotypes from respective background were grouped in the same cluster. It is pertinent to say here that this population structure information is required for conducting association mapping for different traits.

Q-matrix was generated for the population. The average relationship between individuals or lines can be estimated by kinship (K) calculated either from pedigrees or a suitable number of random markers across the entire genome. A composite approach, $Q + K$, that combines information from both Q and K, has been shown to be superior to other methods (Yu *et al.*, 2006). Also, a structured association approach can partially correct the false associations by using a Q-matrix of population membership estimates (Bradbury *et al.*, 2007). Structure based clustering has been carried out in Pomegranate by Alamuti *et al.* (2012), who clustered 738 accessions into 8 main clusters with 4.61 per cent as admixture. Ophir *et al.* (2014) used 480 SNPs to identify two major groups: one composed of accessions from ICI (India, China and Iran) and second with rest of the world. However, this study could not differentiate wild from the cultivated ones, indicating SSRs might be more informative than the SNP markers for resolving population structure.

The results from TASSEL 3.0 revealed that there were many significant associations between the markers used and different traits studied. However, no marker used in the study (GLM) was associated with days to maturity, number of arils per fruit, aril per cent, juice per cent, volume of juice, rind per cent and TSS. Further no markers used in the SMA were associated with days to maturity, pistil length, rind percentage, seed length and seed width. Common markers associated in both GLM and SMA, no marker used in the study was associated with days to maturity, flower breadth, pistil length, number of arils, aril per cent, juice per cent, juice volume, single aril weight, rind per cent, TSS, pH of juice, phenolic content, seed length and seed width.

In the present study, a total of 208 (GLM) and 343 (SMA) markers were found to be associated with various economically important characters (Table 37, 38 and 39). Among the associated markers with R^2 value of 5 % or above is considered as significant threshold for the association between pairs of loci (Abdurakhmonov and Abdugarimov, 2008). In all the marker- trait association (MTA), level of association was significant with a minimum p-value of 0.002.

Common SSR loci (EPS10) linked to the bacterial blight disease severity in both GLM and SMA model, can be used further for selection of the genotypes in the breeding population. Similarly only one SSR marker (PGCT051) has linked to the nine traits viz., leaf length, leaf breadth, flower length, calyx diameter, acidity, maturity index, rind thickness and bacterial blight disease on stem and leaves. Further, three more markers viz., pg14a, pg14b and pg14c linked to major fruit contributing traits such as fruit length, fruit diameter, fruit weight, total aril weight and 100 aril weight, which can be effectively used for selection of superior genotypes over the existing cultivars. Association studies for pomegranate were conducted with local collections, Basaki *et al.* (2011) and Basaki *et al.* 2013) who associated seven SSRs with 20 traits within the Iranian collections. Singh *et al.* (2015) analyzed the association of three traits, by GLM with 44 SSR markers in 88 pomegranate germplasm. Harel *et al.* (2015) also used 76 F2 population with 347 SNP markers.

Once the genes controlling traits of interest or phenotype associated molecular markers have been identified, direct selection for desired genotypes becomes possible through molecular marker analysis. Marker assisted selection (MAS) is especially valuable for the improvement of various traits, which are complex in nature because gene–environment interactions reduce the accuracy of direct phenotype selection.

MAS can significantly improve breeding efficiency by the accurate selection of desired genotypes in early generations or at early growth stages. MAS can also reduce the impact of non genetic factors that can interfere with phenotype selection. Pomegranate has limited genomic information. The results of the study can be valuable finding for pomegranate improvement. However, confirmation has to be made through multi-locational trial in different agro-climatic zones.

Practical implications of the results

- Existing commercial cultivable varieties (Bhagwa and Ganesh) are susceptible to bacterial blight disease. In the present study 11 superior genotypes (PK10-190, PK15-41, PG10-37, PK10-59, PK20-47, PG25-37, PK15-40, PK10-2, PK15-48, PK15-85 and PK20-64) identified for fruit quality traits and disease resistance, can be evaluated in large scale for the benefit of the farming community.
- Common SSR loci (EPS10) linked to the bacterial blight disease severity in both GLM and SMA model, can be further validated to use for selection of the genotypes.
- The SSR marker (PGCT051) has linked to the nine traits viz., leaf length, leaf breadth, flower length, calyx diameter, acidity, maturity index, rind thickness and bacterial blight disease on stem and leaves. Similarly three more markers viz., pg14a, pg14b and pg14c marker trait associations to major fruit contributing traits such as fruit length, fruit diameter, fruit weight, total aril weight and 100 aril weight, which can be validated before their use for selection of superior genotypes for the associated traits.

Future line of work

Based on the information available and results of the present study following future line of work can be proposed to develop productive cultivars with resistance to pest and disease.

1. Large-scale evaluation of the superior progenies identified for bacterial blight resistance at major pomegranate growing regions to confirm their superiority.
2. Large scale evaluation in different locations of the superior progenies identified for better fruit quality traits with tolerance to bacterial blight disease.

3. New source material with large genetic diversity generated for different yield components, fruit quality traits and bacterial blight tolerance can be utilized in future breeding programme.
4. Marker trait association found for important economic traits needs to be validated for confirmation before their use in MAS.
5. Detailed biochemical analysis of bacterial blight resistance/tolerant and susceptible genotypes to understand the factors other than acidity associated with resistance need to be done for future breeding strategy.

6. SUMMARY AND CONCLUSIONS

The present study was conducted during *mrighbahar* and *Ambe bahar* 2014-2015 and 2015-1016 at Horticulture Research and Extension Station, Vijayapur (Tidagundi), University of Horticultural Sciences, Bagalkot to generate information on the pathogenic variability to identify virulent strain and finally to evaluate γ -irradiated seed derived progenies and germplasm for genetic variability, diversity and marker trait association analysis for important morphological and disease resistance in pomegranate. The major findings of the study are presented here

The infected samples of bacterial blight of pomegranate plants were collected from four districts viz., Vijayapur, Bagalkot, Koppal and Chidradurga districts of Karnataka. All diseased samples were collected for the purpose of study of variation in *Xanthomonas axonopodis* pv. *punicae* and each sample was considered as an isolate (*Xap1*, *Xap2*, *Xap3* and *Xap4*). The isolates of *X. axonopodis* pv. *punicae* were characterized based on 16s rRNA, gyrB, ERIC, BOX and Rep-PCR and confirmed that there is not much variation existing between the collected strains and virulence is on par with each other. The Vijayapur strain (*Xap1*) was used to screen the genotypes of the pomegranate for the bacterial blight on leaves both *in vivo* and *in vitro* during the *mrighbahar* season where disease severity is more.

- The experimental material consisting of 475 genotypes were planted in a augmented block design with single replications in both *mrighbahar* and *amb-e-bahar* seasons. The observations were recorded on plant growth, fruit quality traits and bacterial blight disease resistance viz., days to maturity, plant height, plant spread, leaf thickness, leaf length, leaf breadth, flower length, flower breadth, pistil length, fruit length, fruit diameter, fruit weight, calyx length, calyx diameter, total aril weight, number of arils per fruit, 100 aril weight, single aril weight, aril percentage, juice percentage, volume of juice, TSS, pH of juice, acidity, maturity index, phenolic content, anthocyanin, rind weight, rind percentage, seed length, seed width, 100 dry seed weight, bacterial blight on stem, leaves, fruit and disease severity in both seasons. The observations on bacterial blight disease incidence on leaves both *in vivo* and *in vitro* were recorded only during *mrighbahar* season.

- All the traits under study showed a wide range of variation. Analysis of variance pooled across the seasons for different traits indicated the presence of significant amount of high variability among the genotypes and genotype $\times$ season interaction for majority of the traits.
- Both phenotypic and genotypic coefficients of variation were high for the characters viz., phenolic content, anthocyanin, days to maturity, acidity, maturity index, fruit weight, total of aril weight, juice volume, number of arils per fruit, 100 aril weight, rind weight, rind thickness and bacterial blight disease spread on leaves indicating that phenotypic variability is a reliable measure of genotypic variability.
- In general the characters days to maturity, plant spread, plant height, juice percentage, rind thickness, rind percentage, acidity, phenolic content, anthocyanin content, seed width, bacterial blight disease on leaves, stem and disease severity on tree had higher heritability as well as high genetic advance indicating an additive nature of genes and that there is chances of improvement in these characters through selection. Frequency distribution based on the mean data of all the genotypes showed a normal distribution pattern for majority of the traits except for phenolic content, anthocyanin content indicating polygenic inheritance of these traits.
- Out of 20 numerically superior genotypes over the best check Bhagwa, only 11 genotypes showed superiority for selection index score. The genotypes PK20-38, PK10-90 and PK10-204 were moderately susceptible to bacterial blight disease, while PK15-41 and PK10-59 were having high selection index score. These genotypes are to be tested over years and locations to confirm their superiority.
- Positive and significant association was observed between majority of the characters studied, where as acidity was found to be significantly negatively correlated with majority of the characters studied indicating that genotypes having more acidity will show less incidence of the bacterial blight.
- The PCA analysis found to reduce the data of 37 traits into six most significant variables or components, which showed maximum and stable variability among all other characters under study. The results indicated that the total variability is being explained by 37 principal components, where first seven principal components with

eigen values greater than one explained highest variation with 52.66 per cent of the total variability observed. Variations in most of the studied characters like plant height, plant spread, leaf length, number of arils per fruit, total aril weight, rind thickness, fruit length, fruit diameter, fruit weight, volume of juice, 100 aril weight, single aril weight, rind weight, bacterial blight disease on fruit, stem and disease severity on tree was explained by first principal component (PC1), which accounted for 19.22 per cent of the total variation.

- The data of 125 SSR markers were used for identification and evaluation of genetic diversity in 188 pomegranate genotypes. A total of 268 alleles were detected by 125 primers in the 188 genotypes with an average of 2.14 alleles per primer.
- The cluster analysis was done using Squared Euclidean distance (SED) for 188 pomegranate genotypes with SSR data. The dendrogram constructed by UPGMA method. The genetic distance values in the distance matrix ranged from 0.012 to 0.519 suggesting a moderate genetic variation within pomegranate genotypes. The highest genetic distance of 0.519 observed between genotype PG20-9 and Nana genotype and least genetic distance of 0.09 was observed between genotype PK10-60 and genotype PK10-2.
- Population structure was analyzed using STRUCTURE 2.3.3 software and marker trait association was carried out using TASSEL 3.0 software. The results from Structural analysis revealed that there was peak at the log likelihood, $K = 2$ (at $\Delta K = 5126.08$), which suggested two different clusters with each genotype having = 80 per cent affiliation probability in its respective cluster. Based on the different marker systems used the population was grouped into two main clusters leaving some genotypes as admixture.
- Marker trait association (MTA) was performed on a panel of 188 pomegranate genotypes using 125 SSR markers with a TASSEL 3.0 software (Bradbury *et al.*, 2007). A General Linear Model (GLM) and Single Marker Analysis (SMA) was employed to discover genomic region with the different phenotypic traits. The results from TASSEL 3.0 revealed that there were many significant associations between the markers used and different traits studied. However, no marker used in

the study (GLM) was associated with days to maturity, number of arils per fruit, aril per cent, juice per cent, volume of juice, rind per cent and TSS.

- In the present study, a total of 208 (GLM) and 343 (SMA) markers were found to be associated with various economically important characters. Among the associated markers with R^2 value of 5 per cent or above is considered as significant threshold for the estimates to reveal association between pairs of loci.
- In all the marker-trait association (MTA), level of association was significant with a minimum p-value of 0.002.
- The plant height was associated with EPS-07 ($R^2 = 0.083$), ABR11-MP-51 ($R^2 = 0.11$), PGCT051 ($R^2 = 0.056$), PGCT014 ($R^2 = 0.070$), PGCT001 ($R^2 = 0.076$) and EPS04 (0.066), with their respective contribution.
- The EPS-10 is common marker showing association with disease resistance characters such as bacterial blight on leaves, fruit, stem and disease severity on tree with contribution of 10.84, 10.40, 12.66 and 12.71 per cent, respectively, where as the marker PG-20 also associated with disease resistance characters except in bacterial blight on fruit.
- About 208 significant ($P < 0.001$) marker-trait associations in GLM and 343 significant associations were found in the SMA model for the plant growth, fruit quality, bacterial blight disease resistance and its component traits. About 25 associations in GLM and 16 significant associations were found in the SMA model for the acidity. Highest number of markers (20) explaining more than five per cent phenotypic variation were found associated with calyx diameter followed by 17 markers for leaf length, 16 marker each for leaf breadth and maturity index; and 12 markers for rind weight, 11 markers each for plant spread, fruit length and 10 markers each for bacterial blight on leaves and stem and only three markers for disease severity on tree. Similarly in SMA about 32 markers having more than five per cent contribution showed significant association with fruit diameter followed by 29 and 24 markers for fruit length, total aril weight and fruit weight, respectively. Only six markers were found associated with bacterial blight on stem and followed by two marker each associated with bacterial blight on leaves, stem and disease severity, respectively.

Genetic and molecular analysis for growth and fruit quality traits and bacterial blight disease resistance) can be concluded as:

- Significant variability for growth, fruit quality traits and bacterial blight disease resistance amongst the progenies and germplasm was observed, which is attributed to genetic factors as they are growing in the same environment and are of the same age.
- Traits having high heritability and genetic gain indicate high genetic control. This variability can be exploited in fruit improvement programmes through selection and breeding approaches for development of advanced generations.
- Variability and divergence studies suggested that variation can be exploited for any breeding programme of pomegranate on the basis of its important characteristics to obtain heterotic vigour through hybridization. It is also revealed that most of the progenies from different seed sources clustered in the same group.
- Molecular characterization using SSR markers revealed higher diversity in the pomegranate progenies, which almost delineated the progenies of same division into the same major cluster.
- Based on the SSR marker systems used the pomegranate genotypes were clustered using STRUCTURE software leaving some genotypes as admixture. Most of the genotypes from respective seed sources were grouped in the same cluster showing their relationship at the genetic level as determined by molecular markers.
- The results from TASSEL 3 revealed that there were many significant associations between the markers used and different traits studied. It is found that more than one marker were contributing towards the expression of a trait suggesting that there are no major genes that are involved in the expression of the trait but could be due to the action of several genes with small effect.
- Association of marker trait associations using SSR markers as reported above can be very useful in selection and improvement of different traits at an early stage.

- In the end, it may be concluded that evaluation of the pomegranate diversity can be very useful qualitative and quantitative improvement of different traits studied and the marker trait association can aid the pomegranate improvement program.
- These associated markers can be effectively utilized in marker assisted selection.

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Appendix I: Monthly meteorological data for the year 2014-15, 2015-16 and average of past 9 years (2005-2013) of Horticulture Research and Extension Station, Vijayapur (Tidagundi)

[illegible]

Appendix II: Pooled mean performance of pomegranate genotypes for bacterial blight disease resistance on leaves (PDI) during *Mrig bahar*, 2014-15 and 2015-16

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
1	PK10-1	47.99 (43.85)*	55.32 (48.05)	25	PK10-39	33.99 (35.66)	48.65 (44.23)
2	PK10-2	52.65 (46.52)	67.32 (55.13)	26	PK10-42	43.32 (41.16)	49.99 (44.99)
3	PK10-3	62.65 (52.33)	50.65 (45.37)	27	PK10-43	45.99 (42.70)	49.32 (44.61)
4	PK10-5	45.99 (42.7)	54.65 (47.67)	28	PK10-45	50.65 (45.37)	43.32 (41.16)
5	PK10-6	51.32 (45.76)	52.65 (46.52)	29	PK10-48	58.65 (49.98)	50.65 (45.37)
6	PK10-7	28.66 (32.37)	61.32 (51.54)	30	PK10-52	49.99 (44.99)	45.32 (42.32)
7	PK10-8	31.99 (34.44)	47.99 (43.85)	31	PK10-53	33.99 (35.66)	45.99 (42.7)
8	PK10-10	21.99 (27.97)	41.32 (40)	32	PK10-54	32.66 (34.85)	46.66 (43.08)
9	PK10-13	41.32 (40.00)	45.99 (42.7)	33	PK10-55	38.66 (38.44)	47.99 (43.85)
10	PK10-14	47.32 (43.46)	48.65 (44.23)	34	PK10-57	46.66 (43.08)	47.99 (43.85)
11	PK10-15	33.33 (35.26)	50.65 (45.37)	35	PK10-58	29.99 (33.21)	47.99 (43.85)
12	PK10-16	37.32 (37.66)	45.99 (42.7)	36	PK10-59	45.99 (42.7)	43.99 (41.55)
13	PK10-20	62.65 (52.33)	49.99 (44.99)	37	PK10-60	51.32 (45.76)	45.32 (42.32)
14	PK10-21	37.32 (37.66)	50.65 (45.37)	38	PK10-62	57.32 (49.21)	54.65 (47.67)
15	PK10-22	54.65 (47.67)	53.99 (47.29)	39	PK10-63	23.33 (28.88)	41.32 (40)
16	PK10-24	45.99 (42.7)	51.32 (45.76)	40	PK10-64	43.99 (41.55)	58.65 (49.98)
17	PK10-25	59.32 (50.37)	51.32 (45.76)	41	PK10-66	41.32 (40.00)	47.99 (43.85)
18	PK10-28	45.32 (42.32)	51.99 (46.14)	42	PK10-67	47.32 (43.46)	52.65 (46.52)
19	PK10-29	63.98 (53.12)	49.99 (44.99)	43	PK10-70	45.99 (42.7)	47.32 (43.46)
20	PK10-34	41.32 (40.00)	54.65 (47.67)	44	PK10-74	35.32 (36.47)	43.99 (41.55)
21	PK10-35	45.99 (42.70)	54.65 (47.67)	45	PK10-75	42.66 (40.78)	43.99 (41.55)
22	PK10-36	38.66 (38.44)	52.65 (46.52)	46	PK10-76	52.65 (46.52)	51.99 (46.14)
23	PK10-37	39.32 (38.84)	53.32 (46.9)	47	PK10-81	34.66 (36.07)	43.32 (41.16)
24	PK10-38	39.32 (38.84)	51.32 (45.76)	48	PK10-82	44.66 (41.93)	45.99 (42.7)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
49	PK10-83	51.32 (45.76)	41.99 (40.39)	73	PK10-127	62.65 (52.33)	52.65 (46.52)
50	PK10-85	50.65 (45.37)	42.66 (40.78)	74	PK10-131	64.65 (53.52)	52.65 (46.52)
51	PK10-86	41.99 (40.39)	47.32 (43.46)	75	PK10-133	23.99 (29.33)	48.65 (44.23)
52	PK10-87	47.32 (43.46)	43.32 (41.16)	76	PK10-134	63.32 (52.72)	51.32 (45.76)
53	PK10-89	30.66 (33.62)	42.66 (40.78)	77	PK10-136	20 (26.56)	14.66 (22.51)
54	PK10-90	31.33 (34.03)	36.66 (37.26)	78	PK10-138	52.65 (46.52)	40.66 (39.61)
55	PK10-94	59.99 (50.76)	49.32 (44.61)	79	PK10-140	45.32 (42.32)	26.66 (31.09)
56	PK10-95	40.66 (39.61)	51.99 (46.14)	80	PK10-146	54.65 (47.67)	51.99 (46.14)
57	PK10-96	44.66 (41.93)	51.32 (45.76)	81	PK10-147	53.32 (46.9)	53.99 (47.29)
58	PK10-98	55.99 (48.44)	53.32 (46.9)	82	PK10-151	35.32 (36.47)	57.99 (49.6)
59	PK10-103	47.99 (43.85)	50.65 (45.37)	83	PK10-153	50.65 (45.37)	37.99 (38.05)
60	PK10-104	42.66 (40.78)	44.66 (41.93)	84	PK10-157	37.99 (38.05)	40.66 (39.61)
61	PK10-107	55.32 (48.05)	47.32 (43.46)	85	PK10-163	47.32 (43.46)	51.99 (46.14)
62	PK10-108	43.99 (41.55)	41.99 (40.39)	86	PK10-164	50.65 (45.37)	53.99 (47.29)
63	PK10-109	41.32 (40)	46.66 (43.08)	87	PK10-167	60.65 (51.15)	51.32 (45.76)
64	PK10-114	57.99 (49.6)	53.32 (46.9)	88	PK10-170	49.32 (44.61)	53.99 (47.29)
65	PK10-115	57.32 (49.21)	43.99 (41.55)	89	PK10-173	44.66 (41.93)	44.66 (41.93)
66	PK10-117	40.66 (39.61)	42.66 (40.78)	90	PK10-174	51.32 (45.76)	48.65 (44.23)
67	PK10-118	50.65 (45.37)	47.32 (43.46)	91	PK10-175	47.32 (43.46)	51.99 (46.14)
68	PK10-119	51.99 (46.14)	48.65 (44.23)	92	PK10-176	45.99 (42.7)	45.99 (42.7)
69	PK10-120	61.32 (51.54)	40.66 (39.61)	93	PK10-177	35.32 (36.47)	44.66 (41.93)
70	PK10-123	71.98 (58.04)	49.32 (44.61)	94	PK10-178	49.32 (44.61)	48.65 (44.23)
71	PK10-124	55.32 (48.05)	51.32 (45.76)	95	PK10-179	39.32 (38.84)	51.32 (45.76)
72	PK10-126	51.99 (46.14)	53.99 (47.29)	96	PK10-180	48.65 (44.23)	51.99 (46.14)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
97	PK10-181	43.99 (41.55)	44.66 (41.93)	122	PK10-228	47.32 (43.46)	53.99 (47.29)
98	PK10-182	47.32 (43.46)	50.65 (45.37)	123	PK10-229	31.99 (34.44)	49.99 (44.99)
99	PK10-183	59.99 (50.76)	51.99 (46.14)	124	PK15-2	47.32 (43.46)	48.65 (44.23)
100	PK10-184	25.33 (30.22)	57.32 (49.21)	125	PK15-3	49.99 (44.99)	58.65 (49.98)
101	PK10-188	40.66 (39.61)	58.65 (49.98)	126	PK15-7	41.99 (40.39)	52.65 (46.52)
102	PK10-190	38.66 (38.44)	49.99 (44.99)	127	PK15-11	43.32 (41.16)	51.99 (46.14)
103	PK10-191	51.99 (46.14)	52.65 (46.52)	128	PK15-12	41.32 (40)	51.99 (46.14)
104	PK10-197	42.66 (40.78)	53.99 (47.29)	129	PK15-13	39.32 (38.84)	51.99 (46.14)
105	PK10-198	53.32 (46.9)	57.99 (49.6)	130	PK15-14	27.33 (31.52)	49.99 (44.99)
106	PK10-199	34.66 (36.07)	57.32 (49.21)	131	PK15-16	59.32 (50.37)	51.99 (46.14)
107	PK10-201	41.99 (40.39)	51.99 (46.14)	132	PK15-17	37.32 (37.66)	53.32 (46.9)
108	PK10-203	40.66 (39.61)	52.65 (46.52)	133	PK15-19	48.65 (44.23)	46.66 (43.08)
109	PK10-204	45.32 (42.32)	48.65 (44.23)	134	PK15-20	47.32 (43.46)	53.32 (46.9)
110	PK10-206	31.33 (34.03)	49.32 (44.61)	135	PK15-21	46.66 (43.08)	49.32 (44.61)
111	PK10-207	33.99 (35.66)	39.99 (39.23)	136	PK15-22	63.32 (52.72)	55.32 (48.05)
112	PK10-208	55.99 (48.44)	53.99 (47.29)	137	PK15-23	57.32 (49.21)	53.32 (46.9)
113	PK10-209	46.66 (43.08)	46.66 (43.08)	138	PK15-24	55.99 (48.44)	53.32 (46.9)
114	PK10-210	51.99 (46.14)	44.66 (41.93)	139	PK15-27	65.32 (53.92)	56.65 (48.82)
115	PK10-212	37.32 (37.66)	45.32 (42.32)	140	PK15-28	59.99 (50.76)	52.65 (46.52)
116	PK10-213	36.66 (37.26)	49.99 (44.99)	141	PK15-29	53.32 (46.9)	56.65 (48.82)
117	PK10-214	56.65 (48.82)	51.32 (45.76)	142	PK15-30	47.99 (43.85)	57.32 (49.21)
118	PK10-216	47.32 (43.46)	46.66 (43.08)	143	PK15-31	67.32 (55.13)	53.99 (47.29)
119	PK10-219	56.65 (48.82)	51.32 (45.76)	144	PK15-32	60.65 (51.15)	53.32 (46.9)
120	PK10-221	51.32 (45.76)	57.99 (49.6)	145	PK15-36	51.99 (46.14)	39.99 (39.23)
121	PK10-227	51.32 (45.76)	43.32 (41.16)	146	PK15-38	46.66 (43.08)	56.65 (48.82)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
147	PK15-39	33.99 (35.66)	51.99 (46.14)	172	PK15-91	57.32 (49.21)	54.65 (47.67)
148	PK15-40	49.99 (44.99)	44.66 (41.93)	173	PK15-93	46.66 (43.08)	48.65 (44.23)
149	PK15-41	42.66 (40.78)	47.32 (43.46)	174	PK15-94	56.65 (48.82)	51.99 (46.14)
150	PK15-42	51.99 (46.14)	46.66 (43.08)	175	PK15-97	57.99 (49.6)	41.32 (40)
151	PK15-47	49.99 (44.99)	53.32 (46.9)	176	PK15-102	63.98 (53.12)	51.99 (46.14)
152	PK15-48	59.32 (50.37)	51.99 (46.14)	177	PK15-111	44.66 (41.93)	41.32 (40)
153	PK15-49	47.99 (43.85)	55.32 (48.05)	178	PK15-115	47.99 (43.85)	43.99 (41.55)
154	PK15-51	68.65 (55.95)	53.32 (46.9)	179	PK15-120	29.99 (33.21)	53.32 (46.9)
155	PK15-52	55.32 (48.05)	36.66 (37.26)	180	PK15-128	48.65 (44.23)	47.99 (43.85)
156	PK15-56	75.31 (60.21)	60.65 (51.15)	181	PK15-129	61.32 (51.54)	49.32 (44.61)
157	PK15-60	34.66 (36.07)	53.32 (46.9)	182	PK15-132	51.32 (45.76)	35.99 (36.86)
158	PK15-62	53.32 (46.9)	57.32 (49.21)	183	PK15-133	32.66 (34.85)	33.33 (35.26)
159	PK15-63	40.66 (39.61)	55.32 (48.05)	184	PK15-135	59.99 (50.76)	35.32 (36.47)
160	PK15-65	39.32 (38.84)	49.32 (44.61)	185	PK15-136	31.99 (34.44)	41.32 (40)
161	PK15-66	48.65 (44.23)	55.99 (48.44)	186	PK15-138	55.99 (48.44)	49.99 (44.99)
162	PK15-71	53.99 (47.29)	53.32 (46.9)	187	PK15-140	47.32 (43.46)	51.32 (45.76)
163	PK15-72	25.99 (30.65)	39.32 (38.84)	188	PK15-142	39.32 (38.84)	49.99 (44.99)
164	PK15-75	37.99 (38.05)	45.32 (42.32)	189	PK15-147	39.32 (38.84)	35.99 (36.86)
165	PK15-76	49.99 (44.99)	49.32 (44.61)	190	PK15-150	33.33 (35.26)	58.65 (49.98)
166	PK15-78	46.66 (43.08)	52.65 (46.52)	191	PK15-151	31.99 (34.44)	82.65 (65.38)
167	PK15-79	43.99 (41.55)	51.32 (45.76)	192	PK15-155	58.65 (49.98)	61.32 (51.54)
168	PK15-83	51.32 (45.76)	53.99 (47.29)	193	PK15-156	38.66 (38.44)	53.32 (46.9)
169	PK15-85	57.32 (49.21)	53.32 (46.9)	194	PK15-157	45.32 (42.32)	57.32 (49.21)
170	PK15-86	56.65 (48.82)	57.99 (49.6)	195	PK15-161	59.32 (50.37)	46.66 (43.08)
171	PK15-89	59.99 (50.76)	51.32 (45.76)	196	PK15-169	54.65 (47.67)	60.65 (51.15)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
197	PK15-171	63.32 (52.72)	55.32 (48.05)	222	PK20-44	47.99 (43.85)	56.65 (48.82)
198	PK15-172	51.32 (45.76)	35.99 (36.86)	223	PK20-47	43.99 (41.55)	53.32 (46.9)
199	PK15-173	28.66 (32.37)	38.66 (38.44)	224	PK20-48	39.32 (38.84)	49.32 (44.61)
200	PK15-174	64.65 (53.52)	37.32 (37.66)	225	PK20-54	51.99 (46.14)	43.99 (41.55)
201	PK15-175	49.99 (44.99)	31.99 (34.44)	226	PK20-58	49.32 (44.61)	54.65 (47.67)
202	PK15-177	41.99 (40.39)	33.99 (35.66)	227	PK20-61	53.32 (46.9)	50.65 (45.37)
203	PK15-180	59.99 (50.76)	56.65 (48.82)	228	PK20-62	22.66 (28.43)	47.99 (43.85)
204	PK15-182	68.65 (55.95)	52.65 (46.52)	229	PK20-64	33.99 (35.66)	53.99 (47.29)
205	PK20-5	41.32 (40)	47.32 (43.46)	230	PK20-70	10 (18.43)	34.66 (36.07)
206	PK20-8	43.32 (41.16)	50.65 (45.37)	231	PK20-71	59.99 (50.76)	53.32 (46.9)
207	PK20-10	47.99 (43.85)	37.99 (38.05)	232	PK20-76	62.65 (52.33)	53.99 (47.29)
208	PK20-11	43.99 (41.55)	45.32 (42.32)	233	PK20-87	63.98 (53.12)	54.65 (47.67)
209	PK20-15	47.32 (43.46)	47.99 (43.85)	234	PK20-90	43.32 (41.16)	35.99 (36.86)
210	PK20-16	61.32 (51.54)	51.32 (45.76)	235	PK20-92	58.65 (49.98)	47.99 (43.85)
211	PK20-259	32.66 (34.85)	48.65 (44.23)	236	PK20-93	43.99 (41.55)	23.99 (29.33)
212	PK20-27	55.32 (48.05)	53.32 (46.9)	237	PK20-99	48.65 (44.23)	25.33 (30.22)
213	PK20-31	38.66 (38.44)	41.99 (40.39)	238	PK20-101	50.65 (45.37)	60.65 (51.15)
214	PK20-33	42.66 (40.78)	47.99 (43.85)	239	PK20-112	47.99 (43.85)	34.66 (36.07)
215	PK20-34	38.66 (38.44)	50.65 (45.37)	240	PK20-114	28.66 (32.37)	47.32 (43.46)
216	PK20-35	61.98 (51.93)	55.99 (48.44)	241	PK20-115	42.66 (40.78)	32.66 (34.85)
217	PK20-36	53.32 (46.9)	59.99 (50.76)	242	PK20-116	41.32 (40)	38.66 (38.44)
218	PK20-37	51.99 (46.14)	59.32 (50.37)	243	PK20-117	63.32 (52.72)	55.32 (48.05)
219	PK20-38	45.32 (42.32)	55.32 (48.05)	244	PK20-121	56.65 (48.82)	54.65 (47.67)
220	PK20-41	66.65 (54.73)	59.32 (50.37)	245	PK20-122	49.32 (44.61)	45.99 (42.7)
221	PK20-43	53.32 (46.9)	51.99 (46.14)	246	PK20-124	37.32 (37.66)	27.99 (31.94)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
247	PK20-126	58.65 (49.98)	29.99 (33.21)	272	PK20-184	49.99 (44.99)	41.32 (40)
248	PK20-128	59.32 (50.37)	38.66 (38.44)	273	PK20-185	58.65 (49.98)	50.65 (45.37)
249	PK20-129	43.32 (41.16)	32.66 (34.85)	274	PK20-186	51.99 (46.14)	46.66 (43.08)
250	PK20-130	55.99 (48.44)	28.66 (32.37)	275	PK20-191	55.99 (48.44)	53.32 (46.9)
251	PK20-138	58.65 (49.98)	46.66 (43.08)	276	PK20-192	51.99 (46.14)	56.65 (48.82)
252	PK20-140	61.32 (51.54)	54.65 (47.67)	277	PK20-200	47.32 (43.46)	41.32 (40)
253	PK20-144	52.65 (46.52)	38.66 (38.44)	278	PK20-204	54.65 (47.67)	52.65 (46.52)
254	PK20-145	59.32 (50.37)	38.66 (38.44)	279	PK20-214	45.99 (42.7)	49.32 (44.61)
255	PK20-146	42.66 (40.78)	34.66 (36.07)	280	PK20-219	35.99 (36.86)	49.32 (44.61)
256	PK20-147	53.99 (47.29)	27.99 (31.94)	281	PK20-220	39.32 (38.84)	55.32 (48.05)
257	PK20-148	60.65 (51.15)	37.32 (37.66)	282	PK20-221	37.32 (37.66)	43.99 (41.55)
258	PK20-154	61.32 (51.54)	30.66 (33.62)	283	PK20-223	38.66 (38.44)	54.65 (47.67)
259	PK20-157	19.33 (26.08)	41.99 (40.39)	284	PK20-224	62.65 (52.33)	55.99 (48.44)
260	PK20-159	67.98 (55.54)	57.32 (49.21)	285	PK20-227	49.99 (44.99)	59.99 (50.76)
261	PK20-160	66.65 (54.73)	50.65 (45.37)	286	PK20-228	16 (23.58)	23.33 (28.88)
262	PK20-167	53.32 (46.9)	45.32 (42.32)	287	PK20-232	45.99 (42.7)	50.65 (45.37)
263	PK20-170	33.99 (35.66)	45.99 (42.7)	288	PK20-233	57.32 (49.21)	53.99 (47.29)
264	PK20-171	68.65 (55.95)	51.99 (46.14)	289	PK20-234	52.65 (46.52)	52.65 (46.52)
265	PK20-172	50.65 (45.37)	45.99 (42.7)	290	PK20-235	48.65 (44.23)	44.66 (41.93)
266	PK20-174	58.65 (49.98)	54.65 (47.67)	291	PK20-236	49.99 (44.99)	48.65 (44.23)
267	PK20-175	52.65 (46.52)	47.99 (43.85)	292	PK20-237	39.99 (39.23)	47.99 (43.85)
268	PK20-177	29.33 (32.79)	49.32 (44.61)	293	PK20-238	27.33 (31.52)	35.99 (36.86)
269	PK20-179	61.98 (51.93)	45.32 (42.32)	294	PK20-239	51.32 (45.76)	41.32 (40)
270	PK20-182	58.65 (49.98)	38.66 (38.44)	295	PK20-241	48.65 (44.23)	49.32 (44.61)
271	PK20-183	43.32 (41.16)	53.32 (46.9)	296	PK20-243	45.32 (42.32)	42.66 (40.78)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
297	PK20-247	43.32 (41.16)	43.32 (41.16)	322	PK25-71	40.66 (39.61)	61.98 (51.93)
298	PK20-248	40.66 (39.61)	43.32 (41.16)	323	PK25-79	53.32 (46.9)	48.65 (44.23)
299	PK20-259	56.65 (48.82)	47.99 (43.85)	324	PK25-96	47.99 (43.85)	30.66 (33.62)
300	PK20-267	69.98 (56.78)	42.66 (40.78)	325	PK25-98	42.66 (40.78)	57.99 (49.6)
301	PK20-274	65.98 (54.32)	42.66 (40.78)	326	PK25-124	32.66 (34.85)	47.99 (43.85)
302	PK20-276	64.65 (53.52)	41.32 (40)	327	PK25-130	24.66 (29.77)	36.66 (37.26)
303	PK20-278	63.32 (52.72)	58.65 (49.98)	328	PK25-133	47.99 (43.85)	51.99 (46.14)
304	PK20-279	65.32 (53.92)	52.65 (46.52)	329	PK25-134	42.66 (40.78)	36.66 (37.26)
305	PK20-283	61.98 (51.93)	49.32 (44.61)	330	PK25-135	39.32 (38.84)	49.99 (44.99)
306	PK20-284	63.98 (53.12)	53.99 (47.29)	331	PK25-141	41.32 (40)	37.99 (38.05)
307	PK25-7	35.33 (36.47)	34.66 (36.07)	332	PK25-144	49.99 (44.99)	49.99 (44.99)
308	PK25-11	46.66 (43.08)	50.65 (45.37)	333	PK25-149	46.66 (43.08)	49.32 (44.61)
309	PK25-16	35.99 (36.86)	65.98 (54.32)	334	PG10-1	41.32 (40)	51.32 (45.76)
310	PK25-18	51.99 (46.14)	45.99 (42.7)	335	PG10-2	44.66 (41.93)	59.32 (50.37)
311	PK25-19	53.32 (46.9)	57.32 (49.21)	336	PG10-3	47.99 (43.85)	59.99 (50.76)
312	PK25-20	59.99 (50.76)	51.32 (45.76)	337	PG10-4	40.66 (39.61)	54.65 (47.67)
313	PK25-25	42.66 (40.78)	49.32 (44.61)	338	PG10-7	36.66 (37.26)	41.32 (40)
314	PK25-26	40.66 (39.61)	30.66 (33.62)	339	PG10-9	51.99 (46.14)	45.32 (42.32)
315	PK25-29	37.99 (38.05)	25.33 (30.22)	340	PG10-11	51.32 (45.76)	41.32 (40)
316	PK25-37	53.99 (47.29)	37.32 (37.66)	341	PG10-13	46.66 (43.08)	36.66 (37.26)
317	PK25-39	53.32 (46.9)	38.66 (38.44)	342	PG10-18	45.99 (42.7)	46.66 (43.08)
318	PK25-41	49.32 (44.61)	36.66 (37.26)	343	PG10-24	40.66 (39.61)	45.99 (42.7)
319	PK25-56	45.99 (42.7)	49.99 (44.99)	344	PG10-26	56.65 (48.82)	39.99 (39.23)
320	PK25-62	62.65 (52.33)	59.99 (50.76)	345	PG10-28	49.99 (44.99)	54.65 (47.67)
321	PK25-63	68.65 (55.95)	50.65 (45.37)	346	PG10-29	46.66 (43.08)	53.99 (47.29)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
347	PG10-31	39.32 (38.84)	48.65 (44.23)	372	PG15-27	39.32 (38.84)	36.66 (37.26)
348	PG10-32	43.32 (41.16)	32.66 (34.85)	373	PG15-28	49.32 (44.61)	43.32 (41.16)
349	PG10-34	50.65 (45.37)	47.99 (43.85)	374	PG15-29	47.99 (43.85)	41.99 (40.39)
350	PG10-35	38.66 (38.44)	41.32 (40)	375	PG15-35	45.32 (42.32)	33.33 (35.26)
351	PG10-36	44.66 (41.93)	45.99 (42.7)	376	PG15-51	45.99 (42.7)	39.99 (39.23)
352	PG10-37	45.99 (42.7)	41.99 (40.39)	377	PG20-3	51.32 (45.76)	44.66 (41.93)
353	PG10-38	47.32 (43.46)	40.66 (39.61)	378	PG20-8	58.65 (49.98)	45.32 (42.32)
354	PG10-39	43.32 (41.16)	43.99 (41.55)	379	PG20-9	54.65 (47.67)	41.32 (40)
355	PG10-43	44.66 (41.93)	45.32 (42.32)	380	PG20-14	55.32 (48.05)	46.66 (43.08)
356	PG10-46	37.99 (38.05)	47.99 (43.85)	381	PG20-16	55.99 (48.44)	49.99 (44.99)
357	PG10-47	39.99 (39.23)	40.66 (39.61)	382	PG20-17	60.65 (51.15)	51.32 (45.76)
358	PG10-49	33.33 (35.26)	49.32 (44.61)	383	PG20-21	53.99 (47.29)	44.66 (41.93)
359	PG10-50	56.65 (48.82)	51.32 (45.76)	384	PG20-23	50.65 (45.37)	43.99 (41.55)
360	PG10-52	43.32 (41.16)	51.32 (45.76)	385	PG20-25	49.99 (44.99)	46.66 (43.08)
361	PG10-53	46.66 (43.08)	45.99 (42.7)	386	PG20-30	55.32 (48.05)	35.99 (36.86)
362	PG15-4	47.32 (43.46)	33.33 (35.26)	387	PG20-32	49.32 (44.61)	46.66 (43.08)
363	PG15-6	54.65 (47.67)	41.32 (40)	388	PG20-33	49.99 (44.99)	40.66 (39.61)
364	PG15-7	37.32 (37.66)	40.66 (39.61)	389	PG20-34	57.32 (49.21)	45.99 (42.7)
365	PG15-9	47.99 (43.85)	35.32 (36.47)	390	PG20-36	54.65 (47.67)	47.32 (43.46)
366	PG15-10	40.66 (39.61)	22.66 (28.43)	391	PG20-39	44.66 (41.93)	54.65 (47.67)
367	PG15-11	42.66 (40.78)	26.66 (31.09)	392	PG20-47	49.99 (44.99)	58.65 (49.98)
368	PG15-15	41.32 (40)	41.99 (40.39)	393	PG20-48	45.32 (42.32)	38.66 (38.44)
369	PG15-16	42.66 (40.78)	45.99 (42.7)	394	PG20-49	37.99 (38.05)	36.66 (37.26)
370	PG15-19	38.66 (38.44)	45.32 (42.32)	395	PG20-57	40.66 (39.61)	53.99 (47.29)
371	PG15-26	45.32 (42.32)	42.66 (40.78)	396	PG20-63	61.98 (51.93)	46.66 (43.08)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
397	PG20-72	31.33 (34.03)	34.66 (36.07)	422	PG25-15	60.65 (51.15)	46.66 (43.08)
398	PG20-79	42.66 (40.78)	34.66 (36.07)	423	PG25-16	51.32 (45.76)	51.32 (45.76)
399	PG20-80	43.99 (41.55)	10 (18.43)	424	PG25-21	50.65 (45.37)	46.66 (43.08)
400	PG20-83	42.66 (40.78)	53.99 (47.29)	425	PG25-22	44.66 (41.93)	50.65 (45.37)
401	PG20-86	51.32 (45.76)	41.32 (40)	426	PG25-27	42.66 (40.78)	27.33 (31.52)
402	PG20-88	41.32 (40)	33.33 (35.26)	427	PG25-31	51.99 (46.14)	54.65 (47.67)
403	PG20-90	45.32 (42.32)	49.99 (44.99)	428	PG25-33	43.32 (41.16)	47.32 (43.46)
404	PG20-92	39.32 (38.84)	31.99 (34.44)	429	PG25-34	49.32 (44.61)	40.66 (39.61)
405	PG20-93	64.65 (53.52)	29.99 (33.21)	430	PG25-35	40.66 (39.61)	38.66 (38.44)
406	PG20-96	35.32 (36.47)	27.33 (31.52)	431	PG25-36	45.99 (42.7)	43.99 (41.55)
407	PG20-104	68.65 (55.95)	49.32 (44.61)	432	PG25-37	54.65 (47.67)	45.99 (42.7)
408	PG20-105	39.32 (38.84)	60.65 (51.15)	433	PG25-38	47.99 (43.85)	51.32 (45.76)
409	PG20-110	42.66 (40.78)	51.99 (46.14)	434	PG25-40	47.32 (43.46)	47.99 (43.85)
410	PG20-113	52.65 (46.52)	35.99 (36.86)	435	PG25-41	39.99 (39.23)	43.32 (41.16)
411	PG20-122	51.32 (45.76)	51.99 (46.14)	436	PG25-44	39.32 (38.84)	47.32 (43.46)
412	PG20-128	58.65 (49.98)	52.65 (46.52)	437	PG25-45	40.66 (39.61)	53.32 (46.9)
413	PG20-138	57.99 (49.6)	43.99 (41.55)	438	PG25-46	52.65 (46.52)	35.32 (36.47)
414	PG20-144	62.65 (52.33)	49.99 (44.99)	439	PG25-48	11.33 (19.67)	12.66 (20.85)
415	PG20-150	55.99 (48.44)	47.32 (43.46)	440	PG25-49	35.99 (36.86)	31.99 (34.44)
416	PG20-151	63.32 (52.72)	52.65 (46.52)	441	PG25-50	47.99 (43.85)	27.33 (31.52)
417	PG25-4	45.99 (42.7)	23.99 (29.33)	442	PG25-54	46.66 (43.08)	32.66 (34.85)
418	PG25-9	58.65 (49.98)	20.66 (27.04)	443	PG25-55	43.99 (41.55)	27.99 (31.94)
419	PG25-10	62.65 (52.33)	27.99 (31.94)	444	PG25-56	46.66 (43.08)	25.99 (30.65)
420	PG25-11	43.32 (41.16)	27.33 (31.52)	445	PG25-61	41.32 (40)	47.99 (43.85)
421	PG25-12	46.66 (43.08)	33.33 (35.26)	446	PG25-66	47.32 (43.46)	43.32 (41.16)

Contd.....

Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>	Sl. No.	Genotype	<i>In vivo</i>	<i>In vitro</i>
447	PG25-67	45.32 (42.32)*	48.65 (44.23)	462	KRS	50.65 (45.37)	46.66 (43.08)
448	PG25-71	59.99 (50.76)	32.66 (34.85)	463	Dholka	43.32 (41.16)	48.65 (44.23)
449	PG25-74	45.32 (42.32)	31.33 (34.03)	464	P-26	47.99 (43.85)	39.32 (38.84)
450	PG25-79	65.98 (54.32)	44.66 (41.93)	465	P-23	47.32 (43.46)	53.32 (46.9)
451	PG25-80	71.32 (57.62)	41.32 (40)	466	Bedana seedless	33.99 (35.66)	12 (20.27)
452	PG25-82	58.65 (49.98)	44.66 (41.93)	467	P-16	40.66 (39.61)	31.99 (34.44)
453	Early Bhagwa	13.33 (21.41)	14 (21.97)	468	G-137	14 (21.97)	16.66 (24.09)
454	Yarcaud	21.33 (27.5)	19.33 (26.08)	469	Jollore Seedless	31.99 (34.44)	22.66 (28.43)
455	Tobesta	35.99 (36.86)	19.99 (26.56)	470	Patna-5	9.33 (17.79)	14 (21.97)
456	Kabulli yellow	35.99 (36.86)	13.33 (21.41)	471	Kalipathiya	41.99 (40.39)	25.33 (30.22)
457	Jyothi	38.66 (38.44)	12 (20.26)	472	Nana	0 (0)	0 (0)
458	Nimalli	58.65 (49.98)	35.32 (36.47)	473	Ganesh (C1)	46.66 (43.08)	57.32 (49.21)
459	P-13	59.99 (50.76)	45.32 (42.31)	474	Bhagwa(C2)	61.32 (51.54)	59.99 (50.76)
460	Bassein Seedless	27.33 (31.52)	17.33 (24.6)	475	Ruby (C3)	38.66 (38.44)	41.32 (40)
461	Co-White	54.65 (47.67)	47.32 (43.46)		S.Em. $\pm$	2.49	1.91
					C.D. @ 5 %	6.91	5.29
					Mean	(43.39)	(40.56)
					Maximum	(56.11)	(49.17)
					Minimum	(9.52)	(10.18)

* Figures in parentheses indicate arc sine value

Appendix III: Structure matrix for the 188 genotypes taken for association mapping

Sl. No.	Trait	Q ₁	Q ₂	Sl. No.	Trait	Q ₁	Q ₂
1	PK10-1	0.998	0.002	41	PK10-190	0.999	0.001
2	PK10-2	0.999	0.001	42	PK10-191	0.917	0.083
3	PK10-3	0.608	0.392	43	PK10-197	0.987	0.013
4	PK10-5	0.566	0.434	44	PK10-199	0.998	0.002
5	PK10-6	0.999	0.001	45	PK10-201	0.998	0.002
6	PK10-14	0.999	0.001	46	PK10-203	0.999	0.001
7	PK10-15	0.999	0.001	47	PK10-204	0.999	0.001
8	PK10-20	0.999	0.001	48	PK10-206	0.999	0.001
9	PK10-24	0.999	0.001	49	PK10-207	0.923	0.077
10	PK10-29	0.993	0.007	50	PK10-210	0.985	0.015
11	PK10-52	0.998	0.002	51	PK10-212	0.993	0.007
12	PK10-57	0.999	0.001	52	PK10-219	0.998	0.002
13	PK10-59	0.999	0.001	53	PK10-221	0.999	0.001
14	PK10-60	0.999	0.001	54	PK10-227	0.998	0.002
15	PK10-64	0.999	0.001	55	PK10-229	0.996	0.004
16	PK10-70	0.982	0.018	56	PK15-12	0.999	0.001
17	PK10-74	0.999	0.001	57	PK15-13	0.999	0.001
18	PK10-75	0.999	0.001	58	PK15-20	0.998	0.002
19	PK10-76	0.928	0.072	59	PK15-23	0.999	0.001
20	PK10-86	0.999	0.001	60	PK15-31	0.999	0.001
21	PK10-89	0.999	0.001	61	PK15-36	0.998	0.002
22	PK10-90	0.999	0.001	62	PK15-40	0.998	0.002
23	PK10-103	0.999	0.001	63	PK15-41	0.999	0.001
24	PK10-109	0.999	0.001	64	PK15-48	0.999	0.001
25	PK10-117	0.998	0.002	65	PK15-49	0.999	0.001
26	PK10-120	0.998	0.002	66	PK15-56	0.998	0.002
27	PK10-127	0.998	0.002	67	PK15-60	0.998	0.002
28	PK10-133	0.999	0.001	68	PK15-62	0.999	0.001
29	PK10-134	0.859	0.141	69	PK15-65	0.999	0.001
30	PK10-140	0.998	0.002	70	PK15-66	0.999	0.001
31	PK10-147	0.999	0.001	71	PK15-72	0.999	0.001
32	PK10-153	0.998	0.002	72	PK15-78	0.999	0.001
33	PK10-157	0.998	0.002	73	PK15-135	0.999	0.001
34	PK10-164	0.999	0.001	74	PK15-138	0.971	0.029
35	PK10-170	0.997	0.003	75	PK15-147	0.999	0.001
36	PK10-174	0.998	0.002	76	PK15-155	0.999	0.001
37	PK10-176	0.999	0.001	77	PK15-174	0.996	0.004
38	PK10-182	0.999	0.001	78	PK20-5	0.999	0.001
39	PK10-183	0.999	0.001	79	PK20-8	0.999	0.001
40	PK10-184	0.998	0.002	80	PK20-38	0.999	0.001

Contd.....

Sl. No.	Trait	Q ₁	Q ₂	Sl. No.	Trait	Q ₁	Q ₂
81	PK20-41	0.998	0.002	121	PK25-62	0.997	0.003
82	PK20-43	0.999	0.001	122	PK25-96	0.998	0.002
83	PK20-47	0.998	0.002	123	PK25-98	0.998	0.002
84	PK20-48	0.999	0.001	124	PK25-133	0.998	0.002
85	PK20-54	0.999	0.001	125	PK25-134	0.357	0.643
86	PK20-62	0.999	0.001	126	PK25-141	0.995	0.005
87	PK20-64	0.999	0.001	127	PG10-31	0.038	0.962
88	PK20-70	0.998	0.002	128	PG10-34	0.001	0.999
89	PK20-71	0.999	0.001	129	PG10-35	0.001	0.999
90	PK20-87	0.989	0.011	130	PG10-36	0.002	0.998
91	PK20-90	0.998	0.002	131	PG10-37	0.001	0.999
92	PK20-92	0.999	0.001	132	PG10-43	0.001	0.999
93	PK20-93	0.999	0.001	133	PG10-46	0.002	0.998
94	PK20-101	0.998	0.002	134	PG10-50	0.001	0.999
95	PK20-112	0.99	0.01	135	PG10-52	0.001	0.999
96	PK20-115	0.999	0.001	136	PG15-7	0.001	0.999
97	PK20-117	0.999	0.001	137	PG15-10	0.004	0.996
98	PK20-121	0.999	0.001	138	PG20-9	0.008	0.992
99	PK20-129	0.998	0.002	139	PG20-16	0.001	0.999
100	PK20-130	0.997	0.003	140	PG20-23	0.001	0.999
101	PK20-157	0.999	0.001	141	PG20-30	0.001	0.999
102	PK20-159	0.982	0.018	142	PG20-36	0.001	0.999
103	PK20-160	0.998	0.002	143	PG20-63	0.001	0.999
104	PK20-182	0.998	0.002	144	PG20-72	0.002	0.998
105	PK20-183	0.999	0.001	145	PG20-79	0.001	0.999
106	PK20-192	0.998	0.002	146	PG20-86	0.001	0.999
107	PK20-220	0.998	0.002	147	PG20-104	0.342	0.658
108	PK20-238	0.999	0.001	148	PG20-122	0.001	0.999
109	PK20-248	0.999	0.001	149	PG20-128	0.001	0.999
110	PK20-259	0.999	0.001	150	PG20-144	0.001	0.999
111	PK20-274	0.972	0.028	151	PG20-150	0.001	0.999
112	PK20-276	0.999	0.001	152	PG25-11	0.001	0.999
113	PK20-278	0.143	0.857	153	PG25-12	0.001	0.999
114	PK20-283	0.995	0.005	154	PG25-15	0.001	0.999
115	PK20-284	0.999	0.001	155	PG25-37	0.001	0.999
116	PK25-11	0.998	0.002	156	PG25-38	0.001	0.999
117	PK25-16	0.997	0.003	157	PG25-49	0.001	0.999
118	PK25-20	0.998	0.002	158	PG25-80	0.001	0.999
119	PK25-25	0.999	0.001	159	PG25-82	0.001	0.999
120	PK25-37	0.998	0.002	160	Early Bhagwa	0.339	0.661

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Sl. No.	Trait	Q ₁	Q ₂	Sl. No.	Trait	Q ₁	Q ₂
161	Yarcaud	0.452	0.548	175	G-137	0.001	0.999
162	Tobesta	0.337	0.663	176	Jollore Seedless	0.001	0.999
163	Kabulli yellow	0.294	0.706	177	Patna-5	0.259	0.741
164	Jyothi	0.001	0.999	178	Kalipathiya	0.368	0.632
165	Nimalli	0.001	0.999	179	Nana	0.297	0.703
166	P-13	0.008	0.992	180	PK10-63	0.998	0.002
167	Basseian Seedless	0.001	0.999	181	PK10-136	0.998	0.002
168	Co-White	0.477	0.523	182	PK20-228	0.999	0.001
169	KRS	0.002	0.998	183	PK25-7	0.999	0.001
170	Dholka	0.064	0.936	184	PK25-130	0.999	0.001
171	P-26	0.003	0.997	185	PG25-48	0.001	0.999
172	P-23	0.029	0.971	186	Ganesh (C1)	0.206	0.794
173	Bedana seedless	0.211	0.789	187	Bhagwa (C2)	0.168	0.832
174	P-16	0.004	0.996	188	Ruby (C3)	0.192	0.808

EVALUATION OF γ -RAY IRRADIATED SEED DERIVED PROGENIES AND GERMPLASM FOR PLANT GROWTH, FRUIT QUALITY TRAITS, BACTERIAL BLIGHT RESISTANCE AND ANALYSIS OF MARKER TRAIT ASSOCIATION IN POMEGRANATE (*Punica granatum* L.)

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2017

**Dr. NARAYAN MOGER
CHAIRMAN**

ABSTRACT

Present study was conducted during *mrig bahar* and *ambe bahar* 2014-2015 and 2015-2016 at the Horticulture Research and Extension Station, Vijayapur, University of Horticultural Sciences, Bagalkot to generate information on variability of *Xanthomonas axonopodis* pv. *punicae*, identifying virulent strain and to evaluate 475 γ -irradiated seed derived progenies of pomegranate (cv. Bhagwa and Ganesh) and germplasm planted in augmented design. The irradiated seeds at different doses (10, 15 20 and 25 kR) produced dwarf plants, stunted growth, variation in plant growth, fruit quality traits and bacterial blight disease resistance.

Four isolates of *X. axonopodis* pv. *punicae* were characterized with 16s rRNA, gyrB, ERIC, BOX and Rep-PCR. Among the collected isolates, *Xap* I was more virulent and used to screen the genotypes of the pomegranate for the bacterial blight resistance on leaves both *in vivo* and *in vitro* during the *mrig bahar*.

Significant amount of variability was found among the genotypes and genotype $\times$ season interaction for majority of the traits. Twenty genotypes were found superior to check varieties with respect to fruit quality traits. Among which only ten genotypes were found superior over best check variety Bhagwa (25) and remaining ten genotypes found superior over Ganesh and Ruby. The genotype PK10-190 and PK15-41 scored the maximum index score (28/45) followed by PG10-37, PK10-59 and PK20-47 with index score of 27 points. Two genotypes (PK10-59 and PK15-48) were found to be with low bacterial blight disease severity of 32.43 and 30.16 per cent, respectively as compared to Bhagwa (68.95%).

Marker trait association (MTA) was performed with 125 SSR markers revealed many significant associations between the markers and traits. The marker (EPS-10) is common marker showing association with bacterial blight disease resistance, leaf length, leaf breadth, acidity and maturity index. The markers (pg14a, b & c) associated with fruit diameter, fruit weight, total aril weight and 100 aril weight.