MOLECULAR MARKER PROFILING AND ITS ASSOCIATION WITH ECONOMIC TRAITS OF POPULAR INDIAN CULTIVARS OF POMEGRANATE (*Punica granatum* L.)

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CERTIFICATE

This is to certify that the thesis entitled **"MOLECULAR MARKER PROFILING AND ITS ASSOCIATION WITH ECONOMIC TRAITS OF POPULAR INDIAN CULTIVARS OF POMEGRANATE (*Punica granatum* L.)"** submitted by **Ms. NUSRAT PERVEEN** ID. NO. **UHS15PGM603** for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **FRUIT SCIENCE** to the University of Horticultural Sciences, Bagalkot, is a record of research work carried out by her during the period of her study in this university, under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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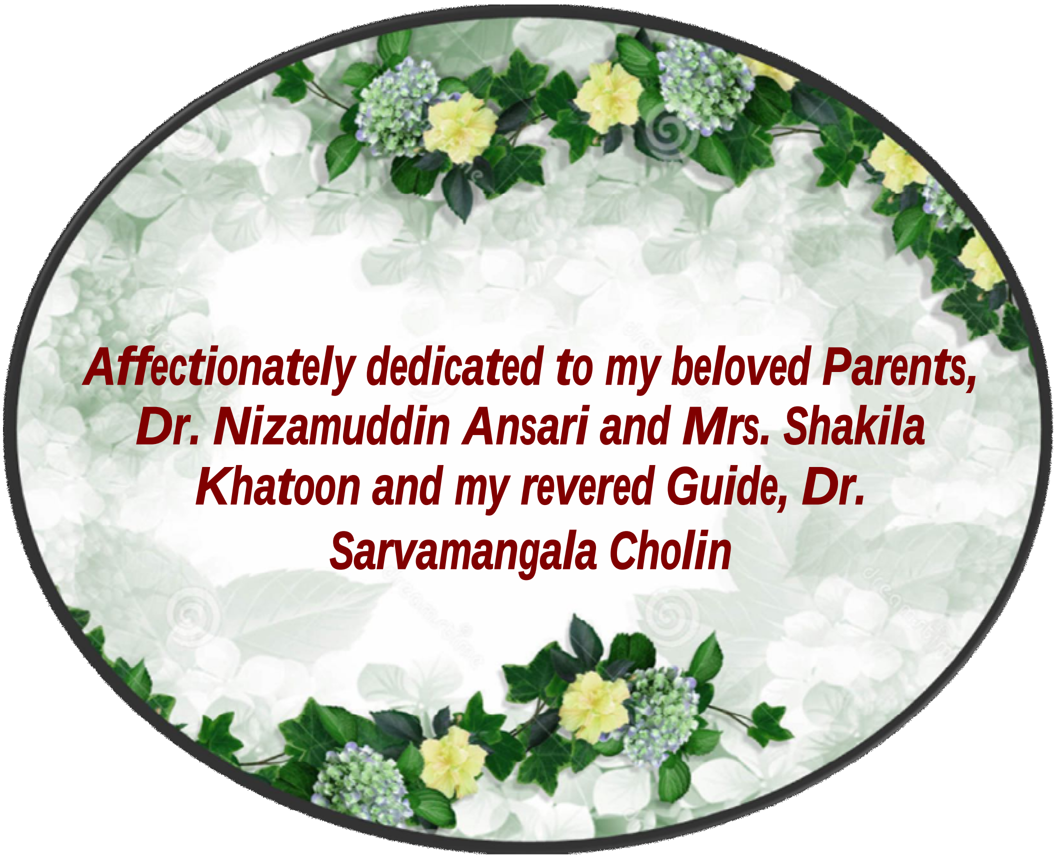

Affectionately dedicated to my beloved Parents, Dr. Nizamuddin Ansari and Mrs. Shakila Khatoon and my revered Guide, Dr. Sarvamangala Cholin

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LIST OF ABBREVIATIONS

mm: Milli meter

ml: Milli litre

µl: Micro litre

pM: Pico moles

mg: Milli gram

g: Gram

mM: Milli molar

M: Molar

N: Normality

nm: Nano meter

min: Minute

no.: Number

TSS: Total Soluble Solids

TA: Titratable acidity

Wt.: weight

1. INTRODUCTION

Pomegranate (*Punica granatum* L.), one of the oldest and beloved cultivated species of fruit crops, is a predominant member of family Lythraceae (earlier Punicaceae), comprising only two species, *Punica granatum* L. and *P. protopunica* Balf. f. 1882. *Punica protopunica* is endemic to Socotra Island (Yemen) and is considered to be the only congeneric relative of *P. granatum* species currently in cultivation (Zukovski, 1950; Mars, 2000; Levin, 2006), and has been suggested as the ancestor of this genus based on its xylem anatomy (Shilkina, 1973). The chromosome number differs among the cultivars and haploid chromosome no. of eight (Sheidai and Noormohammadi, 2005) or nine (Darlington and Janaki Ammal, 1945) has been reported.

The name ‘‘pomegranate’’ follows the Latin nomenclature, *Malum granatum*, meaning ‘‘grainy apple.’’ The generic name *Punica* refers to Pheonicia (Carthage) probably as an outcome of the assumption regarding its origin. Pomegranate and its usage are an integral part of human history, with its utilization spreading across many ancient human cultures as food as well as a medical remedy. Despite this, pomegranate culture has hitherto been restricted and is often considered as a minor crop.

India is by far the largest producer of pomegranate in the world with a total production of 2306.00 thousand MT from an area of 197.00 thousand hectares with a productivity of 12.00 MT/ha (NHB database, 2015-16) and is commercially cultivated mainly in Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Madhya Pradesh Tamil Nadu and Rajasthan.

Pomegranate cultivation is primarily confined to arid and semi-arid regions of the tropics and subtropics with cool winters and hot summers favouring its growth and development. It can withstand frost but temperature below - 10°C can cause damage to the plants. The optimum growth conditions for pomegranate exist in Mediterranean-like climates which include long exposure to sunlight; mild winters with minimal temperatures not lower than 12°C; and dry hot summers without rain during the last stages of the fruit development (Levin, 2006).

The main regions of pomegranate cultivation include Iran, Afghanistan, India, Mediterranean countries (Morocco, Spain, Turkey, Tunisia and Egypt) and Middle Eastern countries (Jbir *et al.*, 2008; Melgarejo *et al.*, 2009). Owing to the adaptability, the possibilities of its expansion to arid and semi-arid zones of the world are enormous, particularly to the regions where salinity and water scarcity are limiting factors for the cultivation of other crops.

Pomegranate is commercially grown for its fully luscious grains called 'arils' which constitute about 55–60% of the total fruit weight and consists of about 75–85% juice and 15–25% seeds (Al-Maiman and Ahmad, 2002). Pomegranate varieties differ in their taste ranging from sweet to sour (Holland *et al.*, 2008) which is directly related to the quality and quantity of the organic acids and sugars present in the fruit. Fruits are widely consumed fresh or processed into juice, syrup, jams and wine (Poyrazoglu *et al.*, 2002). Dried arils, known as anardana, are used as acidulent for culinary purposes.

A recent upsurge witnessed in the demand for pomegranate products is mainly attributed to the medicinal and nutritional values due to the antioxidant properties of this fruit (Gil *et al.*, 2000; Seeram *et al.*, 2008). Pomegranate juice has been found to possess a 3-fold higher antioxidant activity than that of red wine or green tea (Gil *et al.*, 2000), and 2, 6 and 8-fold higher levels than those detected in grape/cranberry, grapefruit and orange juice respectively (Rosenblat and Aviram, 2006). Fruit and its different parts contain about 153 phytochemicals like ellagic acid, catechins and procyanidins, fatty acids, triglycerides, sterols, terpenoides, flavonols *etc.* Apart from fruits, derived products from flowers, bark and leaves are also rich in bioactive phytochemicals like polyphenols mainly including ellagitannin, anthocyanin and punicalagin A and punicalagin B, two polyphenolics unique to this fruit.

Pomegranate possess a huge diversity of pomological traits (Patil and Sanghavi, 1980), however in spite of the presence of significant amount of variability, its utilization in breeding programs has been meagre till date. Any crop improvement programme mainly depends on the choice of superior parents for hybridization for which, evaluation of the physico-chemical parameters and genetic diversity at the molecular level for various popular genotypes of pomegranate grown in India, becomes the prime objective of a breeder in order to exploit the existing variability in this miraculous plant. In addition to this, since pomegranate consumption is driven by both the fresh market and processing industry, it becomes inevitable to acknowledge all of the fruit’s characteristics not only to classify varieties from a botanical point of view, but also to meet demands of different markets for quality fruits.

Morphological and biochemical studies are considered to be a key source of germplasm diversity estimation. Pomegranate crop have routinely been characterized using morphological and or biochemical markers (Ozgen *et al.*, 2008; Tehranifara *et al.*, 2010). Although, these markers are of great significance, they furnish limited information in distinguishing different varieties owing to the environmental plasticity and thus, heretofore, the identification of cultivars using these traits remains difficult and time consuming, eventually less preferred.

In the past few decades, molecular markers have proved to be a powerful tool for assessing genetic variation and phylogenetic relationships among the cultivars of many species of fruit crops. In recent years, increasing efforts have been made to develop molecular markers for characterization and genetic diversity assessment of *Punica granatum* cultivars. Different classes of markers such as AFLP (Amplified Fragment Polymorphism, Moslemi *et al.*, 2010; Yuan *et al.*, 2007), RFLP (Restriction Fragment Length Polymorphism, Melgarejo *et al.*, 2009), RAPD (Random Amplified Polymorphic DNA, Singh *et al.*, 2013; Sarkosh *et al.*, 2009, 2006; Ercisli *et al.*, 2007; Zamani *et al.*, 2007; Durgac *et al.*, 2008; Narzary *et al.*, 2009; Hasnaoui *et al.*, 2010) and SSR (Simple Sequence Repeats, Gadaleta *et al.*, 2007; Pirseyedi *et al.*, 2010; Currò *et al.*, 2010; Ebrahimi *et al.*, 2010, Parvaresh *et al.*, 2012; Lihua *et al.*, 2013; Singh *et al.*, 2015) have been developed in recent years for pomegranate.

Among these markers, microsatellite markers (SSRs) have proven to be very useful for genetic diversity studies in a wide range of plants due to their high polymorphism, abundance, distribution in the whole genome and co-dominant inheritance (Gupta *et al.*, 2000). Furthermore, because of their high reproducibility and reliability these are the markers of choice for efficient and effective selection of desirable clones.

Determination of the range of variability existing in the antioxidant property and other physical and chemical properties among different pomegranate cultivars and finding if there is any association between fruit properties, such as fruit weight, rind thickness *etc.* with juice chemical characteristics, such as titratable acidity, ascorbic acid, sugars *etc.* will provide an opportunity to select the best genotype(s) for cultivation and will help breeders to select and hybridize genotypes with higher levels of desired properties, to develop improved varieties.

Till date, the reports on characterization of pomegranate cultivars grown in different parts of India comprising molecular, morphological and biochemical diversity analysis is scarce. In this regard, the present study, on complete characterization of Indian pomegranate varieties with respect to their fruit morphological and biochemical traits, disease reaction, and molecular marker profiling along with marker-trait association, will be more relevant to provide detailed information about the desirable parental sources for future breeding program. Hence, an attempt has been made to extensively characterize the popular Indian cultivars at morphological, biochemical and molecular level together with screening them for disease reaction in comparison to previously identified resistant wild forms such as Nana and Daru.

The following objectives have been laid down for accomplishment of overall objective of the study:

1. To evaluate the popular cultivars for fruit morphological, biochemical parameters and field screening for bacterial blight and fruit and foliar spot resistance
2. To perform molecular marker profiling of popular Indian cultivars and the wild forms
3. To analyze the morphological and molecular diversity
4. To analyze the marker-trait association for economic traits of pomegranate

2. REVIEW OF LITERATURE

Pomegranate, from time immemorial is being cultivated for its juicy arils and has been acknowledged for its medicinal values in various folk medicines. But recently there has been an increase in the demand owing to its suitability for industrial processing as well as superior pharmacological and therapeutic properties. A large variability is present in domestic, wild and ornamental genotypes of pomegranate in terms of physico-chemical properties and traits like resistance to biotic and abiotic stresses. In order to identify the varieties suitable for processing or pharmaceutics and select superior and diverse parents for hybridization, complete characterization of existing cultivars adapted to Indian conditions would be a paramount. Since most of the fruit and nutritional quality parameters are polygenic and highly influenced by environment, there is a need to evaluate these cultivars at molecular level, for the effective selection of parents in breeding program. Hence, an attempt has been made in the present investigation to study in detail the popular genotypes of India from a morphological, biochemical and molecular point of view for identifying the best variety among the popular varieties for different characters as well to establish the importance of wild forms as source of disease resistance. A brief review of available literature pertaining to the investigation has been delineated in this chapter under the following sub-headings:

2.1 Genetic variability for morphological and biochemical characters

2.2 Bacterial blight, foliar and fruit spot resistance in pomegranate

2.3 Genomics and molecular markers

2.4 Marker-Trait association for economic traits in pomegranate

2.1. Genetic variability for morphological and biochemical characters

Identification of cultivars of fruit crops based on morphological and physiological descriptions has been practiced by the horticulturists since long. The main advantage of this method lies in its being simple, rapid and inexpensive. Pomegranate, being cross pollinated in nature is highly heterozygous and exhibits wide variations in various fruit and aril characters. The literature reviewed for studying the morphological and biochemical parameters like fruit weight, fruit volume, fruit colour, aril colour, titratable acidity, TSS, sugars, anthocyanin *etc.*, has been presented here under.

The traits that can be visually scored or whose inheritance could be followed with naked eyes are considered as morphological markers.

Bailey (1917) and Hodgson (1917) first recognized the significance of the use of morphological characters and incorporated in their description the characters like rind and petal colour and size of pomegranate tree. However, for characterization of pomegranate varieties they relied mainly on the acid content of fruits.

Nath and Randhawa (1959) carried out a comprehensive morphological study in seven pomegranate varieties namely, Dholka, Double Flower, GBG-1 (Ganesh), Japanese Dwar, Khandhari, Muskat White and Patiala. In their study they observed that a combination of several traits would be useful for characterization; however no single character can be depended upon to identify a pomegranate cultivar. They concluded that traits like evergreen or deciduous nature, size of tree, colour and shape of fruit *etc.* could be used for characterizing pomegranate varieties.

Sawant (1973) reported G-137 to possess heavier fruits (289.90 g) and seeds 219.40 g) and the high juiciness (88.70%). Choudhari and Shirsath (1976) found a relation between TSS and colour and shape of fruit. The red coloured fruits with red markings and ridged appearance were found to contain higher TSS and hard seeds.

Malhotara *et al.* (1983) assessed 21 pomegranate cultivars for fruit colour, shape, weight, length, width, percentage of fruit cracking, rind thickness and seed parameters like seed colour, No. of seeds per fruit and juice percentage. They found, cultivar Kazki and Achik Dana to be big fruited (124.70 g and 114.90 g respectively) were as Afghan Kandhari seedling was reported to be small fruited (56.80 g).

Purohit (1985) was the first to give the technique and criteria for testing seed hardiness. He categorized seven commercial cultivars of pomegranate, Bedana, Bassein Seedless, Ganesh and Dholka as soft seeded with testa thickness less than 0.50 mm, whole seed density, less than 0.60 g/ml, testa density less than 0.40 g/ml and testa weight less than 50% of whole seed weight. Further, Purohit (1986) quantitatively determined the soft seediness of Alandi, Ganesh and Muskat and found that Ganesh was having softest seeds among them.

Kaulgud *et al.* (1997) attempted the development of descriptor for pomegranate. Subsequently they listed 53 pomegranate cultivars with respect to morphology, morpho-agronomy, physico-chemical characteristics and grouped them based on the nature of foliage (evergreen or deciduous), tree habit (erect, semi-spreading, spreading), flowering flush and fruit composition.

Despite of the ease and simplicity of execution, morphological characters cannot be relied on owing to its amenability to change with environmental conditions, also in most cases, the measurements and descriptions vary from person to person. Furthermore, the existence of same cultivar with different names in different areas makes cultivar identification more complicated and often less authentic. Another important lacuna associated with morphological characterization is the time and expense involved in evaluating morphological traits (Laxminarayan, 1980).

Melgarejo *et al.* (2000) analyzed individual organic acids and sugar compositions of 40 Spanish pomegranate cultivars for two consecutive seasons. They found citric acid to be the predominant acid with a range of 0.142–2.317 g/100 g followed by Malic acid with a range of 0.135–0.176 g/100 g. Total sugars ranged between 11.43 g/100 g and 13.5 g/100 g with an average Fructose content of 6.58 g/100 g and that of glucose 6.14 g/100 g.

Al-Maiman and Ahmad (2002) studied the Physico-chemical properties of pomegranate cultivar Taifi. The edible portion of fruit (57.51% of total fruit wt.) was comprised 63.58% of juice and 36.21% of seeds. Total protein, ascorbic acid, fat and phenolic compounds in seeds was reported to be 4.06, 0.23, 0.15, 2.92%, respectively and glucose was found to be the major sugar (53.5%) followed by fructose (46.6%).

Poyrazoglu *et al.* (2002) analyzed organic acids and phenolic compounds in juice of thirteen pomegranate varieties obtained from four different growing regions of Turkey. Total titratable acidity varied between 4.58 and 17.30 g/L (average of 9.82g/L), and total sugars between 139.6 and 160.6 g/L (average of 148.75 g/L). Among the organic acids analyzed, citric acid was found to be the predominant one with a range of 0.33–8.96 g/L followed by l-Malic acid with a range of 0.56–6.86 g/L. Tartaric, oxalic, (-) - quinic and succinic acids ranged between 0.28–2.83, 0.02–6.72, 0.00–0.82 and 0.00–1.54 g/L, respectively. Furthermore, Gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, o- and p-coumaric acids, catechin, phloridzin and quercetin were the phenolic compounds identified in fresh pomegranate juices.

Fadavi *et al.* (2005) analyzed ten pomegranate varieties for their per cent of skin, juice, seed, lipid content and moisture, and sugars, minerals, vitamin C, protein, pH, titratable acidity (TA) and soluble solid content (SSC) of juices. Predominant sugars were fructose (3.50 to 5.96g/100g) and glucose (3.40 to 6.40g/100g). Among minerals highest content of K, Na, Ca and Mg was found in fruit juice. The average concentration of vitamin C, protein, TA, SSC and pH ranged between 0.09–0.40 mg/100 g, 0.29–1.93 %, 4.0–24.50 g/L, 10.0–16.50 °Brix and 2.90–4.21, respectively.

Ozgen *et al.* (2008) evaluated the chemical parameters *viz.* total phenolics (TP), total monomeric anthocyanins (TMA), soluble solids (TSS), titratable acidity (TA), individual sugars and organic acids and antioxidant properties of six pomegranate cultivars obtained from Mediterranean region of Turkey. The antioxidant capacities averaged 5.60 and 7.35 mmol TE/l by the TEAC and FRAP methods. Variability among cultivars was observed to be greatest for TMA content (CV 132%) ranging from 6.10 to 219 mg cy3-Gluc l1. Levels of FRAP, TEAC, TP, and TMA were found to be strongly correlated (r = 0.82–0.96). The major sugars were fructose (6.40 g/100 ml) and glucose (6.8 g/100 ml) while the major acids were citric (1.78 g/100 ml) and malic (0.12 g/100 ml).

Akbarpour *et al.* (2009) reported a wide range of variation for physical and chemical properties of pomegranate such as fruit fresh weight, volume and density, peel thickness, soluble solids (TSS), titratable acidity (TA), EC, pH, vitamin C, ellagic acid content of juice and peel, total antioxidant activity of peel and juice *etc.* by evaluating twelve Iranian cultivars. Fruit weight ranged from 103.38 to 505.00 g and fruit volume from 99.41 to 547.88 cm. Among the chemical properties, for reducing sugars, the range was 13.89 to 29.83 g/100 ml, total soluble solids ranged from 15.17 to 22.03 (°Brix) and for titratable acidity the range was between 0.35% and 3.36% in pomegranate juices. They also evaluated other chemical compounds such as ellagic acid of juice and peel (1-2.38 mg/100 ml and 10-50.00 mg/100 g, respectively) as measured by HPLC and total antioxidant activity measured by FRAP ranged from 225.17-705.50 (mmol/100 g) and 157.33-419.33 (mmol/100 ml) in peel and juice, respectively.

Al-Said *et al.* (2009) determined physical and chemical properties of four locally grown pomegranate cultivars of the Sultanate of Oman. Significant differences in fruit size and skin colour, aril size and colour, juice content and titratable acidity were found among the cultivars. Pomegranate cultivars with higher aril toughness yielded less juice per 100 g seeds. ‘Wild’ pomegranate with characteristic intense ‘sweet and sour’ taste in comparison with the other cultivars studied was reported to have significantly very low sugar: acid ratio.

Elfalleh *et al.* (2009) studied physic-chemical properties and DPPH-ABTS scavenging activities of Peels and fresh extracts of pomegranate of six local ecotypes. In their findings they reported fructose (ca. 7 g/100 ml) and glucose (ca. 8 g/100 ml) to be major sugars in pomegranate. Soluble protein content in juice extracts were approx 7 g/l (Bradford assays) and 22% (Kjeldhal assays) from dry pulp. They also studied mineral contents in peel and juice and found it to be 9.4390.93 and 9.4691.05 mg/100 ml respectively for phosphorus and 210.86910.70 and 271.94960.59 mg/100 g for potassium. High antioxidant content in peels was reported. Free radical scavenging activity of peel was found to be about 3.5890.38 mg/ml. The antioxidant capacity value determined by ABTS was 7.36490.403 mMTrolox equivalent antioxidant capacity/100 g dry weight. By these findings they concluded that bio-active compounds from the peel might be potential resources for the development of antioxidant function dietary food.

Islam *et al.* (2009) performed pomological and morphological characterization of pomegranates grown in the Black Sea region and observed considerable variations in fruit traits. Fruit weight ranged between123 g and 295 g, skin thickness 2.95 mm and 6.42 mm, seed number per fruit was reported to be 103-667, total soluble solids 12% and 15%, fruit size was found to be medium to large, flavor was sweet or sweet-sour and the skin color generally reddish-yellow.

Hassan *et al.* (2012) evaluated chemical and antioxidant properties of thirty two pomegranate cultivars obtained from different regions in Egypt. Juice content, total soluble solids (°Brix), titratable acidity (TA), pH and vitamin C content was reported to range from 43.0 to 75.0, 12.27 to 20.33, 2.81% and 0.30 %, 2.91-4.53 and 2.77-9.48 mg/100 ml, respectively.

Tehranifar *et al.* (2010) conducted a study to investigate the physic-chemical properties and antioxidant activity of twenty pomegranate cultivars grown in Iran. They reported a wide range of variation in fruit weight, skin percentage, aril percentage and juice percentage of the cultivars ranging from 196.89–315.28 g, 32.28–59.82%, 37.59–65% and 26.95–46.55%, respectively. The total soluble solids content was found to vary from 11.37 (°Brix) to 15.07 (°Brix), pH values were in range of 3.16 to 4.09, titratable acidity content from 0.33 g 100 g^{-1} to 2.44 g 100 g^{-1} and total sugars content from 13.23 g 100 g^{-1} to 21.72 g 100 g^{-1}. A great variation in ascorbic acid was also reported with the value ranging from 9.91mg 100 g^{-1} to 20.92 mg 100 g^{-1}. The total anthocyanin content cultivars were between 5.56 mg 100 g^{-1} and 30.11mg 100 g^{-1}. The level of total phenolics also varied from 295.79mg 100 g^{-1} to 985.37mg 100 g^{-1}. The antioxidant activity of pomegranate cultivars was in range of 15.59 and 40.72%. Based on these findings they concluded that the cultivar was the main factor which affects the physic-chemical properties and antioxidant activity in pomegranates.

Ferrara *et al.* (2011) conducted a study to evaluate the morpho-pomological and chemical parameters of eight pomegranate genotypes of central area of Apulia region (Southeastern Italy). Significant differences were observed for various parameters investigated, among the pomegranate genotypes *viz.* fruit weight ranged from 168.90 g (SouMol) to 574.90 g (SouOst), ºBrix from 14.70 (ComTri) to 18.0 (SouMol) and titratable acidity from 5.40 (ComMol) to 25.00 (SouTri) g/L. The cultivar SouMol recorded the highest polyphenols (97.10 mg/L) and vitamin C (236.30 mg/L) contents. Oil content of the seeds ranged between 5.90% and 10.30%. Based on these findings they suggested that AdeSgi is the best genotype for fresh market and SouOst for the juice industry.

Hasnaoui *et al.* (2011a) observed a strong correlation between sourness and citric acid content of sour pomegranate while malic acid was reported to be most prevalent in sweet ones. Total sugar content of pomegranate fruits, ranged between

17.77 and 19.98g/100g for the sour cultivars and between 13.13 and 16.55g/100g for the sweet ones essentially consisting of fructose and glucose.

Hasnaoui *et al.* (2011b) studied organic acids, sugars, and anthocyanin content of 30 Tunisian accessions of pomegranate using high performance liquid chromatography. Malic acid was found to be the major organic acid (>50%) followed by citric acid (>22%), while among sugars, fructose and glucose were predominant in pomegranate juice contributing 53.9 and 43.4% of the total sugar content, respectively. The total anthocyanin content ranged from 9–115 mg/L of juice with six compounds in the range of, cyanidin-3,5-diglucoside (3.10–74.40mg/L), delphinidin-3-glucoside (0.70–22.0 mg/L), cyanidin-3-glucoside (0.80–21.0 mg/L), pelargonidin-3-glucoside (0.50–16.10 mg/L), pelargonidin-3,5-diglucoside (0.0–11.80 mg/L),and delphinidin-3,5-diglucoside (0.0–5.40 mg/L). Based on principle component and cluster analyses it was suggested that the composition of pomegranate fruits is determined by cultivar rather than geographical location.

Rawat *et al.* (2012) characterized the morpho-physico-chemical properties of wild pomegranate (*Punica protopunica* L.) of Garhwal, Himalaya. The chemical analysis of fruits showed that the fruits contain 71.35% juice, 12.00% TSS, 2.43% total sugars. The malic acid and vitamin C content of fruits were recorded to be 4.17% and 5.83 mg/100 g of fruit pulp, respectively. The results obtained in this study revealed that the fruits of wild selections were more acidic than those of commercial cultivars.

Khadivi-Khub *et al.* (2014) evaluated pomological and leaf characters of 87 local accessions of pomegranate. They observed wide variations in parameters like fruit weight ranging from 69.77 to 341.91 g, 100 aril fresh weight from 14.44 to 42.42 g, total soluble solids from 10.80 to 17.60% and titratable acidity from 0.40 to 4.15%. They also reported high positive correlation between fruit weight and fruit dimensions and a negative correlation between titratable acidity and pH. On the basis of their findings they recommended soft seeded pomegranates as parent for developing high-juice cultivars owing to their significantly higher juice content.

Okatan *et al.* (2015) studied pomological and chemical traits of seventeen pomegranate genotypes selected from Narlidere district (Bitlis) of turkey. They observed considerable variation in fruit weight, aril weight, fruit length and fruit width ranging from 99.77 (N-15) to 515.97 g (N-05), 14.16 (N-01) to 41.92 g (N-10), 51.03 (N-15) to 90.99 mm (N-05) and 58.99 (N-03) to 103.11 mm (N-05) among genotypes, respectively. Considerable variations were also reported in chemical parameters *viz.* soluble solid content (SSC), titratable acidity (TA), pH and juice yield of genotypes varying between 5.96 (N-02) to 9.13% (N-03), 0.12 (N-12) to 0.91% (N-14), 2.51 (N-14) to 4.52 (N-10) and 48.58 (N-06) to72.07% (N-01), respectively. Promising genotypes are important genetic resources for use in future pomegranate breeding activities.

Radunic´*et al.* (2015) evaluated the physical and chemical properties of eight pomegranate accessions which included seven cultivars and one wild genotype, collected from the Mediterranean region of Croatia. The study revealed high variability in fruit weight and size, calyx and peel properties, number of arils per fruit, total aril weight, and aril and juice yield. Variables that define sweet taste, such as low total acidity (TA; 0.37–0.59%), high total soluble solids content (TSS; 12.50–15.0%) and their ratio (TSS/TA), were evaluated, and results generally aligned with sweetness classification of the fruit. They reported that pomegranate fruits have high variability in total phenolic content (1985.60–2948.70 mg/L). HPLC-MALDI-TOF/MS analysis showed that accessions with dark red arils contain highest total anthocyanin content, with cyanidin 3-glucoside being the most abundant compound.

Li *et al.* (2015) investigated the physico-chemical characteristics, polyphenol compositions and antioxidant potentials of pomegranate aril juices of ten cultivars from four different regions of China. The results demonstrated that the soluble solid, reducing sugar, titratable acid of the cultivars ranged from 13.97–16.30 ºBrix, 62.82–110.70 g/L, 2.65–36.62 g/L, respectively. The total polyphenols, flavonoids, tannin and anthocyanin concentrations were recorded to be 3.15–7.43 mg GaE/mL, 0.045–0.335 mg QuE/mL, 0.540–2.531 mg TaE/mL, and 0.004–0.160 mg CyE/mL, respectively. They reported that sugar-acid ratio, titratable acid content, total flavonoid concentration and DPPH scavenging capacity were influenced mainly by sweet and sour cultivar type, while there was environmental effect on soluble solid content and total anthocyanin concentration. Further, they found that the temperature during maturity period and latitude of growing region has a significant effect on polyphenol and antioxidant potential of pomegranate juice.

Inheritance pattern in pomegranate

Jalikop and Kumar (1990) reported that red pigmentation in the petiole base, leaf margin, flower bud and fruit rind in the variety 'Ganesh' is governed by a single dominant gene while its recessive form confers yellow pigmentation in these plant parts of Kabul Yellow. TSS content in the juice has been found to be positively and significantly associated with acidity (Kerale and Desai, 2000).

Meena *et al.* (2003) reported a significantly higher heritability and genetic advance for fruit weight, weight of 100 arils, acidity of juice and 100 seeds weight and emphasized that these traits can be used effectively as a selection criteria in breeding programmes. Hard seediness and red and pink aril colour are dominant to soft-seediness and white aril colour where soft-seediness is governed by multiple genes (Jalikop *et al.*, 2005).

Jalikop *et al.* (2007) demonstrated that fruit acidity is monogenically controlled whereas sourness or higher acidity is dominant over sweet or low acidity, and is controlled by a major gene SS while a few modifiers with small effects caused the fluctuations.

Jalikop *et al.* (2010) found that an increase in intensity of aril colour resulted in reduction in severity of aril browning whereas an increase in aril browning was observed with a rise in TSS. Hence, it is desirable to strike a balance between aril colour and TSS level when developing aril browning free variety is the objective.

2.2 Bacterial blight, foliar and fruit spot resistance in pomegranate

Bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* (Xap) is the major threat to pomegranate cultivation and alone causes up to 60 to 80% losses in pomegranate production in India (Mondal and Mani, 2009). Bhagwa, the most important cultivar and is highly susceptible to Bacterial blight.

This disease was first reported in India from Delhi (Hingorani and Mehta, 1952), and Subsequently from Karnataka (Hingorani and Singh, 1959), Himachal Pradesh (Sohi *et al*., 1964), Haryana (Kanwar, 1976), Maharashtra (Kamble, 1990; Dhandar *et al*., 2004; Sharma *et al*., 2008) and Punjab (Rani and Verma, 2001).

The disease is locally known as 'Kyararoga', 'Telkatdaag' or 'oily spot' in Maharashtra. Until 2001-2002 it was a minor disease, after which it suddenly assumed epidemic proportions in Bellary and Vijayapur districts of Karnataka (Yenjerappa *et al*., 2004).

Bacterial blight of pomegranate has assumed epidemic proportions (40-85% severity) in northern Karnataka (Vijayapur, Bellary, Bagalkot) posing a serious threat to its cultivation in the state (Jamadar *et al*., 2011).

Symptoms on leaves appears as small, irregular water soaked spots which are initially translucent and later turn light brown to dark brown surrounded by prominent water soaked margins. The spots coalesce to form large patches and in severe infections defoliation occurs. Girdling at the point of infection is quite conspicuous and plant parts above the infected site become yellow and dry up. This kind of symptoms is commonly referred as twig blight or nodal blight. On fruits initially oily spots appears which later on turn dark brown to black raised spots on the pericarp with L or Y shaped cracks (Bora and Kataki, 2014).

The bacterium has been reported to survive on the tree and in the fallen leaves and fruits (for more than 120 days) during off-season (Arjunan *et al*., 1999). However, the main damage is caused by the planting material *i.e.* infected cuttings. The bacteria primarily spread through infested planting material while cutting implements and rain splashes are responsible for secondary spread. Ravikumar *et al*. (2006) reported neem (*Azadirachta indica*) and bael (*Aegle marmelos*) plants to be the possible alternative hosts for this pathogen.

High humidity with high temperature is congenial for the occurrence of bacterial blight and the survivability of the pathogen is not affected even at temperatures around 40 °C (Rawal, 2006) in contrast, low temperatures during winter with no rainfall suppress the disease.

Yenjerappa *et al.* (2006) reported that temperatures around 31.8 to 35.6°C with sufficient rainfall during July to October is favourable for disease development and spread, hence selection of hasta-bahar crop by pruning in September-October is highly beneficial in escaping the blight incidence in pomegranate.

A rest period given to pomegranate plants rejuvenates the host and suppresses the pathogen. Yenjerappa *et al.* (2006) betoned a rest period of four to six months after the harvest is necessary to avoid the disease. Rawal (2006) reported resistant sources such as ‘PG-7, 8, 9, 10 and 11’ from Tamil Nadu, ‘Russian seedless’, ‘RS 75’, ‘K3’, ‘Gul-e-shah Red’, ‘H-3’, ‘Kandhari’, ‘Ganganagar’, ‘HS-80-29’ and ‘Kong’ in Hissar.

Development of varieties resistant to bacterial blight has become a paramount to defend the pomegranate industry from collapsing. Work to screen hybrids for this disease has been initiated in 2003 (Jalikop *et al*., 2006).

Hybrids of Daru and Ganesh, which are susceptible, recorded a mean disease score of 3.8 indicating that the resistance in Daru is controlled by recessive alleles. While hybrids of Nana, a miniature pomegranate resistant to blight, having ornamental value, (Nath and Randhawa, 1959) with Ganesh exhibited little susceptibility (0.42) suggesting the role of incomplete dominant alleles in determining the bacterial blight resistance. Tri hybrids (Ganesh x Nana) x Daru also produced some disease symptom (0.38), implying that the resistance of Daru was recessive to the resistance of Nana and both are valuable donors for bacterial blight resistance (Jalikop *et al.*, 2006).

Mondal *et al.* (2012) developed a polymerase chain reaction (PCR) based detection technique for Xap using primers designed from gyrBgene. They synthesized a primer set KM5 and KKM6 based on sequence alignment of 530 nucleotides of C-terminus region in the gyrB genes from 15 different bacterial strains. The primer set was further validated for amplification of 491 bp of gyrB gene. The authors claimed that the technique could detect pathogen in infected pomegranate plant samples including leaf, fruit and stem within 3 hr, at a detection limit 0.1 ng µl-1 template DNA.

Sharma *et al.* (2012) sequenced the genome of LMG 859, reference strain of *X. axonopodis* pv. *punicae*, using the Roche 454 GS (FLX Titanium) pyro sequencing platform (Macrogen, Republic of Korea) and reported a 4.94-Mb genome sequence. The authors posited that draft genome will aid in comparative genomics, epidemiological studies, and quarantine of this devastating pathogen of pomegranate.

Raghuwanshi *et al.* (2013) conducted a field survey in the major pomegranate growing regions of Western Maharashtra that revealed a high prevalence of bacterial blight incidence in Solapur, Sangli and Nashik districts. Four different isolates of *X. axonopodis* pv. *punicae* were obtained from highly infected plant materials collected during the field survey, out of these Nashik isolate was found to be most virulent. Inter Simple Sequence Repeat (ISSR) analysis separated these isolates into different clusters with Akkalkot-Solapur isolate being most divergent, whereas a similarity was observed between Deola-Nashik and Sangamner-Ahmednagar isolates.

Raghuwanshi *et al.* (2013) further reported that complete control in all four isolates was observed with Bordeaux mixture (1%); Captan (0.25%) + Copper oxychloride (0.3%), captan (0.25%) + Copper hydroxide (0.3%), Bromopol (500 ppm) + Copper oxychloride (0.3%), Streptocycline (250 ppm) + Copper hydroxide (0.3%), Streptocycline (500 ppm) + Copper hydroxide (0.3%) during in vitro study.

Eknath *et al.* (2015) assessed the genetic diversity of seven strains of *X. axonopodis* by using RAPD markers and differentiated them into seven major region representing Malegaon, Nampur, Satana, Khakurdi, Ramnagar, Rahuri and Nashik region. The genetic similarity within seven strains of *X. axonopodis* ranged between 72.72-100 %. Genetic relationship of strains *X. axonopodis* was also established and showed 72.72 %, 72.72 %, 72.72 % 92.30%, 94.11% and 100 % similarity with region of Malegaon, Nampur, Satana, Khakurdi, Ramnagar, Rahuri and Nashik region. Average polymorphism in strains of *X. axanopodis* based on RAPD analysis was 84.09 %.

2.3 Genomics and molecular markers

Durgac *et al.* (2008) determined the fruit characteristics and RAPD band patterns of six local cultivars, (‘ncekabuk’, ‘Ek_inar’, ‘Kannarı’, ‘Katırba_ı’,‘erife’ and ‘Tatlınar’) from Hatay, Turkey. Their results confirmed an existence of great level of morphological variation. The UPGMA cluster of fruit characteristics obtained in their analysis indicated that ‘Katırba_ı’ and ‘Kannarı’ were similar to each other and were separated from the rest of cultivars. Twenty-two RAPD primers that were used in the study generated total of 106 reproducible bands of which, 22% were polymorphic. The UPGMA dendrogram of RAPD data showed that ‘Tatlınar’ and ‘_erife’ were very closely related while ‘_ncekabuk’ was distinct from the other cultivars. Hence, discrepancies were detected between results of morphological and molecular data. Thus, they concluded that diversity among the fruit characteristics were not good indicators of genetic relatedness making molecular tools more valuable for study of such similarities.

Narzary *et al*. (2009) used PCR methods *viz.* directed amplification of minisatellite DNA (DAMD) and random amplification of polymorphic DNA (RAPD) to study genetic diversity among the wild genotypes of the *P. granatum* in India. The study was performed with forty-nine accessions representing two regions of Western Himalaya. Similarity coefficient value was reported to vary from 0.08 to 0.79 across different accessions. Their results indicated that DAMD (97.08%) revealed more polymorphism in comparison to RAPD (93.72%) suggesting the efficiency of these methods in unravelling the genetic variations in wild pomegranates in Western Himalayas.

Hasnaoui *et al*. (2010) developed 11 microstallite markers (SSR) for pomegranate and evaluated them on a set of 27 pomegranate accessions sampled in Tunisia. The markers displayed a total of 25 alleles, with number of alleles per locus ranging between 1 and 4, and observed heterozygosity from 0.037 and 0.592. The authors suggested that this set of SSR markers can be very useful for studies on genetic diversity assessment of germplasm, cultivars/varieties fingerprinting and pedigree analysis of pomegranate.

Mir *et al*. (2010) investigated the magnitude of variability in different morphological characters of ten pomegranate cultivars *viz.* Kabuli Kandhari, Chawla, Ganesh, Mridula, Jyoti, G-137, Dholka, Bedana, Kandhari grown in Karewa belts of Kashmir. They reported a high range of variability for plant height (cm), plant spread (cm), duration of flowering (days), fruit weight (g), fruit volume (cm3), number of seeds per fruit and cracking per cent. Phenotypic coefficient of variation was observed to be higher than genotypic coefficient of variation for all the characters under test. High heritability and high magnitude of genetic advance was recorded for plant height (cm), fruit weight (g), number of seeds per fruit and fruit set percentage. While, low heritability was recorded in days taken from flower opening to fruitlet development, ascorbic acid (mg/ 100ml), juice content (%), and number of fruits per plant and Anar butterfly incidence (%). Based on the results they concluded that three cultivars, Dholka, Bedana and Kandhari were best in terms of plant height, fruit set per cent, fruit volume and marketable produce and hence can be used for further breeding programme.

Pirseyedi *et al*. (2010) reported isolation and characterization of 12 polymorphic microsatellite markers from a repeat-enriched genomic library of pomegranate and assessment of the genetic diversity of these loci in 60 genotypes of pomegranate. All loci were found to be variable and the number of polymorphic alleles per locus ranged from two to five with an average of 2.9. The observed and expected heterozygosities ranged from 0.15 to 0.87 and 0.29 to 0.65, respectively. The polymorphic information content ranged from 0.26 to 0.61 (average: 0.43). They claimed this to be the first report on use of polymorphic microsatellite markers for *P. granatum* L.

Soriano *et al*. (2011) developed 117 microsatellite loci from a CT/AG-enriched pomegranate genomic library and analyzed them using eleven accessions. The polymorphism information content (PIC) value across all loci was reported to range between 0.09 and 0.71, with an average of 0.37. These markers could be useful tool for genetic diversity studies, mapping, and genotyping of pomegranate.

Ercisli *et al*. (2011) determined the genetic variability among 23 wild grown pomegranates collected from different parts of CoruhValley in Turkey using RAPD primers. Among eighty-six RAPD primers used, 12 were reported to produce reliable polymorphic patterns and generated 145 RAPD bands with band size ranging between 250 and 2400 bp. This study proves the suitability of RAPD technique as a tool for the management of plant collections and provides the scientific basis for selection and management of germplasm.

Wetzstein *et al*. (2011) evaluated the compositional changes, and fruit attributes like fruit volume and weight, aril weight and number, pericarp weight, seed weight, and juice/pulp content, fruits of variable sizes of cultivar Wonderful. Correlation studies indicated that fruit volume, fruit weight, and total aril weight are closely correlated and any of these characteristics can be used as an indicator of fruit size. They found that number of arils per fruit was highly correlated with fruit size with larger fruit containing greater numbers of arils in contrast to this the individual average aril weight, showed no significant relationship to fruit size. The authors suggested that crop production strategies aiming at increasing number of aril may prove to be a mean to obtain larger size fruits.

Ono *et al.* (2011) performed shotgun transcriptome sequencing of pomegranate fruit peel cDNA using RNA-Seq on the Illumina Genome Analyzer platform. Over 100 million raw sequence reads were obtained and assembled into 9,839 transcriptome assemblies (TAs) (>200 bp). Candidate genes for hydrolyzable tannin, anthocyanin, flavonoid, terpenoid and fatty acid biosynthesis and/or regulation were identified. Three lipid transfer proteins were obtained that may contribute to IgE reactivity of pomegranate fruit extracts. Furthermore, 115 SSR markers were identified from the pomegranate fruit peel transcriptome and primers were designed for 77 SSR markers. The pomegranate fruit peel transcriptome set provides a valuable platform for natural product biosynthetic gene and SSR marker discovery in pomegranate

Alamuti *et al.* (2012) used twelve simple sequence repeat (SSR) markers to evaluate the genetic background of 738 pomegranate accessions, belonging to the Iranian National Pomegranate Collection, from 23 provinces of Iran. Forty-three alleles were obtained with a mean of 3.59 alleles per locus with locus ABRII-MP26 showing the highest level of polymorphism. The mean values of expected heterozygosity and polymorphic information content were reported to be 0.521 and 0.458, respectively. They concluded that SSR markers are effective tools for conducting genetic diversity studies in pomegranate and developing strategies for germplasm management.

Parvaresh *et al.* (2012) studied the genetic diversity and phylogenetic analysis among 75 pomegranate genotypes from Iran, Japan, Turkmenistan, Russia, Italy and USA using microsatellite markers. Out of 25 pairs of primers used, 10 showed 12 polymorphic loci and revealed 38 alleles with an average of 3.16 alleles per locus and the mean of polymorphic information content (PIC) obtained was 0.35. Iranian genotypes were found to have highest genetic diversity supporting the hypothesis that Iran is the center origin of pomegranate.

Derazmahalleh *et al.* (2013) in a study tested seven microsatellite markers (SSR) on 194 accessions of the sweet pomegranate germplasm obtained from the Iranian National Pomegranate Base Collection which were collected from 23 different regions of Iran. All markers showed significant polymorphism among the studied samples. A total number of 29 alleles were observed, with the number of alleles per locus ranging between 2 to 8. The average polymorphism information content and heterozygosity of these markers were 0.807 and 0.847 respectively. Their result proves the suitability of microsatellite markers for genetic diversity studies.

Raina *et al.* (2013) used 47 SSR markers to assess the genetic diversity in thirty pomegranate genotypes including local cultivars, strains and exotic accessions, being maintained at Punjab Agricultural University, Ludhiana. A total of 3,154 alleles were amplified with an average of 105 alleles per genotype. The number of alleles detected in the study ranged from 1to 4 with an average of 2.15 alleles per primer pair. The polymorphism information content (PIC) ranged between 0.00 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 revealed some genotypes from same province clustered in one group *viz.* genotypes Kali Shirin, AnarShirin, ShirinAnar, AnarShirin, Mohamad Ali, AnarMoherab 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 collections made from Punjab. The study proved SSR markers to be an excellent genetic marker system for allelic profiling of genotypes in pomegranate germplasm.

Ferrara *et al.* (2014) carried out a two year study to evaluate 13 pomegranate genotypes, sweet and sour, of Italian and Israeli origin collected in Puglia region of South-eastern Italy, using SSR markers. They observed significant differences for many of the parameters investigated, in particular fruit and aril size and maturity index of fruits, titratable acidity, total polyphenol content and antioxidant activity of juices and oil content of seeds. Genetic analysis on the pomegranate collection identified 53 SSR loci able to distinguish different genotypes and estimate genetic distances. In the dendrogram, pomegranate genotypes clustered according to both geographical origin and morphological–chemical traits (skin colour, size, taste and polyphenolic content), suggesting that SSR markers could be effective to detect the genetic diversity of pomegranate. In general, morphological, chemical and genetic parameters allowed clear distinction between the Italian and Israeli genotypes.

Ismail *et al.* (2014) characterized six Egyptian pomegranate cultivars using physical and chemical characteristics of the fruit and two molecular markers; Inter simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP). ISSR and AFLP markers showed 53.00 and 90.70% level of polymorphism across cultivars respectively. Genetic similarity matrices estimated from ISSR and AFLP data, revealed similarity coefficients ranging from 0.77 to 0.94 and 0.33 to 0.73, respectively. Wide variations in fruit characteristics were also recorded *viz.* fruit weight ranging between 185 g to 479.4, firmness from 71.84 to 79.98 Newton, fruit peel thickness from 0.30 mm to 0.60 mm and total arils weight from 87.5 to 275 g. The volume of juice ranged from 62.41 to 71.81 ml/100 g arils with pH values varying between 2.9 to 3.3 and anthocyanin content from 1.47 to 10.03. The study confirmed that Egyptain cultivars of pomegranate have a wide variation in the morphological and chemical characteristics required for both fresh fruit and industrial purpose.

Nafees *et al.* (2015) conducted a study to estimate the morphological and molecular diversity in 42 pomegranate accessions collected from 10 different regions of Pakistan using 13 morphological traits of fruits and 29 SSR markers. Principal Component Analysis (PCA) of fruit length (mm), fruit diameter (mm), crown length (mm), hull thickness (mm), arils and seeds dimensions (mm) explained 93.9% of the total morphological diversity in first six principal components. Genetic analysis revealed the average value of MAF, GD, HZ and PIC to be 0.5981, 0.497 0.404 and 0.425, respectively. Among the primers used, the primer POM_AAC1 was found to be highly polymorphic with maximum PIC value of 0.55. The authors reported existence of a very high morpho-genetic diversity in selected pomegranate accessions and recommended the use of molecular markers for germplasm characterization as most of the accessions having similar morphological traits, showed dissimilarity for molecular relationship.

Sinjare (2015) used 12 SSR markers to evaluate the genetic diversity among 11 pomegranate cultivars collected from Kurdistan region of Iraq particularly. Seven out of 12 primers were reported to be polymorphic with a total of 25 alleles and an average of 2.08 alleles per locus. The mean values of expected (He) heterozygosity and content average of polymorphic information were 0.3315 and 0.2572 respectively and the genetic distance among the 11 varieties were found to range from 0.0606 to 0.2683.

2.4 Marker-Trait association for economic traits in pomegranate

Basaki *et al.* (2011) used microsatellite markers to identify informative markers associated with traits like 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. They used 30 primers out of which, seven pairs were found to be polymorphic producing 23 alleles in 202 pomegranate genotypes.

Their results confirmed a significant and positive correlation among these traits and marker MP26 was found to be linked with most traits studied in their research. Since all the used SSR loci, particularly, MP26 showed significant association with the studied traits, they concluded that it is possible to use these markers along with morphological traits in pomegranate breeding programs for identification of suitable parents for generating mapping populations and developing hybrid varieties.

Rania *et al.* (2012) characterized a set of 32 Tunisian pomegranate cultivars using specific microsatellite (SSRs) markers. Thirteen SSR primers used in this study produced polymorphic profiles representing a total of 46 genotypes and a total of 40 microsatellites ranging from 112 bp to 250 bp were scored. The number of detected alleles per locus varied from 1 to 6 with an average of 3 alleles per locus. The derived Neighbour-joining (NJ) dendrogram was constructed using DAS genetic distances which exhibited a genetic diversity structured independently from the geographical origin of cultivars and their denomination suggesting that Tunisian pomegranate cultivars may be characterized by a common genetic basis despite of their phenotypic divergences. A cultivar's identification key has also been established based on the multi-locus genotypes which permit unambiguous differentiation between varieties.

Hasnaoui *et al.* (2012) developed four polymorphic SSR markers and along with 11 previously published SSRs, used them to investigate molecular diversity of 33 pomegranate ecotypes. Twenty-two distinctive genotypes were identified based on the multi-locus profiles. Measure of allele richness (2.83 per locus) and heterozygosity (He=0.245; Ho=0.243), revealed a quite low genetic diversity reflecting the narrow genetic background of the plant material. The results of ordination and cluster analysis suggested that almost all the Tunisian cultivars share similar genetic background, and are possibly derived from a small number of introductions in ancient times. The authors recommended these SSR markers as a powerful tool for marker assisted selection (MAS) program and for QTL studies.

Nemati *et al.* (2012) evaluated the diversity among 31 Iranian pomegranate cultivars using fruit morphological characteristics and AFLP markers. Seven AFLP primer combinations were used to amplify a total of 112 polymorphic fragments (47.26%). AFLP data revealed low genetic diversity among cultivars. The relationship between fruit characteristics was analyzed using the principal component analysis (PCA). The cluster analysis based on both fruit characteristics and AFLP data depicted that cultivars were not grouped according to their geographic origins. Furthermore, the correlation between the diversity matrix based on fruit characteristics and Dice's genetic similarity coefficient was insignificant (r=0.06). The results obtained from this study can improve the conservation and management of pomegranate germplasm resources and could be helpful in optimizing breeding programs.

Noormohammadi *et al.* (2012) used three molecular markers including RAPD, ISSR and SSR to evaluate the genetic diversity of thirty six Iranian pomegranate genotypes. Based on molecular data, genetic parameters were calculated which consisted of effective alleles (Ne), Nei genetic diversity (H), Shannon index (I) and polymorphic information content (PIC). SSR markers being co-dominant in nature showed the highest value of genetic parameters aforementioned except PIC value among all markers. Combined data of three markers showed higher genetic diversity than two ISSR and RAPD markers. Principle Component Analysis (PCA) based on the first two components confirmed clustering. The homonymous, synonymous and/or mislabeled genotypes were identified using three molecular markers. The analysis of molecular variance (AMOVA) indicated no significant genetic variation (p= 0.27) between pomegranate genotypes in different localities (seven locality groups). Only 2% of overall genetic variation was due to differences among locality groups while 98% of variation was due to differences within the group.

Harel-Beja *et al.* (2015) reported the construction of a genetic map of pomegranate enriched with quantitative trait loci (QTLs) for seven traits using an F2 population generated from a cross between Nana and Black. The map includes 1092 SNP markers and covers 1141 centi morgans (cM) with an average of 1.17 cM between markers over 11 linkage groups. They identified twenty-five QTLs for fruit traits like total soluble solids (TSS), fruit weight and perimeter, seed hardness, aril color and weight, and plant height. Further, using a set of 346 SNPs, an association study was conducted where 233 of these SNPs, were mapped on the genetic map and were found to be distributed in the different linkage groups (LGs).These common markers were used to anchor the associated traits to the map. This genetic map will provide a useful resource for further genomics and genetic studies of pomegranate along with serving as a reservoir of markers for fruit improvement.

Singh *et al.* (2015) studied genetic diversity to explore the association mapping strategies for bacterial blight resistance in 87 accessions of pomegranate of Indian origin using 44 publicly available microsatellite markers. They identified 4 microsatellite markers linked with fruit weight, titratable acidity and bacterial blight severity. Marker PGCT001 was found to be associated with both fruit weight and bacterial blight. They clustered Daru, a resistant wild source for bacterial blight, as the closest relative of cultivated pomegranate. Their research demonstrated the effectiveness of microsatellites markers in developing population structure among the wild and cultivar collection of pomegranates for future use in association mapping studies.

3. MATERIAL AND METHODS

3.1 Plant material

A total of 25 Indian pomegranate genotypes, including four mutant lines and two wild forms along with popular Indian cultivars were selected for the present study, details of which are provided in the Table 1. All the 25 genotypes were subjected to molecular characterization, while only 23 popular genotypes (excluding Daru and Nana) were selected for fruit morphological & biochemical characterization. The genotypes were also screened for foliar, fruit spot and bacterial blight disease under natural field conditions.

3.2 The experimental work flow followed to accomplish the objectives of the present study have been detailed under following sub-headings

3.2.1 Phenotypic characterization

3.2.2 Biochemical analyses

3.2.3 Screening for foliar and fruit spot and bacterial blight

3.2.4 Molecular marker profiling

3.2.5 Marker-Trait Association

3.2.1 Phenotypic characterization

For phenotyping, hasta-bahar fruits (Feb-April, 2017) were selected as this is the most preferred bahar under Northern Karnataka region owing to lesser incidence of bacterial blight during this season. Fruits of selected cultivars were collected from UHS, Bagalkot pomegranate orchard, HRES, Tidagundi (Vijayapur) and IIHR, Hesaragatta, Bengaluru. Pomegranate genotypes studied in the present investigation and the collection site of respective genotypes are listed under Table 1. Twenty three popular pomegranate genotypes were subjected to fruit morphological and biochemical characterization. Fruits, after collection were carried to the laboratory and kept at 4°C

Table 1. List and source of collection of different pomegranate genotypes utilized in the present study

Sl. No.	Cultivar	Source of collection
1	Amlidana	IIHR, BENGALURU
2	Bhagwa	UHS, BAGALKOT
3	CO-1	HRES,TIDAGUNDI
4	Daru	IIHR, BENGALURU
5	Dholka	HRES, TIDAGUNDI
6	Early Bhagwa	UHS, BAGALKOT
7	G-137	HRES, TIDAGUNDI
8	Ganesh	UHS, BAGALKOT
9	Kabul Yellow	IIHR, BENGALURU
10	Kaladagi Local	UHS, BAGALKOT
11	KRS	HRES, TIDAGUNDI
12	Mridula	HRES, TIDAGUNDI
13	Nana	UHS, BAGALKOT
14	P-23	HRES, TIDAGUNDI
15	P-26	HRES, TIDAGUNDI
16	PhuleArakta	UHS, BAGALKOT
17	Ruby	HRES, TIDAGUNDI
18	Super Bhagwa	UHS, BAGALKOT
19	Tobesto	HRES, TIDAGUNDI
20	UHSP 23	HRES, TIDAGUNDI
21	UHSP 57	HRES, TIDAGUNDI
22	UHSP 81	HRES, TIDAGUNDI
23	UHSP 125	HRES, TIDAGUNDI
24	Wonderful	UHS, BAGALKOT
25	Yercaud	HRES, TIDAGUNDI

until analysis of biochemical parameters using the standard protocol as described hereunder. Six replicates were maintained for each analysis and all the reagents, solvents and standards were of analytical grade and for the molecular marker analysis, the reagents were of molecular grade.

3.2.1.1 Climatic conditions

Bagalkot is located in the northern region of Karnataka and positioned at 16°12′N, 75°45′E the average elevation in this area reaches approximately 610 m. The climate is warm and dry throughout the year and rainfall is scarce with an average annual rainfall of 518 mm and belongs to semi-arid region.

3.2.1.2 Phenotyping for fruit morphological characters

Three plants were randomly selected per genotype with three fruits from each plant, thus, nine fruits of each genotype were individually analysed for morphological characters. The list of observations for morphological and biochemical parameters is detailed in Table 2. Weight of the fruit was taken using a precision balance with an accuracy of 0.001g. Fruit volume was calculated by liquid displacement method. The length and diameter of the fruit and calyx were measured using digital vernier callipers with 0.001 mm accuracy.

The measurement of fruit length (mm) was done in the polar axis, *i.e.* between the apex and the end of stem. The maximum width of the fruit (mm), measured in the direction perpendicular to the polar axis, was taken as diameter. After recording the external morphological traits, the arils were separated manually from the fruits, and total number of arils was counted. Peel thickness and other parameters like length and breadth of arils and seeds were measured using the digital Vernier callipers. The measurement of rind thickness (mm) was performed on two opposite faces of the fruit in equatorial zone. Moisture per cent of arils was determined by drying the arils at 60°C in hot air oven until reaching a constant weight. The juice of arils were analysed for biochemical parameters.

Table 2. List of observations recorded for morphological and biochemical characters in different pomegranate genotypes

Sl. No.	Characters	Particulars
Morphological parameters		
1	Fruit weight (g)	Precision balance
2	Fruit length (mm)	Digital vernier callipers with 0.001mm accuracy
3	Fruit diameter (mm)	Digital vernier calipers with 0.001mm accuracy
4	Fruit length/width	Ratio calculated
5	Fruit volume (cm^3)	Liquid displacement methods
6	Fresh wt. of 100 arils (g)	Precision balance
7	Dry wt. of 100 arils (g)	Precision balance
8	Moisture %	Oven drying arils at 60°C until constant weight
9	Crown length (mm)	Digital vernier callipers with 0.001mm accuracy
10	Peel weight (g)	Precision balance
11	Aril weight (g)	Precision balance
12	Seed %	Percent ratio calculated
13	Skin %	Percent ratio calculated
14	Total No. of Arils/fruit	Manual counting
15	Aril length (mm)	Digital vernier callipers with 0.001mm accuracy
16	Aril width (mm)	Digital vernier callipers with 0.001mm accuracy
17	Seed length (mm)	Digital vernier calipers with 0.001mm accuracy
18	Seed width (mm)	Digital vernier callipers with 0.001mm accuracy
19	Rind thickness (mm)	Digital vernier calipers with 0.001mm accuracy
20	Red coverage of Peel (%)	Visual observation
21-23	Fruit Colour	Hunter’s colour Lab, Colorimeter- L a b
24-26	Aril Colour	Hunter’s colour Lab, Colorimeter- L a b
27	Days to flowering	Days counted from pruning to flowering
28	Days to maturity	Days counted from flowering to harvesting
Biochemical parameters		
29	Anthocyanin content (mg/L)	pH differential method
30	Ascorbic Acid (mg/100gm)	Dye (dichlorophenol indophenol) binding method
31	Titratable Acidity (%)	Titration method with 0.1 N NaOH (pH 8.1)
32	pH of the Juice	pH-meter
33	Fruit Juiciness % (per 100gm aril wt.)	Extracted juice from 100 arils and measured as weight/weight with aril wt.
34	Total Sugars (%)	Phenol Sulphuric Acid method
35	Reducing Sugars (%)	Dinitrosalicylic acid (DNS) method
36	Non-Reducing Sugars (%)	(Total Sugars - Non-Reducing Sugars)
37	TSS (°Brix)	Refractometer

Fruit and aril colour

Colour measurements were performed using a colorimeter (Hunterlab Colorflex EZ). Skin colour measurements were taken along the equatorial axis of each fruit. Three readings of each colour index in the Hunter scale (L, a, b) were taken per fruit, making a total of 27 measurements per cultivar. Similarly, nine measurements of aril colour were also taken per cultivar. The instrument was standardized during each sample measurement with a black and a white tile, and the colour values represented whiteness or brightness/darkness (L), redness/greenness (a) and yellowness/blueness (b).

Estimation of qualitative traits:

Three traits *viz.* juice colour, seed hardiness and shape of the fruit were estimated qualitatively and were subjected to frequency distribution. Seed hardiness and shape of fruit were estimated based on pomegranate descriptor (DUS descriptor NBPGR).

3.2.2 Biochemical estimation

3.2.2.1 Estimation of juice biochemical parameters

Estimation of fruit juiciness, pH and total soluble solids

Fruit juiciness percentage was determined by extracting the contents of 100 g of arils in six replicates per genotype using an electric juice extractor. The pH values were measured using a pH-meter. The TSS contents were recorded in an HI9680I refractometer (0-85%) at 26.50 °C with values being expressed as °Brix.

Estimation of Titratable acidity: The titratable acidity was determined in accordance with procedure given by AOAC (1984).

Reagents: 0.1 N Sodium hydroxide solution (pH 8.1) and phenolphthalein indicator.

Procedure

Ten ml of pomegranate juice sample was diluted to 100 ml with distilled water from which 10 ml sample solution was taken in a beaker and to it 2-3 drops of phenolphthalein indicator was added. The sample solution with phenolphthalein indicator was further titrated against 0.1 N NaOH till the appearance of pink colour as end point.

Calculation

Titratable acidity (TA) was calculated as per cent and express as g citric acid per 100 ml of sample. (Equivalent weight of citric acid = 64)

$$\text{TA (\%)} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Vol. made(ml)} \times \text{Eqv. wt. of acid} \times 100}{\text{Vol. of sample taken (ml)} \times \text{Wt. or vol. of sample taken} \times 1000}$$

Estimation of ascorbic acid: Ascorbic acid was estimated using Dye (2, 6-dichlorophenol indophenol) binding method using Oxalic acid as titrating medium.

Reagents: Oxalic acid (4%), dye solution (42 mg sodium bicarbonate was weighed into a small volume of distilled water, into this 52 mg of 2, 6-dichlorophenol indophenol was dissolved and the volume was made up to 200 ml with distilled water)

Working standards: 10 ml of the stock solution was diluted to 100 ml with 4% oxalic acid. The concentration of working standard was 100µg/ml.

Procedure

Five ml of working standard was pipette into a 100 ml conical flask and to this solution 10 ml of 4% oxalic acid was added and was titrated against the dye till the appearance of end point *i.e.* pink colour persisting for few minutes (V1). Further 5 g sample was extracted in 4% oxalic acid and volume was made up to 100 ml and centrifuged. Five ml of the supernatant was taken and added with 10 ml of 4% oxalic acid and titrated against the dye till appearance of end point (V2).

Calculation

$$\text{Ascorbic acid (mg/100g sample)} = \frac{0.5\text{mg x V2 x 100ml x 100}}{\text{V1ml x 5ml x weight of sample}}$$

Estimation of Anthocyanin

Total anthocyanin was estimated by pH differential method using two buffer systems; potassium chloride buffer, pH 1.0 (25 mM) and sodium acetate buffer, pH 4.5 (0.4 M) (Giusti and Wrolstad, 2001). The samples were diluted by a potassium chloride buffer until the absorbance of the sample at 510 nm wavelength was within the linear range of the spectrophotometer (Cecil 2010 UV–visible). This dilution factor was later used to dilute the sample with the sodium acetate buffer. The wavelength reading was performed after 15 min of incubation, four times per sample, diluted in two different buffers and at two wavelengths *viz.* 510 nm and 700 nm.

The total anthocyanins content was calculated by using following formula:

Total anthocyanins = [(A × MW × DF × 100)/MA]

Where, A = (A510 - A700) pH1.0 - (A510 - A700) pH4.5; MW: molecular weight (449.2); DF: dilution factor; MA: molar absorptive coefficient of cyaniding-3-glucoside (26.900).

Results were expressed as mg/L of juice.

Estimation of Sugars: Both the total sugars and reducing sugars were estimated for 23 popular Indian genotypes selected for the present study and the standard graph was used for the estimation of sample values and converted to percentage.

Estimation of total sugars:

Total sugars were estimated using phenol sulphuric acid method (Dubois *et al.*, 1956; Krishnaveni *et al.*, 1984), which is based on the principle that in hot acidic medium glucose is dehydrated to hydroxymethyl furfural that in turn forms a green coloured product with phenol and has absorption maximum at 490 nm. The protocol followed is as follows:

Reagents and standards

Phenol 5%: Redistilled (reagent grade) phenol (50 g) dissolved in water and diluted to one litre. Sulphuric acid 96% reagent grade. *Standard glucose:* Stock—100 mg in 100 ml of water. Working standard—10 ml of stock diluted to 100 ml with distilled water.

Procedure

1. 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standards were pipette out into a series of test tubes. 25 µl of the sample solution was pipette out in a separate test tube. Volume in each tube was made up to 1 ml with distilled water. Blank consisted of 1 ml of water.

2. One ml of phenol solution was then added to each tube followed by 5 ml of 96% sulphuric acid. The contents were then left at room temperature to lower down the temperature of the contents in tube.

3. Readings were taken in spectrophotometer at 490 nm and amount of total carbohydrate present in the sample solution was calculated by using the standard graph.

Calculation

Absorbance corresponding to 0.1 ml of the test = x mg of glucose 00 ml of the sample solution contains = x /0.1 X 100 mg of glucose= % of total carbohydrate present.

Estimation of reducing sugars

Reducing sugars were estimated using dinitrosalicylic acid method (Miller, 1972). The protocol followed is as follows:

Reagents and standards

Dinitrosalicylic Acid Reagent (*DNS Reagent*): Prepared by dissolving 1g dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphite by continuous stirring in 100 ml 1% NaOH and stored at 4°C.

40 % Rochelle salt solution (Potassium sodium tartrate).

Standard glucose

Stock- 100 mg glucose dissolved in 100 ml water

Working standard- 10 ml of stock diluted to 100 ml with distilled water and stored at 4°C after adding few drops of toluene.

Sample preparation

100 mg sample was used to extract sugars with hot 80% ethanol twice (5 ml each time). The supernatant was collected and ethanol was evaporated by keeping it on a water bath at 80 °C further 10 ml water was added to dissolve the sugars.

Procedure

1. 0.5 to 2 ml of the extracted sample was pipette out in series of test tubes and the volume was equalized to 2 ml in each test tube with distilled water followed by addition of 2 ml of DNS reagent.

2. The contents were heated in a boiling water bath for 5 min and 1 ml of 40% Rochelle salt solution was added when the contents of the tubes were still warm. After the contents cooled down to room temperature, the intensity of dark red colour was read at 510 nm.

3. Standard graph was plotted by running a series of standards using glucose (0–500 µg), with concentration of the standard on the x-axis versus absorbance on the y-axis.

Calculation

The amount of reducing sugars present in the sample was calculated using the standard graph expressed in terms of % of reducing sugars present.

Non-reducing sugar was calculated by subtracting reducing sugar from total sugar for all the selected genotypes in six replications and their mean value was considered for statistical analysis.

Non-reducing sugars = Total sugars - Reducing sugars

3.2.3 Screening for foliar and fruit spot and bacterial blight

Symptoms

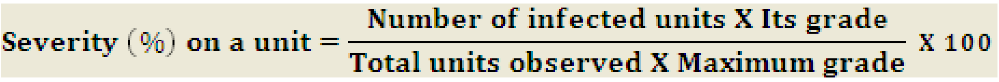
Symptoms on the leaf appears as small 2 to 5 mm, irregular, prominent water soaked spots which in later stages become necrotic with light to dark brown centre surrounded by prominent water soaked margins. At the advance stage of the disease, the individual spots coalesce giving an eventual blighted appearance. Lesions on the stem are dark brown to black that often leads to cracking and breaking of branches. On fruits, the lesions appear as irregular, shining brown to black spots with Y or L shaped cracking or splitting of pericarp (Arjunan *et al.*, 1999; Rawal, 2006).

For recording disease severity on leaves, five twigs were selected each from all four sides of the plant and on fruits, five fruits each from all four direction of the plant were examined.The disease severity was recorded on a 1-5 scale as follows:

Table 3. Scoring system for severity grade (bacterial blight)

Sl. No	Severity Grade to be allotted	Observation method on different units	Resistance types
0	0	Disease not seen	Immune
1	1-10	Disease not easily visible, very few units/plant found diseased.	Resistance
2	11-25	Disease visible easily in each direction, but most (75%) of the units look healthy	Moderately resistance
3	26-50	Both disease and healthy units are equally observed	Moderately susceptible
4	51-75	Disease seen very easily, with only some healthy units	susceptible
5	76-100	Almost all units are diseased with few healthy units.	Highly susceptible

Severity of disease was calculated using the following formula



$$\text{Severity (\%) on a unit} = \frac{\text{Number of infected units X Its grade}}{\text{Total units observed X Maximum grade}} \text{ X } 100$$

Foliar spots

Five plants per genotype were selected randomly and leaves on 5 twigs in different directions were used to determine the disease incidence. Total number of healthy and infected leaves was counted and % disease severity was calculated by the following formula:

$$\frac{\text{Total No. of infected leaves}}{\text{Total No. of leaves}} \text{ X } 100$$

Fruit spots

Total no. of fruits per plant was counted on 3 selected plants and no. of healthy and infected fruits were counted. The percent disease infection was calculated by the following formula:

$$\frac{\text{Total No. of infected fruits}}{\text{Total No. of fruits}} \text{ X } 100$$

Statistical Analysis

Analysis of variance

The analysis of variance (ANOVA) for all the traits was carried out by using the mean phenotypic data.

The structure of ANOVA is as follows

Source of variation	d.f.	MSS	Expected value of MSS	Cal F.
Replication	(r-1)	M_1	-	
Genotypes	(g-1)	M_2	$\sigma^2e+ r\sigma^2g$	M_2/M_3
Error	(r-1) (g-1)	M_3	σ^2e	
Total	(rg-1)	$M_1+M_2+M_3$		

Mean and range

The mean and range of all the selected traits were calculated based on replicated means of each cultivar.

i. Mean $(\bar{X}) = \frac{\text{Sum of the observations of all the plants}}{\text{Number of plants}}$

ii. Range = The minimum and maximum values for each trait

Frequency distribution:

Three qualitative traits *viz.* juice colour, seed hardiness and fruit shape were subjected to frequency distribution using SPSS ver.16 software.

Estimation of genetic variability component

a) Coefficient of variability

Both genotypic and phenotypic coefficients of variability were computed for all the traits under study according to the method suggested by Burton and Devane (1953).

i) Genotypic coefficient of variability (GCV)

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

ii) Phenotypic coefficient of variability (PCV)

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

Where,

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviation

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

GCV and PCV values were classified as low, moderate and high as indicated by Sivasubramanian and Menon (1973) as follows:

0-10% : Low

10-20% : Moderate

20% and above : High

b) Heritability (h^2b.s)

Heritability in broad sense was computed as the ratio of genetic variance to the total phenotypic variance and expressed as percentage.

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

Where,

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

The heritability percentage was categorized as low, moderate and high as given by Robinson *et al.* (1949).

0-30% : Low

30-60% : Moderate

60% and above : High

c) Genetic advance (GA)

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

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

Where,

h^2 = Heritability in broad sense

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

σ_p = Phenotypic standard deviation

d) Genetic advance as per cent of mean (GAM)

$$\text{GAM} = \text{GA}/(\text{X x } 100$$

Where,

GA= Genetic advance

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

Genetic advance as per cent mean was categorized as low, moderate and high as given by Johnson *et al.* (1955).

It is as follows.

0-10%	: Low
10-20%	: Moderate
20% and above	: High

Principal component analysis

Principal component analysis is a variable-reduction technique that aims at reducing a large set of variables into a smaller set of ‘artificial variables’, called principal components, which account for most of the variance in the original variables. A total of 35 quantitative traits were subjected to PCA analysis using SPSS (version 16.0) software by Factor analysis. Principal components were obtained by extraction method of PCA and only the principal components showing Eigen value of >3 were considered. The linearity of the variables was checked using scree plot.

Correlation analysis

The correlation coefficients were computed to determine the degree of association between various morphological as well as biochemical character.

Phenotypic correlations were computed by using the formula given by Webber and Moorthy (1952).

$$r_p = \frac{CovXYp}{\sqrt{\sigma_p^2 \, x \, X \, \sigma_p^2 y}}$$

Where,

r_p= Phenotypic correlation

$CovXY_p$= Phenotypic covariance between the characters ‘x’ and ‘y’

$\sigma_p{}^2x$ and $\sigma_p{}^2y$ = Phenotypic variance of the characters ‘x’ and ‘y’ respectively

Further, the phenotypic correlation coefficients were compared against Table value at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance (Fisher and Yates, 1963).

Path Coefficient analysis

Path coefficient analysis was carried out separately for morphological and biochemical characters by using the correlation coefficients to determine the direct and indirect effects of all the components on fruit weight or total fruit yield/plant as suggested by Wright (1921) and illustrated by Dewey and Lu (1959).

Path coefficients were obtained by solving the simultaneous equations, which express the basic relationship between correlations and path coefficients. The equations were as follows

r1.y = P1y + r1.2 P2y + r1.3 P3y + ... + r1.k Pky

r2.y = r2.1 P1y + P2y + r2.3 P3y +. . . . . . . + r2.kPky

...

...

rk-1.y = rk-1.1P1y + r k-1.2 P2y + rk-1.3 P3y +. . . . . . . + Pk-1y

Where, r1.y to rk-1.y denote the correlation coefficients between independent characters 1 to k-1 and dependent character ‘y’, r1.2 to rk-2.k-1 denote the correlation coefficients between all possible combinations of independent characters. P1y to Pk-1y denote the direct effects of characters 1 to k-1 on character y.

Genetic diversity analysis

Multivariate analysis using D^2 statistics

Mahalanobis D^2 – statistics was used to assess the genetic divergence between 25 pomegranate cultivars by using the software Indostat version 5.1.

The generalized distance between any two populations is defined as,

$$D = \Sigma \lambda ij \delta i \delta j$$

Where,

λij = The reciprocal matrix to the common dispersion matrix

δi = The difference between the two mean values of the two populations for ith character ($\mu i1$- $\mu i2$)

δj = The difference between the mean values of the two populations for the jth character ($\mu j1 - \mu j2$)

μ = Vector mean values for all the characters

The formula for the estimation of distance, D^2 from samples:

$$D^2p = d1\ (S\text{-}1)\ d$$

Where,

D^2p = Square of the distance considering P variables.

d1 = (Xi1 – Xi2)

X = Vector of mean values of all the characters

S-1 = inverse of variance covariance matrix

Formula for computation of D^2 values, which requires inversion of the matrix, becomes complicated especially when the number of variables under consideration is more. Hence, the original correlated unstandardized variables (Xi) were transformed to standardized uncorrelated variables (Yi) so that the computation of D^2 values reduce to simple summation of squares of the differences between values of transformed variables of the two population i.e., D^2i.

From the newly transformed uncorrelated variables, the square of the distance was computed using the following formula,

$$\overline{D^2} = \Sigma\ (\overline{Y}i1 - Yi2)^2$$

Where,

$\overline{Yi1}$ = Vector of transformed mean values, for first genotype

$\overline{Yi2}$ = Vector of transformed mean values, for second genotype

The square root of the D^2 values gives the generalized distance (D) between the two populations. The D^2 values were arranged in a matrix form. The significance of D^2 values between any two clusters was tested using the following formula,

$$F = \frac{(n1 + n2 - p - 1)}{(n1 + n2 - 2)\ P} \text{ x } \frac{(n1\ n2)\ D^2}{(n1 + n2)}$$

This computed F was compared with Table F value at 5 per cent and 1 per cent levels of significance with P(number of characters) and (n1 + n2 – p – 1) degrees of freedom

Determination of population constellation

All the n (n –1)/2 D^2 values were considered for determining the population constellation. This was realized by using Tocher’s method. The criterion used in clustering by this is that any two varieties belonging to the same cluster, should at least, on an average, show a smaller D^2 value than those belonging to different clusters. As per the device it was to start with two closely associated population and finds a third population, which had the smallest average D^2 from these two. Similarly, the fourth was chosen to have a smallest average D^2 from the first three and so on. The permissible increase in D^2 values for clustering into the same group was fixed approximately nearer the maximum D^2 value shown by a population to the nearest population. This procedure was continued till D^2 values of all the pairs of genotypes were exhausted. After the formation of the clusters inter and intra group distances were calculated. The square root of the average D^2 values obtained from the above represents the distance (D) between and within clusters.

3.4 Molecular marker profiling

3.4.1 Collection of samples and DNA extraction

Leaf samples of the selected genotypes were collected from the field, wrapped in aluminium foil and properly labelled. The samples were then carried in ice basket to the laboratory where they were stored at −80°C till extraction.

DNA extraction was performed using the cetyltrimethyl ammonium bromide (CTAB) method described by Doyle and Doyle (1987) with some modifications.

3.4.2 The protocol followed is as follows:

1. 100 mg of leaf samples was ground to a fine powder in liquid nitrogen along with 1 mg of polyvinylpyrrolidone (PVP) (v/v).

2. The powdered samples were then transferred to 2 ml Eppendorf tube, mixed with 500 µl of extraction buffer (3% CTAB; 100 mMTris–HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.3 M NaCl; 4% polyvinylpyrrolidone; 2% β-mercaptoethanol) and gently shaken to mix the buffer with the sample. Samples were then incubated at 65°C for 45 minutes in water bath

3. Samples were cooled to room temperature and to this mixture equal volume of chloroform/isoamylalcohol (24:1) was added, mixed thoroughly and centrifuged at 15000 rpm for 15 minutes at room temperature. This step was repeated twice.

4. The clear supernatant was collected and transferred to 1.5 ml Eppendorf tube and to this, 200 µl potassium acetate and 500 µl of chilled isopropanol was added and kept overnight at -20°C.

6. The DNA was pelleted by centrifugation at 15 000 rpm for 15 minutes at 4°C.

7. The supernatant was then decanted and DNA Pellets were washed with 70% ethanol and centrifuged at 10,000 rpm for 5 minutes at 4°C. This step was repeated twice.

8. The ethanol was then poured out and pellets were air dried for 3-4 hours. Afterwards the dried pellets were dissolved in 150 µl of TE buffer (10 Mm Tris–HCl, pH 8.0, 1 mM EDTA, pH 8.0). The extracted DNA was then stored at −20°C for further use.

3.4.3 Qualitative and Quantitative estimation of DNA

DNA concentration was determined by measuring the absorbance at 260 and 280 nm in a Nanospectometer and the purity of the DNA was estimated by the ratio of absorbance at 260 nm to that of 280 nm (A260/A280). A ratio of in and around 1.8 represented pure DNA. Qualitative analysis of DNA was done by running the extracted genomic DNA samples on 0.8% agarose gel using 0.5X TAE buffer (10 mMTris-HCl and 1 mM EDTA, pH. 8.0). The gels were stained with ethidium bromide and visualized under UV fluorescence in Gel Documentation system

3.4.4 Genotyping and Amplification

Seventy two microsatellite markers were used in this study. Working DNA of concentration 50 ng was prepared from stock DNA of known concentration (quantified using nanospectrometer). For polymerase chain reaction, 24 polymorphic microsatellite markers (listed in Table 4) were diluted to a concentration of 10 Pico moles/µl from the stock. PCR was performed for 10 µl reaction mixtures, the details of components of PCR reaction mixture alongwith their concentration and the PCR protocol followed is presented hereunder.

Table 4. List of polymorphic primers used for molecular characterization of pomegranate genotypes

Sl. No.	Locus	Forward primer	Reverse primer	Reference
1	ABRII-MP26	TTTCTCGAAGAATTGGGTAA	CTGAGTAAGCTGAGGCTGAT	Pirseyedi *et al*., 2010
2	PGKVR031	CCCTAACAATTATCCCATTTGGT	GTGCGTCCAAAAGCTTTCAT	Ravishankar *et al*.,2015
3	PGKVR038	CACTTTGCCTGCCAAAATCT	CTCGCCATCTTCCCAATCTA	Ravishankar *et al.*, 2015
4	PGKVR092	GGTGTTGGCGATGAAGATTT	GTCGAAGGTCGTGGAATCAT	Ravishankar *et al.*, 2015
5	PGKVR094	ATGCTCGGTGGAATGACTCT	CTTCCCGACTCGACTTGAAC	Ravishankar *et al.*, 2015
6	PGKVR100	CCCATCGATCATCTTTCGTT	CGTGTGGAAAGTCATCGACA	Ravishankar *et al.*, 2015
7	PGKVR101	TTCAACACCTTCGTGCCATA	TACCACGCAGAGCACAACTC	Ravishankar *et al.*, 2015
8	PGKVR105	GGAAGAGTTGGACTGGGAGA	CCGCTCCTTCTACTCCTCCT	Ravishankar *et al.*, 2015
9	PGKVR112	TGGAGACGTTTCCCAAGTTT	GGGCTCACACCAAAAGAAAA	Ravishankar *et al.*, 2015
10	PGKVR113	CCTCCCCCTTGTCCTTTATC	ATCCAAGTTTGACCGTCTGG	Ravishankar *et al.*, 2015
11	PGKVR114	AGGGAGATTATGCGGGAGTT	CCGAGCTCTACTCCCTCTCC	Ravishankar *et al.*, 2015
12	PGKVR115	TCCCCCAAAAACAAGTTCAG	GGGTTCACTCCGAAAAGAGA	Ravishankar *et al.*, 2015
13	PGKVR119	CGCTTGGTATTCGTTGCTTT	GTCCTTCGCGCACATTTACT	Ravishankar *et al.*, 2015
14	PGKVR122	TGGTTTCATCGAATGAATGG	TGCCTTGCTTTCCCTCTACT	Ravishankar *et al.*, 2015
15	PGKVR126	CGGTTATCTCAAGTGCAACG	TGTCGGAACATATTCCACTGA	Ravishankar *et al.*, 2015
16	PGKVR127	TGTCACGGTTTTAGGTGCAA	GGGAGTGTGATGGAAGAGGA	Ravishankar *et al.*, 2015
17	PGKVR132	GAGGGTGACTGAGCTGAAGG	CCCATTCACGTGTCTCTCCT	Ravishankar *et al.*, 2015
18	PGKVR137	GCCGACACAAGAGTCCAGTT	CTCCATCCATCGGAGTCTCT	Ravishankar *et al.*, 2015
19	PGKVR139	TCCCGAATCAAAGAGAGGAA	CGTCTCTGTCAGGAGGAAGAA	Ravishankar *et al.*, 2015
20	PGKVR149	CGATGAGCAGAGCAACGATA	TCTGAACGTCTGTCCCATTTC	Ravishankar *et al.*, 2015
21	PGKVR152	ATCCCAAAAGGCAGATTTCC	CGGCTCGACGAAGAACTAAC	Ravishankar *et al.*, 2015
22	PGKVR153	TTTGGAAGTTTGACTGGAGGA	GCATTCTGGGATTGGAAGAA	Ravishankar *et al.*, 2015
23	PGKVR154	GGCAATCAGTGCCATGAATA	CCAATCGAATTGAATGCTCA	Ravishankar *et al.*, 2015
24	PGKVR165	TGGGCCATTTATAGGCAAAG	CTTTTTCCCGGTTTCTCCTC	Ravishankar *et al.*, 2015

Table 5. PCR reactions for molecular markers used in the present study

Components	Concentration	PCR reaction (10 µl)
Primers (F+R)	10 Pm/µl	0.5
PCR master mix	2 X	5
Template (DNA)	50ng/ µl	1
Nuclease free water		3.5
Total		**10.0 µl**

Table 6. PCR protocol followed for microsatellite primers for different pomegranate genotypes

Sl.No.	Steps	Microsatellite Primers		Cycles
		Temperature (°C)	Time	
1	Initial denaturation	94	5 min	40 cycles
2	Denaturation	94	30 sec	
3	Annealing	60*	30 sec	
4	Primer extension	72	45 sec	
5	Final extension	72	10 min	
6	Hold	4	∞	

*Specific annealing temperature as per the Primers

3.4.5 Electrophoresis

PCR products were first checked for amplification on 1.5% Agarose and amplified products were then size separated on 3.5% - 4.0% Agarose for polymorphism check. The bands were scored for each allele by allele sizing. Scoring was done only for polymorphic markers.

Molecular marker and allelic diversity

Molecular marker diversity

A total of 72 SSR markers available in the public database were selected for the study out of which, 24 markers exhibiting polymorphism across the 25 pomegranate genotypes were used to screen the genotypes. Scoring was done primer wise based on the difference of size of allelic polymorphism for the respective alleles across 25 pomegranate germplasm.

Each polymorphic marker was characterized for number of alleles per locus and gene diversity using 25 pomegranate germplasm.

For the purpose of assessing genetic diversity leading to the preparation of a dendrogram, gels were scored by allele sizing and the respective size of alleles were used to compute pair-wise dissimilarity Coefficients (Jaccard, 1908), with 1000 bootstrap value and the dissimilarity matrix thus obtained was subjected to cluster analysis using the UPGMA (un-weighted pair-group method with arithmetic average) algorithm on Darwin version 6.0 software. The diagonal matrix was then submitted to cluster analysis using the maximum likelihood method and a genetic distance of dendrogram was built with the help of bar diagram.

He- expected heterozygosity for a genetic marker was calculated as:

$He = 1 - pi^2$, where pi is the allele frequency of the i^{th} allele

The expected heterozygosity of each loci was defined as $He= 1–Spi^2$, where pi is the frequency of the i^{th} allele (Nei 1978). The observed heterozygosity was calculated according to Nei (1978), *i.e.,* it was given as Ho = No. of accessions harbouring heterozygous genotypes at the i^{th} allele/No. of total accessions.

3.2.4 Marker-trait association:

Single marker analysis (SMA)

Single marker analysis was performed to tag potential SSR markers linked to the phenotypic data of fruit morphological and biochemical traits among the diverse pomegranate genotypes and genotypic data of 24 markers. The statistical methods based on step-wise linear regression method (Haley and Knott, 1992) as suggested Collard *et al.* (2005) using SPSS Version 16.0 software.

4. EXPERIMENTAL RESULTS

The present study was conducted with an objective of performing a complete characterization of popular Indian genotypes of pomegranate at morphological, biochemical and molecular levels, to identify the genotypes superior for different traits. Twenty five genotypes in total were selected for the study out of which two (Daru and Nana) are wild forms of pomegranate. The location of collection of these genotypes has already been presented in the material and methods chapter. All the 25 genotypes were subjected to molecular characterization, while only 23 popular genotypes (excluding Daru and Nana) were selected for morphological and biochemical characterization. The study was carried out at University of Horticultural Sciences, Bagalkot, Karnataka. The details of the results of the experiments have been presented under the following sub-headings.

4.1 Phenotypic characterization

4.2 Molecular characterization

4.3 Marker- Trait association

4.1 Phenotypic characterization

A total of 37 morphological and biochemical traits were recorded for the study, for the fruits obtained during hastha-bahar (Feb-April, 2017). The replicated data were subjected to various statistical analysis such as analysis of variance (ANOVA), analysis for genetic variability and heritability components, principal component analysis (PCA), correlation and path coefficient analysis, Mahalanobi's D^2 analysis. The details of the results are discussed in this chapter accordingly.

4.1.1 Analysis of Variance (ANOVA)

All the 37 traits including morphological and biochemical traits were subjected to Analysis of Variance (ANOVA), the result of which has been presented in Table 7. For ANOVA the source of variation was partitioned between genotypes and between replications (within genotypes).

Table 7. Analysis of Variance (ANOVA) for morphological and biochemical traits evaluated for different pomegranate genotypes

Sl. No.	Characters	Mean Sum of Squares			F Cal	
		Replication (2)	Treatment (22)	Error (44)	Replication	Treatment
1	Fruit weight (gm)	1817.21	35307.54	332.26	5.46**	106.26***
2	Fruit length (mm)	8.93	966.86	21.97	0.40	44.008***
3	Fruit diameter (mm)	0.85	584.91	33.81	0.00	17.29***
4	Fruit length/width	0.00	0.03	0.00	0.70	5.61***
5	Fruit volume (cm^3)	854.28	37516.31	441.99	1.90	84.88***
6	Fresh wt. of 100 arils	9.98	236.20	3.03	3.29*	77.82***
7	Dry wt. of 100 arils	0.09	16.21	0.05	1.90	341.06***
8	Moisture %	4.06	85.36	2.43	1.70	35.11***
9	Crown length (mm)	0.54	30.10	2.13	0.30	14.15***
10	Peel weight (g)	23.93	4839.65	32.89	0.70	147.13***
11	Aril weight (g)	1648.50	15070.67	379.13	4.35*	39.75***
12	Seed %	59.54	119.15	19.19	3.10	6.20***
13	Skin %	59.54	119.15	19.19	3.10	6.20***
14	Total No. of Arils/fruit	9017.58	164807.52	1805.40	4.99*	91.28***
15	Aril length (mm)	0.28	5.85	0.23	1.20	25.64***
16	Aril width (mm)	0.41	6.65	0.33	1.20	19.88***
17	Seed length (mm)	0.71	1.83	0.11	6.34**	16.22***
18	Seed width (mm)	0.13	0.15	0.06	2.10	2.43**
19	Rind thickness (mm)	0.98	2.09	0.43	2.30	4.90***
20	Red coverage of Peel (%)	73.51	1167.72	31.95	2.30	36.54***
21	Days to flowering	0.00	1854.31	5339.10	0.00	0.347
22	Days to maturity	0.00	2229.90	5501.11	0.00	0.405
Biochemical traits						
23	Anthocyanin content (mg/L)	34.53	742.94	8.53	4.04*	87.13***
24	Ascorbic Acid (mg/100gm)	5.48	144.07	6.66	0.80	21.62***
25	Titratable Acidity (%)	0.00	0.18	0.00	0.80	172.25***
26	pH of the Juice	0.01	0.31	0.01	1.30	28.38***
27	Fruit Juiciness % (per 100gm aril wt.)	10.79	381.42	16.97	0.60	22.47***
28	Total Sugars (%)	38.92	706.83	20.27	1.90	34.87***
29	Reducing Sugars (%)	18.98	213.01	16.47	1.20	12.93***
30	Non-Reducing Sugars (%)	3.96	413.17	28.44	0.10	14.52***
31	TSS (°Brix)	0.94	11.01	0.50	1.90	21.83***
Colour parameters						
32	Fruit Colour (L)	6.51	245.55	31.93	0.20	7.68***
33	Fruit Colour(a)	33.84	470.82	31.19	1.10	15.09***
34	Fruit Colour(b)	27.72	195.56	18.29	1.50	10.69***
35	Aril Colour(L)	6.59	518.51	3.29	2.00	157.82***
36	Aril Colour(a)	1.39	140.88	1.41	1.00	132.11**
37	Aril Colour(b)	1.32	139.73	1.03	1.30	135.29***

*-Significance at 5% Probability, **-Significance at 1% Probability,
***-Significance at 0.1% Probability

Analysis of variance revealed significant variation between genotypes for almost all the traits at 1% level of significance, under study.

4.1.2 Mean, range, CV and CD

Mean value with their standard error mean (SEm), their respective range, coefficients of variation (CV %) and CD for different morphological and biochemical characters of 23 popular genotypes of pomegranate has been presented in Table 8. A very wide range of variation was observed in several fruit characters like fruit weight where the fruits weighing from 56.01 g (UHSP 23) to 505.00 g (Ganesh) with an average of 195.91 g were observed. Similar trends were observed in traits like fruit volume which varied in the range of 62.00 (UHSP 23) to 527.78 cm^3 (Ganesh) with mean value of 200.23 cm^3, among the genotypes. Fruit length and diameter has a bearing on the size of the fruit which is one of the important criteria for grading of fruits in pomegranate. Fruit length showed a remarkable variation in the range of 47.06-112.35 mm as well as fruit diameter varied from 42.43-95.87 mm with an average value of 69.22 mm and 68.64 mm respectively. Peel weight which contributes to the fruit weight, also showed a wide range among the genotypes, varying from 24.33-192.44 g with an average of 68.57 g, while the thickness of rind ranged from 1.44-4.37 mm with mean value of 3.39 mm

A marked variation was also observed for range in various aril parameters like total no. of arils/fruit (150.67-1077.89 g), total aril weight (31.67-312.55 g), 100 arils fresh weight (11.45-46.89 g) and 100 arils dry weight (1.15-9.62 g) with mean values of 453.71, 127.35 g, 25.53 g, 5.10 g respectively. Moisture per cent in arils of different genotypes showed comparatively narrow range of variation ranging from 73.94-93.26% with a mean value of 80.50%.

There was a great variation in the L, a, b values for fruit and aril colour (Table 8). The mean ‘L’ value which represents lightness or brightness, ranged from 42.62-74.90 with a mean value of 55.23, for fruit colour while, for aril colour its value ranged from 17.91-60.99 with a mean value of 39.20, among the genotypes. Similarly, with regard to ‘a’ values (red colour) the values ranged from 2.15-41.01and 4.08-34.48 for fruit and aril colours respectively while the means were 27.03 for fruit colour and 33.24 for aril colour. Also, ‘b’ values (yellowness), ranged from 17.88-48.99 with an average of 31.10 for fruit colour and 3.73-29.33 with an average of 16.07 for aril colour.

Table 8. Mean, range, CV and CD for morphological and biochemical traits evaluated for different pomegranate genotypes

Sl. No.	Traits	Mean ± SEm	Range	CV	CD
1	Fruit weight (gm)	195.91±10.52	56.01-505.00	9.30	30.10
2	Fruit length (mm)	69.22±2.71	47.06-112.35	6.77	7.74
3	Fruit diameter (mm)	68.64±3.36	42.43-95.87	8.47	9.60
4	Fruit length/breadth	1.01±0.04	0.89-1.18	6.75	0.11
5	Fruit volume (cm^3)	200.23±12.14	62.00-527.78	10.50	34.71
6	Fresh wt. of 100 arils (g)	25.53±1.01	11.45-46.89	6.82	2.88
7	Dry wt. of 100 arils (g)	5.10±0.13	1.15-9.62	4.28	0.36
8	Moisture %	80.50±0.66	73.94-93.26	7.97	2.57
9	Crown length (mm)	14.31±0.84	8.92-20.05	10.19	2.41
10	Peel weight (g)	68.57±3.31	24.33-192.44	8.37	9.47
11	Aril weight (g)	127.35±11.24	31.67-312.55	15.29	32.15
12	Seed %	64.39±2.53	55.88-81.40	6.8	7.23
13	Skin %	35.61±2.53	18.60-44.12	12.3	7.23
14	Total no. of arils/fruit	453.71±24.53	150.67-1077.89	9.37	70.15
15	Aril length (mm)	8.68±0.28	5.02-11.48	5.50	0.79
16	Aril width (mm)	5.69±0.33	2.52-8.63	10.17	0.96
17	Seed length (mm)	6.59±0.19	4.85-7.76	5.10	0.55
18	Seed width (mm)	2.92±0.14	2.48-3.32	8.45	0.41
19	Rind thickness (mm)	3.39±0.38	1.44-4.37	19.25	1.08
20	Red coverage of Peel (%)	67.52±3.26	13.33-90.00	8.37	9.33
21	Days to flowering	39.85±1.07	32.00-50.00	NS	NS
22	Days to maturity	167.07±0.86	147.33-186.00	NS	NS
23	Fruit colour L	55.23±3.20	42.62-74.90	10.05	12.74
24	Fruit colour a	27.03±3.23	2.15-41.01	12.12	12.18
25	Fruit colour b	31.10±2.49	17.88-48.99	13.90	12.27
26	Aril colour L	39.20±1.06	17.91-60.99	4.72	9.48
27	Aril colour a	33.24±0.61	4.08-34.48	3.52	9.48
28	Aril colour b	16.07±0.59	3.73-29.33	5.32	2.24
29	Anthocyanin content (mg/L)	18.53±1.69	4.35-61.44	15.76	4.82
30	TSS (°Brix)	13.39±0.41	9.62-17.95	5.30	1.17
31	Ascorbic Acid (mg/100gm)	27.34±1.49	20.24-50.00	9.44	4.26
32	Titratable Acidity (%)	0.48±0.02	0.32-2.74	4.19	0.03
33	pH of the Juice	3.45±0.06	2.53-3.99	3.04	0.17
34	Fruit Juiciness% (/100gm aril wt.)	58.39±2.38	29.07-77.33	7.06	6.80
35	Total Sugars (%)	12.08±0.30	8.66-15.69	3.01	0.6
36	Reducing Sugars (%)	10.78±0.32	7.55-14.33	3.65	0.32
37	Non-Reducing Sugars (%)	1.30±0.33	0.79-2.55	30.82	0.33

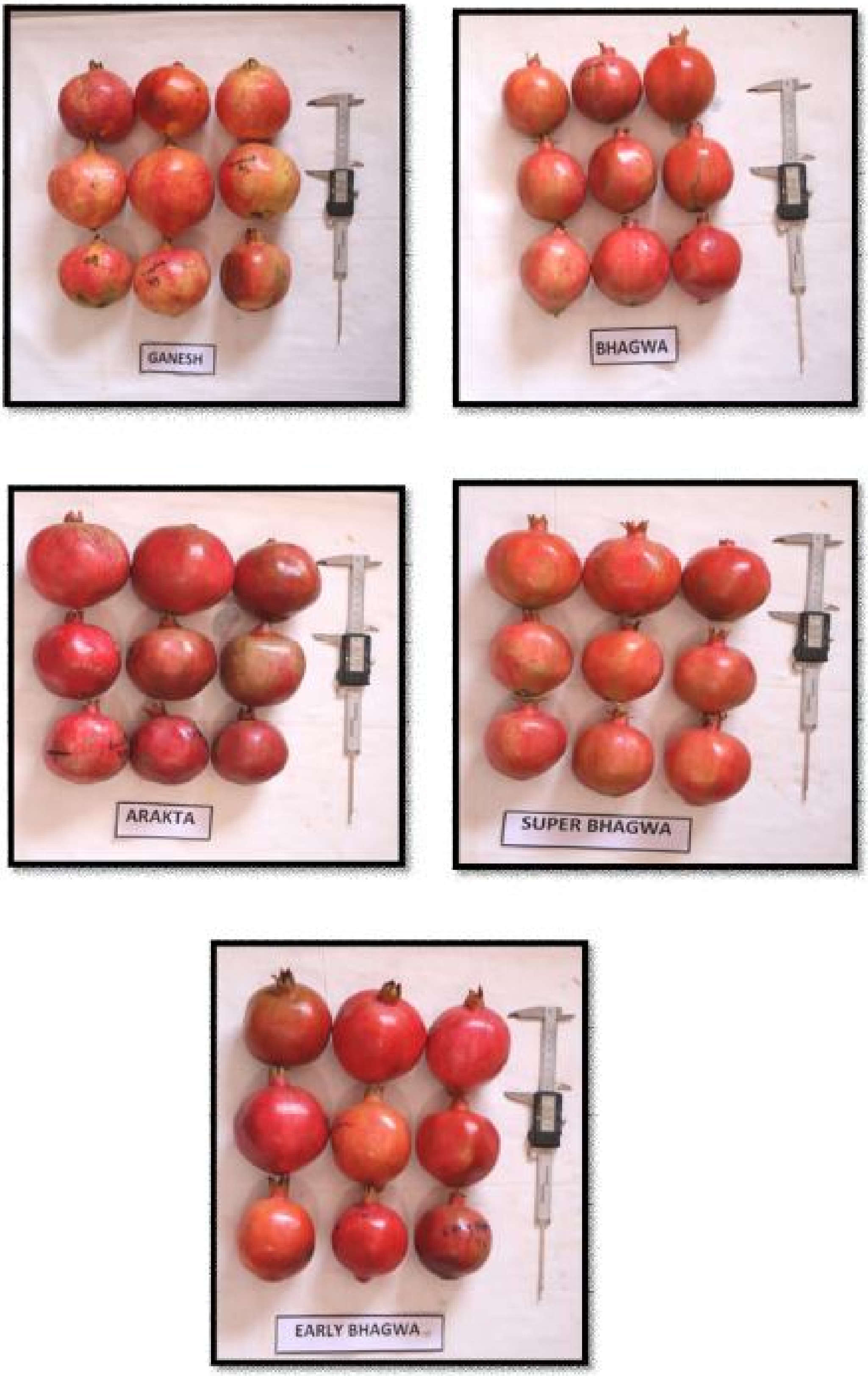


Plate 1: Morphological diversity among the pomegranate genotypes (fruits)

Plate 1: *Contd...*

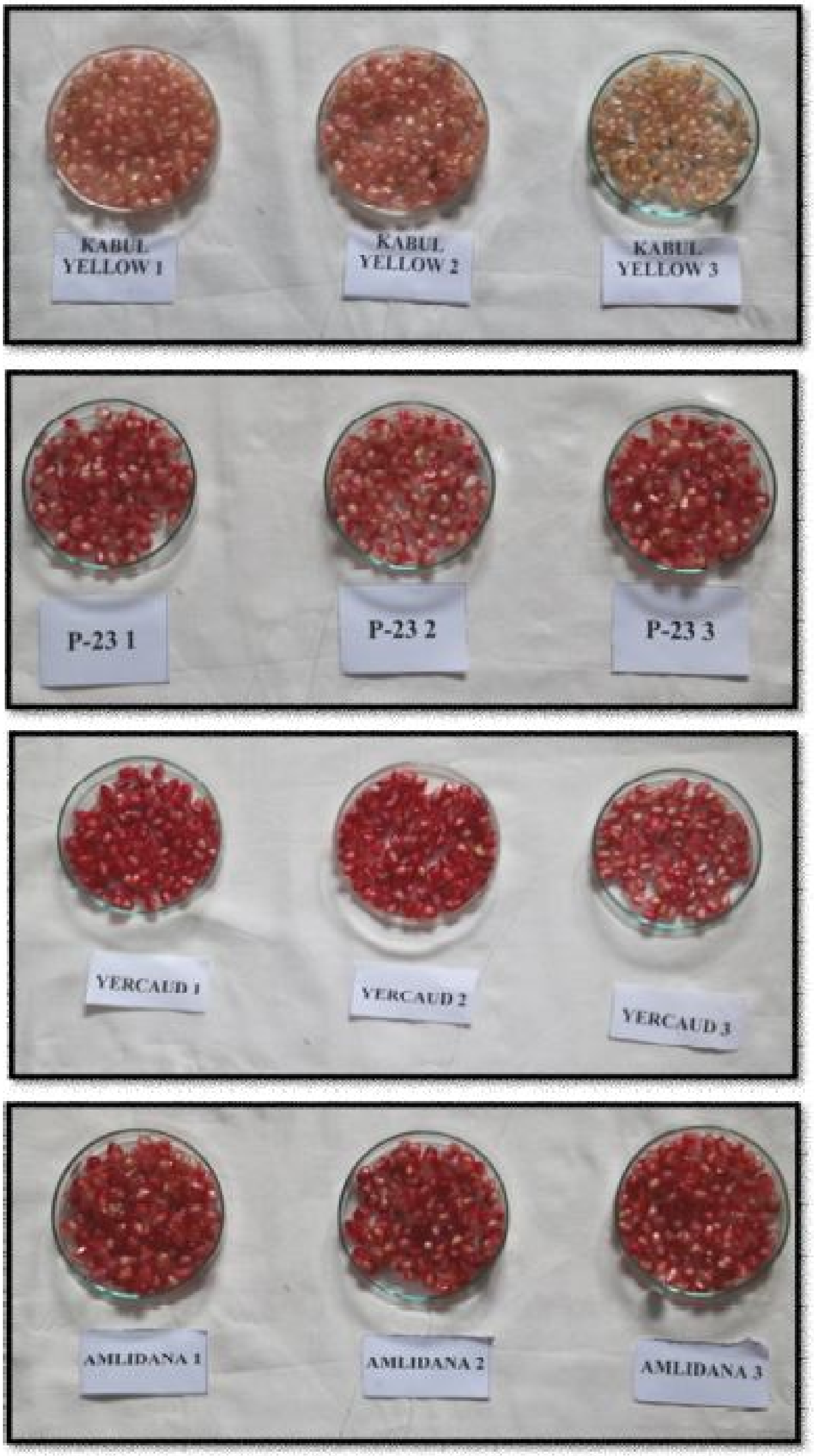


Plate 2: Morphological diversity among the pomegranate genotypes (arils)

Plate 2: *Contd...*

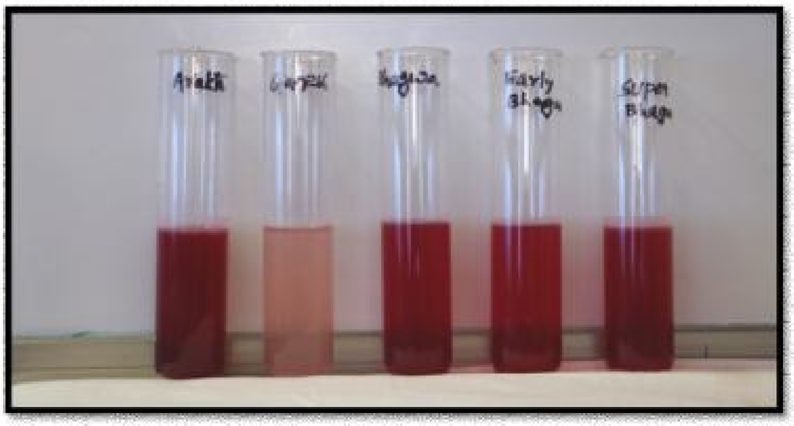


Plate 3: Variation in juice colour among different pomegranate genotypes

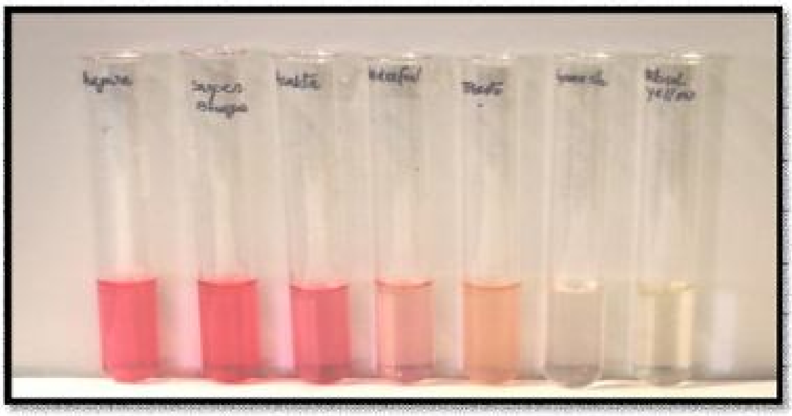


Plate 4: Estimation of anthocyanin in different pomegranate genotypes

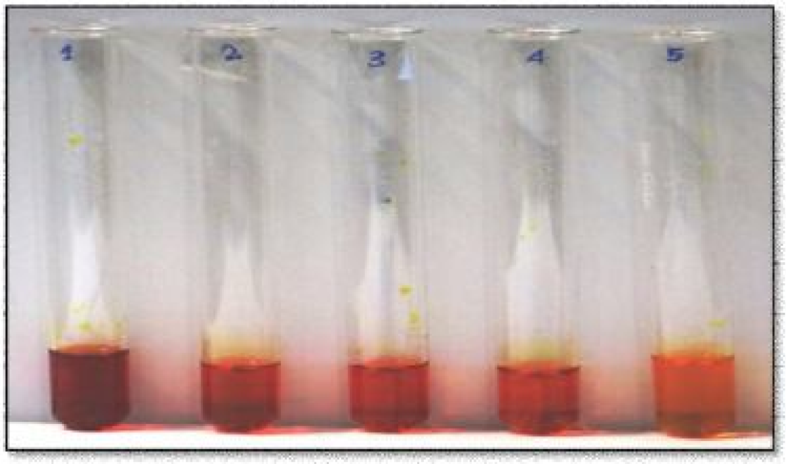


Plate 5: Estimation of reducing sugars in different pomegranate genotypes

Among the genotypes, there was a greater variation in the mean values for different traits. Mean value for individual traits of each genotype is provided in Table 9 (a-c). With regard to fruit parameters like weight, volume, length, diameter and peel weight, Ganesh was found to be the most superior over other genotypes with significantly higher values for all the aforementioned traits *viz.* 505.00 g, 527.78 cm^3, 112.35 mm, 95.87 mm, and 192.44 g respectively. Bhagwa, the most popular variety of India especially for northern Karnataka, was found to have an average fruit weight of 285.33 g, significantly lower than it's improved clones Early Bhagwa (350.22 g) and Super Bhagwa (306.44 g). All the mutants studied showed a significantly lower fruit weight than Ganesh and Bhagwa, with UHSP 23 having the lowest mean fruit weight (56.00g) and peel weight (24.33 g) among all the cultivars included in the study while, fruit volume was lowest for UHSP 81 (42.43 cm^3).

For aril parameters like aril weight, total no. of arils/ fruit, fresh weight of aril, dry weight of aril and for moisture percentage, mean values were 127.35 g, 453.71 g, 25.53 g, 5.10g and 80.50% respectively. Among the genotypes of the present investigation, a wide variation was found for these traits as evident from the range for these traits. The highest aril weight was recorded for Ganesh (312.55 g) followed by Phule Arakta (255.55 g) while lowest was for UHSP 23 (31.67 g) followed by UHSP 125 (42.50 g). Total no. of arils/ fruit, aril length and aril width were also highest for Ganesh being 1,077.89, 11.48 mm, 8.63 mm respectively. However, unlike the weight of aril, the lowest no. of arils per fruit was recorded for UHSP 125 (150.66) followed by UHSP 81 (157.67), while aril length and width were lowest for CO-1 being 5.02 mm and 2.52 mm respectively. Seed length was highest in Dholka (7.76 mm) while the lowest in UHSP 23 (4.85 mm). Moreover for seed width the highest mean value was recorded in Bhagwa (3.32 mm) while lowest in Dholka (2.48 mm).

Among the biochemical parameters, range of anthocyanin content varied between 4.35 mg/l and 61.44 mg/l and per cent juiciness of fruits was found to be varying between 29.07% and 77.33%. The minimum content of reducing sugar was found to be 6.29% (Amlidana) and the highest among the genotypes was 33.99% (Wonderful). TSS values also showed a great range of variation, ranging from 9.62°Brix (Ruby) to 17.95 °Brix (Wonderful) among different genotypes with a mean value of

Table 9 (a). Mean values for individual morphological and biochemical traits of different pomegranate genotype

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit diameter (mm)	Fruit Length/width	Fruit Volume (cm^3)	Fresh wt. of 100 arils	Dry wt. of 100 arils	Moisture %	Crown length (mm)	Peel weight (g)
Amlidana	187.56	70.57	72.80	0.97	189.44	32.55	8.03	75.33	20.05	34.00
Bhagwa	285.33	90.12	76.33	1.18	260.56	24.67	5.48	77.79	10.77	95.89
CO-1	148.65	59.24	65.97	0.90	115.00	22.50	5.86	73.96	13.06	65.00
Dholka	207.50	71.16	75.99	0.94	231.67	20.67	4.14	79.97	19.44	67.00
Early Bhagwa	350.22	100.34	85.70	1.17	358.33	32.33	7.34	77.30	15.13	121.56
G-137	227.03	67.07	73.50	0.91	235.00	22.33	5.61	74.88	19.04	95.00
Ganesh	505.00	112.35	95.87	1.17	527.78	46.89	9.62	79.48	12.83	192.44
Kabul Yellow	134.44	61.37	64.61	0.95	141.95	30.00	7.59	74.70	13.30	45.00
Kaladagi Local	94.70	55.44	57.44	0.97	105.00	17.17	4.05	76.41	15.83	31.67
KRS	186.10	67.03	68.40	0.98	151.67	14.67	3.53	75.94	12.63	50.33
Mridula	96.93	50.52	56.55	0.89	113.33	16.67	3.35	79.90	12.61	30.00
P-23	134.89	59.39	61.98	0.96	112.11	32.22	6.79	78.93	13.20	53.89
P-26	179.78	67.35	69.20	0.98	182.28	36.44	6.59	81.92	13.74	79.22
PhuleArakta	374.22	94.50	86.41	1.09	377.78	32.22	5.91	81.66	8.92	118.67
Ruby	135.56	61.77	64.54	0.96	139.17	15.53	2.54	83.64	19.36	54.67
Super Bhagwa	306.45	97.86	94.14	1.07	313.89	30.56	5.54	81.87	13.60	110.89
Tobesto	225.60	72.00	67.50	1.07	279.67	35.00	7.19	79.46	12.80	70.00
UHSP 23	56.01	47.06	45.85	1.03	62.00	15.01	1.70	88.67	10.48	24.33
UHSP 57	145.76	52.96	54.25	0.98	148.33	23.98	1.62	93.22	12.51	38.67
UHSP 81	79.87	47.41	42.43	1.12	96.67	11.45	1.70	85.15	10.89	30.33
UHSP 125	71.50	53.93	51.43	1.05	93.33	16.97	1.15	93.22	13.78	29.00
Wonderful	202.67	67.72	76.60	0.89	196.67	32.00	6.65	79.22	18.81	80.67
Yearcaud	170.22	64.84	71.18	0.91	173.61	25.45	5.33	79.06	16.31	58.78
C.D.	30.10	7.74	9.60	0.11	34.71	2.88	0.36	2.57	2.41	9.47
SE(m)	10.52	2.71	3.36	0.04	12.14	1.01	0.13	0.90	0.84	3.31

Table 9 (b). Mean values for individual morphological and biochemical traits of different pomegranate

Treatment	Aril Wt. (g)	Seed %	Skin %	Total no. of arils/fruit	Aril Length (mm)	Aril width (mm)	Seed length (mm)	Seed width (mm)	Rind thickness (mm)	Red coverage %
Amlidana	153.56	81.40	18.60	317.44	9.63	6.72	5.89	3.04	1.44	47.50
Bhagwa	189.44	66.31	33.69	656.11	8.11	4.51	6.84	3.32	3.66	80.00
CO-1	83.65	56.15	43.85	320.33	5.02	2.52	7.09	2.85	4.10	62.50
Dholka	140.50	67.67	32.33	685.33	9.03	5.32	7.76	2.48	3.42	47.50
Early Bhagwa	228.67	65.15	34.85	710.00	8.33	6.75	7.53	2.86	4.18	85.83
G-137	132.03	58.11	41.89	559.67	9.00	5.47	7.18	3.04	4.33	53.33
Ganesh	312.55	61.89	38.11	1077.89	11.48	8.63	7.28	2.89	4.19	56.67
Kabul Yellow	89.44	66.31	33.69	283.00	9.74	6.99	5.59	2.62	2.73	13.33
Kaladagi Local	63.03	66.19	33.81	351.83	8.36	5.85	6.66	2.73	2.91	66.67
KRS	135.77	72.33	27.67	574.33	8.66	5.28	5.77	2.66	3.83	67.50
Mridula	66.93	68.80	31.20	330.33	8.72	4.81	7.52	2.89	4.06	80.00
P-23	81.00	58.33	41.67	465.22	9.56	6.84	6.73	3.21	2.29	90.00
P-26	100.56	55.88	44.12	383.37	10.08	6.38	6.61	3.11	3.90	85.00
PhuleArakta	255.55	68.08	31.92	767.78	8.08	4.59	6.84	2.76	2.18	85.00
Ruby	80.89	59.50	40.50	499.78	7.58	4.27	7.11	2.74	4.37	67.50
Super Bhagwa	195.56	63.48	36.52	671.89	9.81	7.14	6.61	2.92	3.85	83.33
Tobesto	155.60	68.97	31.03	231.33	9.85	8.00	6.77	3.25	4.30	57.50
UHSP 23	31.67	56.61	43.39	168.67	6.41	3.75	4.85	2.88	2.66	80.00
UHSP 57	107.10	73.46	26.54	187.67	7.11	4.17	4.85	2.60	2.58	78.67
UHSP 81	49.54	61.73	38.27	157.67	7.46	4.76	6.02	3.13	2.46	88.67
UHSP 125	42.50	59.32	40.68	150.67	8.48	4.81	6.32	3.10	2.96	80.00
Wonderful	122.00	59.79	40.21	502.33	10.35	7.57	6.82	2.97	3.73	67.50
Yearcaud	111.44	65.45	34.55	382.67	8.82	5.69	6.86	3.07	3.89	29.00
C.D.	32.15	7.23	7.23	70.15	0.79	0.96	0.55	0.41	1.08	9.33
SE(m)	11.24	2.53	2.53	24.53	0.28	0.33	0.19	0.14	0.38	3.26

Table 9 (c). Mean values for individual morphological and biochemical traits of different pomegranate

Genotypes	Anthocyanin (mg/L)	TSS (°Brix)	Ascorbic Acid (mg/100gm)	Titratable Acidity (%)	pH of the Juice	Fruit Juiciness %	Total Sugars (%)	Reducing Sugars (%)	Non Reducing Sugars (%)
Amlidana	7.31	15.56	50.00	2.74	2.53	54.89	13.87	12.29	1.58
Bhagwa	24.71	13.22	24.68	0.34	3.67	60.22	12.23	10.64	1.59
CO-1	15.36	14.53	26.79	0.38	3.54	58.37	12.42	11.21	1.21
Dholka	14.16	11.55	36.31	0.34	3.29	62.67	10.68	9.50	1.18
Early Bhagwa	8.35	13.95	23.81	0.40	3.70	68.00	10.82	8.27	2.55
G-137	12.36	12.47	42.26	0.35	3.07	39.40	11.68	10.19	1.50
Ganesh	5.20	15.53	25.60	0.32	3.99	66.22	13.46	12.28	1.18
Kabul Yellow	4.35	14.40	25.00	0.33	3.55	62.44	12.75	11.95	0.79
Kaladagi Local	4.68	14.30	25.60	0.36	3.26	66.67	12.38	11.33	1.05
KRS	10.55	12.50	29.17	0.35	3.49	61.52	10.63	9.64	0.99
Mridula	11.36	10.52	25.00	0.38	3.54	56.00	9.58	8.61	0.98
P-23	12.69	14.83	26.78	0.36	3.26	64.00	13.88	12.73	1.14
P-26	24.24	13.67	26.19	0.37	3.40	59.67	12.61	11.10	1.51
PhuleArakta	17.07	14.75	25.59	0.35	3.78	72.44	13.29	11.95	1.34
Ruby	12.26	9.62	20.24	0.33	3.90	71.55	8.66	7.55	1.10
Super Bhagwa	9.69	12.55	22.02	0.34	3.70	61.33	11.44	10.32	1.12
Tobesto	21.49	15.55	23.81	0.36	3.55	41.54	13.18	12.11	1.07
UHSP 23	26.15	11.65	22.04	0.44	3.34	29.07	11.38	9.98	1.41
UHSP 57	61.44	11.13	22.27	0.46	3.35	52.07	11.39	9.61	1.78
UHSP 81	57.85	12.86	22.26	0.64	3.13	50.65	11.71	10.51	1.20
UHSP 125	43.58	12.44	23.84	0.47	3.64	46.95	11.60	10.52	1.08
Wonderful	15.03	17.95	29.76	0.32	3.59	77.33	15.69	14.33	1.37
Yearcaud	6.41	12.43	29.83	0.32	3.02	60.00	12.38	11.23	1.15
C.D.	4.82	1.17	4.26	0.03	0.17	6.80	0.60	0.65	0.66
SE(m)	1.69	0.41	1.49	0.01	0.06	2.38	0.21	0.23	0.23

13.39°Brix. pH of the juice varied from 2.53-3.99, with a mean value of 3.45, indicating the acidic nature of pomegranate juice.

Variations for mean value of these nine biochemical traits were also high among the studied cultivars. The cultivar Amlidana showed a mean ascorbic acid content of 50.00 mg/g highest among all the genotypes while its pH was lowest the 2.53 among all, indicating highly acidic nature of the cultivar. Mean anthocyanin was highest for UHSP 57 **(**61.44 mg/L) followed by UHSP 81 **(**57.853 mg/L), and it was found to be lowest in Kabul Yellow (4.34 mg/L) as explained by light colour of its aril and fruit. For reducing sugars, average value was highest for Wonderful (34.00 %). pH of the juice was highest for Ganesh (3.99) showing its less acidic nature.

Coefficient of Variation (CV) was found to be in the acceptable range and was below 20% for almost all the traits including biochemical and morphological parameters in the present investigation.

4.1.3 Genetic Variability and Heritability Analysis

The genotypes included in the study exhibited a wide range of variation with respect to traits under study (comprising of most important economic traits), as evident from the range as well as mean value of each trait across the genotype. Hence, for better comprehension of these variations, the data obtained was subjected to variability estimates like genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) as well as the heritability analysis (h^2 broad sense) as heritability is one of the most important selection index for breeders.

The genetic variability and heritability for selected characters is presented in the Table 10. With respect to genetic variability components of the quantitative traits, all the studied characters revealed moderate to high Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) along with heritability components such as heritability (h^2 in broad sense) and Genetic advance (GA). In order to compare the GA among various traits, genetic advance as percent mean (GAM) was calculated from GA with the population mean for respective traits (Table 10).

Table 10. Genetic variability and heritability for morphological and biochemical traits evaluated for different pomegranate genotypes

Sl. No.	Traits	GCV (%)	PCV (%)	h^2b.s (%)	GAM
1	Fruit weight (g)	55.11	55.89	97.23	111.95
2	Fruit length (mm)	25.64	26.52	93.48	51.07
3	Fruit diameter (mm)	19.75	21.49	84.46	37.38
4	Fruit shape	8.37	10.75	60.59	13.42
5	Fruit volume (cm^3)	55.52	56.51	96.55	112.38
6	Fresh wt. of 100 arils	34.53	35.2	96.24	69.78
7	Dry wt. of 100 arils	45.52	45.72	99.13	93.35
8	Moisture %	26.88	28.04	91.92	53.09
9	Crown length (mm)	21.34	23.65	81.43	39.67
10	Peel weight (g)	58.38	58.98	97.99	119.05
11	Aril weight (g)	54.95	57.04	92.81	109.06
12	Seed %	8.97	11.25	63.45	14.71
13	Skin %	16.21	20.35	63.45	26.60
14	Total No. of Arils/fruit	51.38	52.22	96.78	104.12
15	Aril length (mm)	15.77	16.7	89.15	30.66
16	Aril width (mm)	25.52	27.47	86.29	48.83
17	Seed length (mm)	11.48	12.56	83.54	21.61
18	Seed width (mm)	5.84	10.27	32.28	6.83
19	Rind thickness (mm)	21.98	29.22	56.57	34.05
20	Red coverage of Peel (%)	28.82	30.01	92.22	57.01
21	Anthocyanin content (mg/L)	84.42	85.88	96.63	170.95
22	TSS (°Brix)	13.98	14.95	87.41	26.92
23	Ascorbic Acid (mg/100g)	24.75	26.49	87.3	47.64
24	Titratable Acidity (%)	109.47	110.42	98.28	223.56
25	pH of the Juice	9.17	9.66	90.13	17.94
26	Fruit Juiciness % (per 100gm aril wt.)	18.88	20.15	87.75	36.42
27	Total Sugars (%)	52.93	55.22	91.86	104.5
28	Reducing Sugars (%)	51.23	57.31	79.91	94.34
29	Non Reducing Sugars (%)	88.59	97.92	81.85	165.1

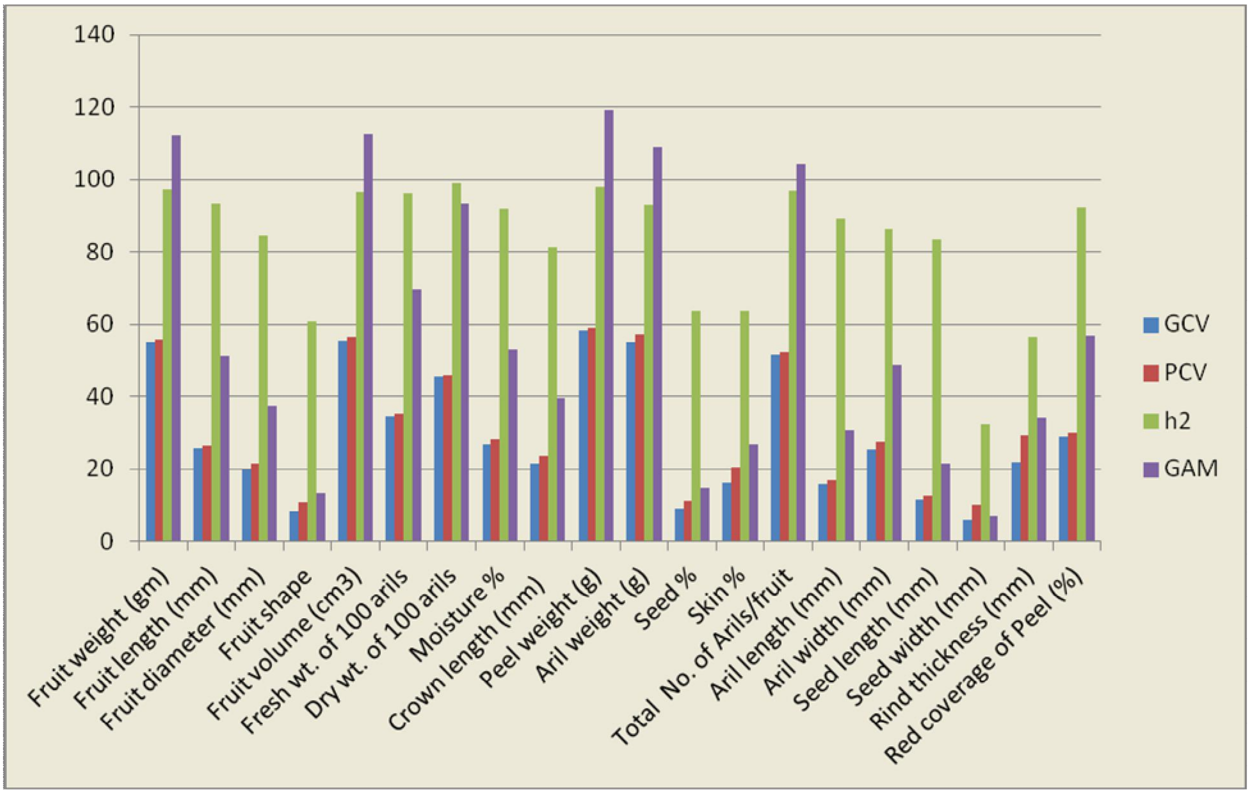


Fig. 1(a): Genetic variability and heritability of 26 morphological traits of pomegranate genotypes

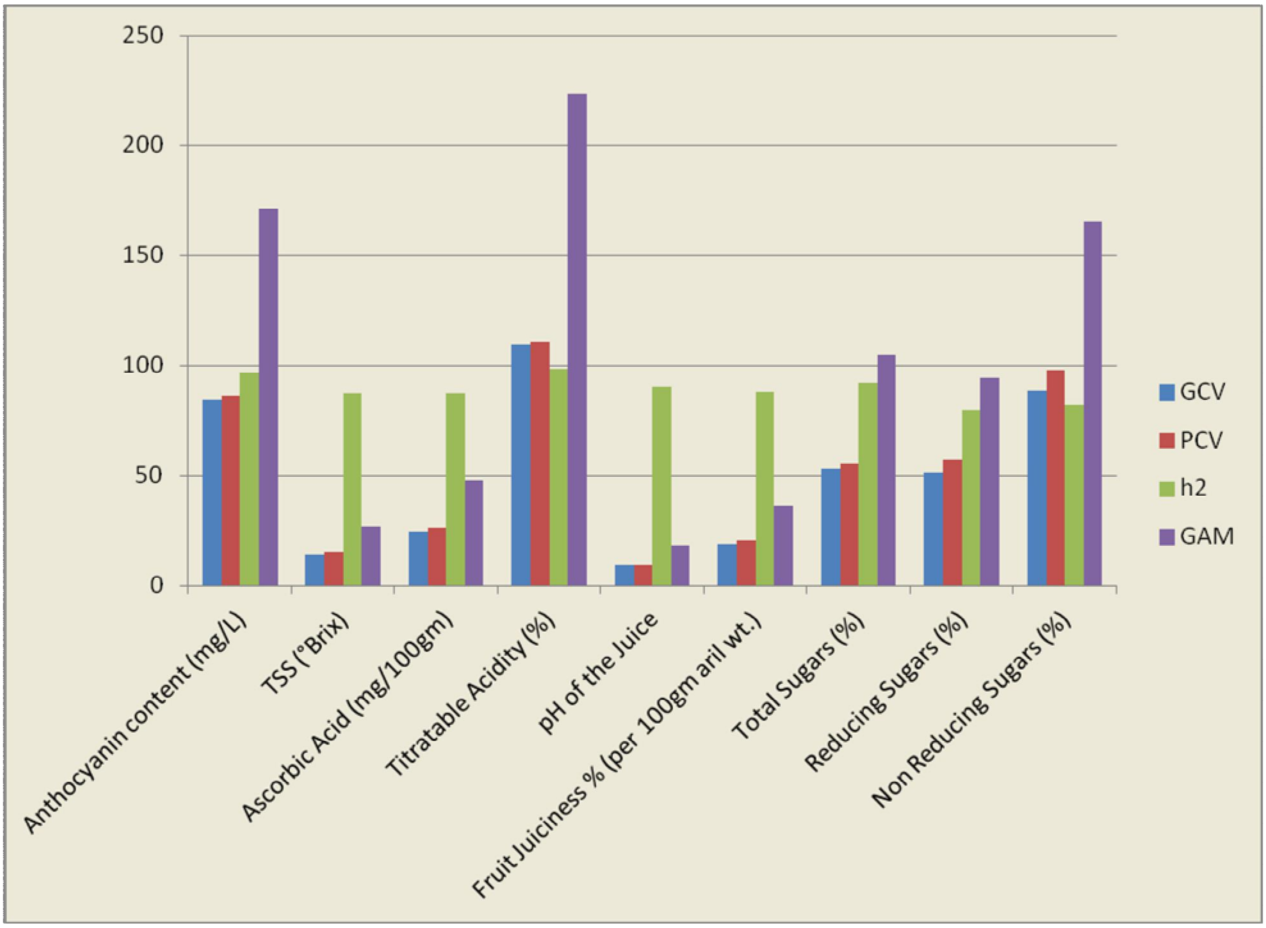


Fig. 1(b): Genetic variability and heritability of 9 biochemical traits of pomegranate genotypes

Most of the traits exhibited a heritability of more than 60% depicting their higher heritability. Traits like fruit weight, fruit volume, fresh wt. of 100 arils, dry wt. of 100 arils, peel weight, total no. of arils/fruit and titratable acidity demonstrated a heritability of greater than 95%. Among the traits analysed, highest heritability of 99.13% was recorded in dry wt. of 100 arils followed by titratable acidity with 98.28%, while, lowest was observed for seed width (32.28%).

With respect to fruit parameters, higher values of both PCV and GCV were observed for fruit and aril traits like fruit weight, fruit volume, fresh weight of 100 arils, dry weight of 100 arils, total aril weight and biochemical traits like titratable acidity (%), anthocyanin content and reducing and non-reducing sugars. Whereas, low to moderate level of variation was recorded for TSS, pH and seed parameters like seed length and width. Among these traits, the highest values for both GCV and PCV were observed for titratable acidity being 109.47% and 110.42% respectively.

On comparing the genetic advance as per cent mean (GAM) among the morphological traits, the highest GAM was observed for peel weight (119.05) followed by fruit volume (112.38), fruit weight (111.95) and aril weight (109.06). Among the biochemical traits, the highest GAM was exhibited by titratable acidity (223.56) followed by anthocyanin content (170.95) and non-reducing sugars (165.10). Whereas the lowest values of GAM among morphological and biochemical traits were observed for seed width (6.83) and pH of the juice (17.94) respectively.

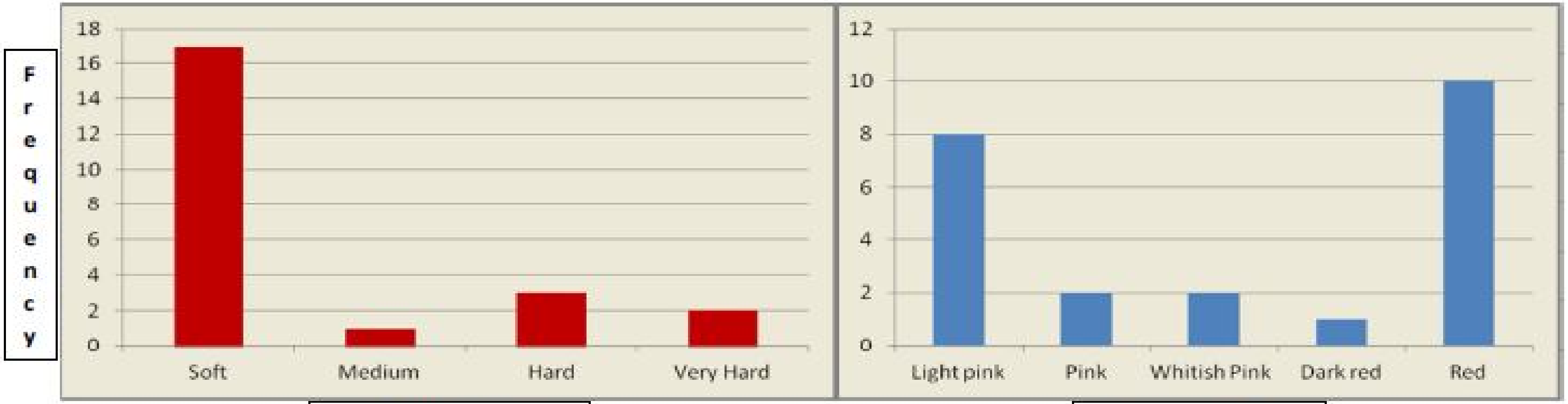
4.1.4 Frequency distribution

In the present investigation, along with quantitative estimation of 37 traits, three traits were also recorded qualitatively *viz.* seed hardiness, fruit shape, and juice colour (Table 11). The data obtained was subjected to frequency distribution, in order to know the frequency of various types of genotypes for the respective traits.

Among the genotypes, the highest frequency was observed for soft seeds as depicted in the frequency distribution graph (Fig. 2). Two cultivars under study, CO-1 and Yercaud had very hard seeds, while Amlidana was found to be hard seeded. The commercial cultivars like Bhagwa, Ganesh and Arakta were in the category of soft seeds.

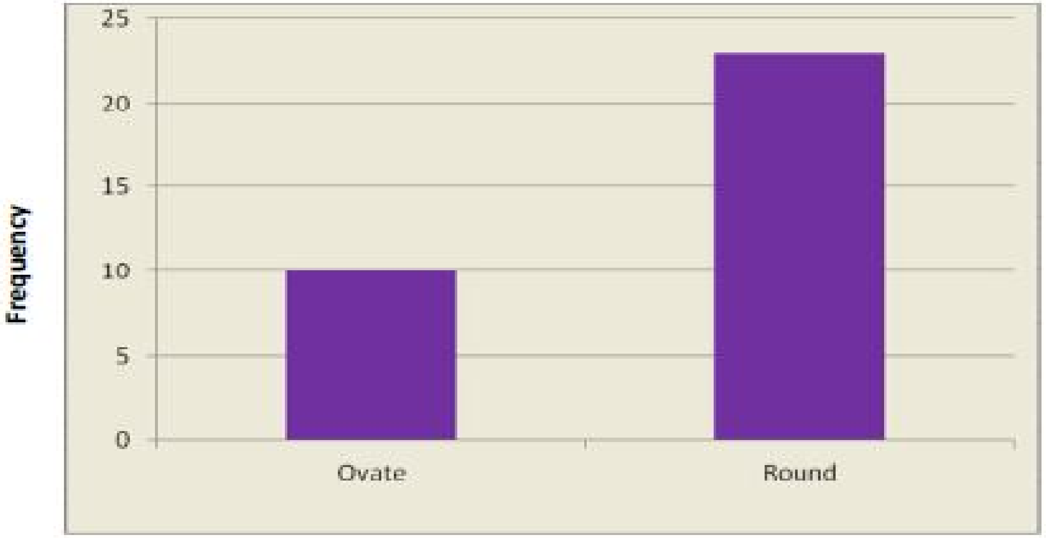
Table 11. Description of the qualitative traits evaluated for different pomegranate genotypes

Sl. No.	Genotypes	Seed Hardiness	Colour of Juice	Shape of Fruit
1	Amlidana	Hard	Light pink	Round
2	Bhagwa	Soft	Dark red	Ovate
3	CO-1	very Hard	Light Pink	Round
4	Dholka	Soft	Light Pink	Round
5	Early Bhagwa	Soft	Red	Ovate
6	G-137	Soft	Light Pink	Round
7	Ganesh	Soft	Pinkish White	Ovate
8	Kabul Yellow	Hard	Whitish Pink	Round
9	Kaladagi Local	Soft	Pink	Round
10	KRS	Soft	Light Pink	Round
11	Mridula	Soft	Red	Round
12	P-23	Soft	Light Pink	Round
13	P-26	Soft	Red	Round
14	Phule Arakta	Soft	Red	Ovate
15	Ruby	Medium	Light Pink	Round
16	Super Bhagwa	Soft	Red	Ovate
17	Tobesto	Hard	Pink	Ovate
18	UHSP 23	Soft	Red	Ovate
19	UHSP 57	Soft	Red	Ovate
20	UHSP 81	Soft	Red	Ovate
21	UHSP 125	Soft	Red	Ovate
22	Wonderful	Soft	Red	Round
23	Yearcaud	Very hard	Light Pink	Round



Seed hardiness

Juice Colour



Fruit shape

Fig. 2: Frequency distribution for different qualitative traits of fruit among pomegranate genotypes

Fruit shape (on the basis of fruit length/width) was found to be either round or ovate for all the cultivars studied with the highest frequency of round fruited type (Fig. 2).

Juice colour of the cultivars varied from whitish-pink to dark red where the highest frequency was observed for red colour juice. Juice colour of Bhagwa was found to be dark red in colour.

4.1.5 Screening for bacterial blight and foliar and fruit spots

All the 25 genotypes selected for study were screened for bacterial blight and foliar and fruit spots under field conditions while, for Daru and Nana cultivars only foliar spots were recorded. The score was given as per the procedure given in the materials and methods chapter of this dissertation.

Table 12 shows % severity of different pomegranate genotypes used in the study to bacterial blight. Highest % severity was recorded for the cultivar Bhagwa, the most popular cultivar of pomegranate in India, followed by its clones Early Bhagwa and Super Bhagwa. Ganesh exhibited a severity% of 31.17 % while, Daru and Nana showed lowest severity% among the studied genotypes with 4.68 and 4.22% severity respectively. For foliar and fruit spots, other than that of bacterial blight, Tobesto showed the highest incidence of 33.38% and 21.05% respectively. While the lowest incidence was recorded for Daru with 4.89% foliar spots and Bhagwa for fruit spots *i.e.* 2.86% (Table 13).

4.1.6 Principal Component Analysis (PCA)

PCA is a variable reduction technique aiming at reduction of a larger set of variables into a smaller set, known as ‘principal components’, such that these principal component accounts for most of the variance present in the original variables. Thus, PCA helps in identification of characters explaining as much of total variation in the original variables but with a very few components, reducing the dimension of the problem.

Table 12. Bacterial blight severity percentage in different pomegranate genotypes

Sl. No.	Genotypes	Severity %	Remarks
1	Amlidana	32.87	Moderately Susceptible
2	Bhagwa	49.39	Susceptible
3	CO-1	38.39	Moderately Susceptible
4	Daru	4.68	Tolerant
5	Dholka	32.55	Moderately Susceptible
6	Early Bhagwa	45.26	Moderately Susceptible
7	G-137	32.75	Moderately Susceptible
8	Ganesh	31.17	Moderately Susceptible
9	Kabul Yellow	28.89	Moderately Susceptible
10	Kaladagi Local	28.02	Moderately Susceptible
11	KRS	31.65	Moderately Susceptible
12	Mridula	29.64	Moderately Susceptible
13	Nana	4.22	Tolerant
14	P-23	26.66	Moderately Susceptible
15	P-26	30.62	Moderately Susceptible
16	PhuleArakta	40.05	Moderately Susceptible
17	Ruby	33.55	Moderately Susceptible
18	Super Bhagwa	44.49	Moderately Susceptible
19	Tobesto	37.83	Moderately Susceptible
20	UHSP 23	40.70	Moderately Susceptible
21	UHSP 57	37.26	Moderately Susceptible
22	UHSP 81	43.43	Moderately Susceptible
23	UHSP 125	37.91	Moderately Susceptible
24	Wonderful	20.22	Slightly Susceptible
25	Yercaud	36.94	Moderately Susceptible

Table 13. Severity percentage of different pomegranate genotypes for fruit and foliar spots

Sl. No.	Genotypes	% Severity	
		Foliar Spots	Fruit Spots
1	Amlidana	20.14	11.11
2	Bhagwa	26.72	2.86
3	CO-1	30.83	12.50
4	Daru	4.89	-
5	Dholka	18.26	16.00
6	Early Bhagwa	20.88	2.99
7	G-137	28.89	30.77
8	Ganesh	20.95	10.53
9	Kabul Yellow	19.94	20.69
10	Kaladagi Local	25.09	4.17
11	KRS	22.83	10.53
12	Mridula	24.07	8.89
13	Nana	7.67	-
14	P-23	24.85	14.71
15	P-26	24.42	12.86
16	Phule Arakta	19.01	5.56
17	Ruby	30.74	5.56
18	Super Bhagwa	22.00	8.57
19	Tobesto	33.38	21.05
20	UHSP 23	24.12	17.39
21	UHSP 57	17.78	17.65
22	UHSP 81	20.83	11.76
23	UHSP 125	27.31	13.04
24	Wonderful	24.21	3.41
25	Yercaud	20.22	5.56

A total of 35 phenotypic traits which included both morphological as well as biochemical parameters were subjected to principal component analysis (PCA). The results of PCA indicated that the total variability was being explained by 35 principal components out of which the components less than 3.0 Eigen values were ignored. A principal component loading of more than 0.50 was considered significant for each factor. The first three Principal components with Eigen values greater than 3 explained highest variation with 55.37% of the total variability observed (Table 14).

Furthermore, the component matrix of PCA analysis from extraction method, indicated that the first component (PC1) related to 12 characters (Table 15), including fruit weight, fruit volume, fruit length, fruit diameter, peel weight, aril weight, total number of arils, aril weight, fresh weight of 100 arils, dry weight of 100 arils, aril length, aril width and anthocyanin content, accounting for 27.77% of the total variation. Out of these 12 characters, 11 showed positive loadings with the highest positive loading exhibited by fruit weight followed by fruit volume, fruit length, aril weight, peel weight and fruit diameter with greater than 0.90 loadings.

The second principal component (PC2) which explained 16.66 % of total variation was dominated by 10 characters most of which were biochemical traits like total and reducing sugars, moisture per cent, as well as the fruit and aril colour parameters.

Moreover, the characters related to skin and seed percentage, pH, titratable acidity, ascorbic acid and rind thickness performed as the third main factor (PC3) and explained 10.93% of the total variance. The variation in anthocyanin was found to be explained by both PC1 and PC2 with higher loading in PC2.

4.1.7 Correlation Analysis

In the present study, a total of 31 quantitative traits were included for the statistical analysis, of which the morphological traits (22) and biochemical traits (9) were separately analysed for correlation. The Tables 16(a) and 16(b) represent phenotypic correlation of morphological (22) and Biochemical (9), characters respectively.

Table 14. Principal component analysis (PCA) for different quantitative characters in pomegranate genotypes

Component	Initial Eigen values		
	Total	% of Variance	Cumulative %
1	9.72	27.78	27.78
2	5.83	16.67	44.44
3	3.83	10.93	55.38
4	3	8.56	63.93
5	1.99	5.67	69.61
6	1.84	5.25	74.86
7	1.42	4.06	78.92
8	1.11	3.18	82.09
9	0.95	2.72	84.81
10	0.78	2.22	87.04
11	0.74	2.12	89.16
12	0.64	1.84	91
13	0.58	1.65	92.65
14	0.45	1.27	93.92
15	0.37	1.05	94.97
16	0.31	0.9	95.87
17	0.3	0.86	96.73
18	0.24	0.67	97.4
19	0.18	0.51	97.92
20	0.16	0.45	98.37
21	0.13	0.38	98.75
22	0.1	0.29	99.04
23	0.09	0.26	99.3
24	0.07	0.2	99.5
25	0.06	0.16	99.66
26	0.05	0.14	99.8
27	0.04	0.11	99.91
28	0.01	0.03	99.95
29	0.01	0.03	99.97
30	0.01	0.01	99.99
31	0	0.01	99.99
32	0	0.01	100
33	0	0	100
34	0	0	100
35	0	0	100

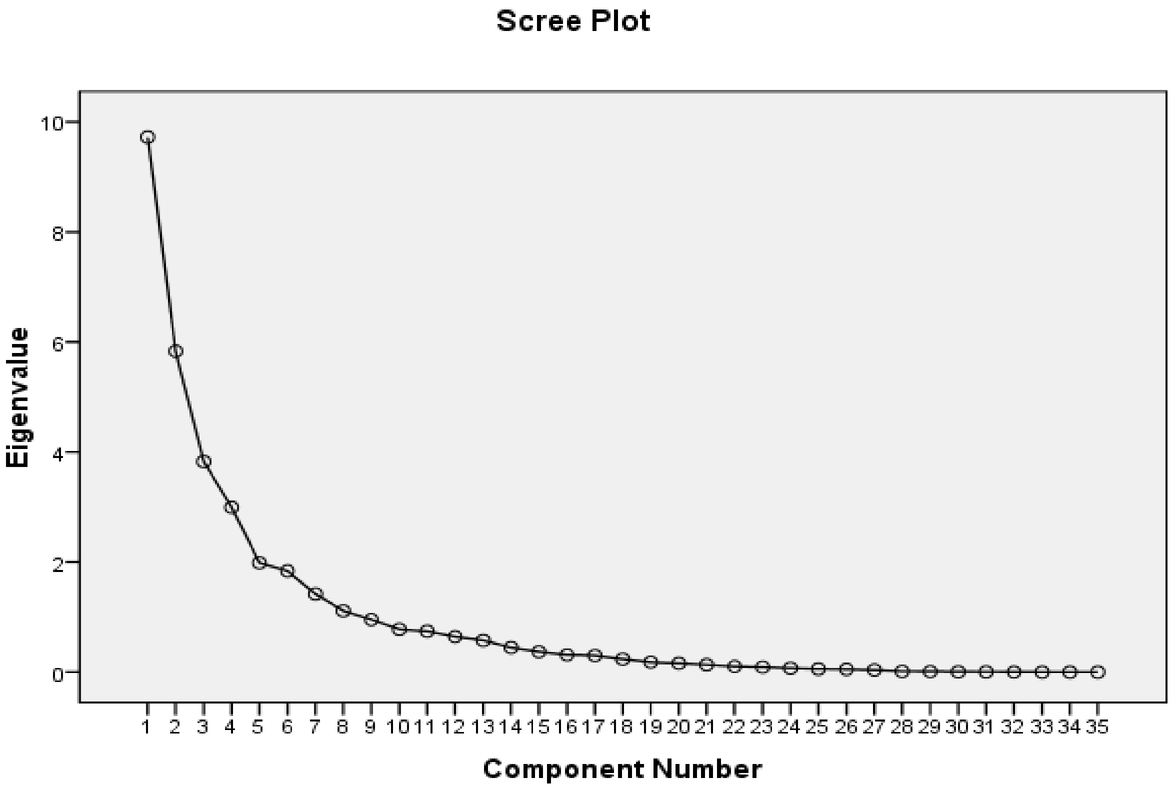


Fig. 3: Scree Plot Showing number of principal components and respective Eigen Values for the 35 quantitative traits by PCA for 23 pomegranate genotypes

With regard to the morphological traits investigated during the study, fruit length was found to have significantly high positive correlation with traits like fruit weight, aril weight, fruit volume, fruit diameter, peel weight and total no. of arils per fruit, in a decreasing order with respect to the strength of the correlation. Fruit length was also found to have significant positive correlation with aril and seed parameters like fresh and dry weights of 100 arils, aril length, aril width and seed length, the correlation with seed width was positive but not significant.

Almost similar trends were observed for correlation between fruit diameter and the other traits, which in addition, also exhibited significant positive correlation with rind thickness not observed in case of fruit length. Fruit length by width ratio, which determines the fruit shape was found to be significantly and positively correlated with fruit length but not with fruit diameter and had a significant negative correlation with crown length.

Fruit volume had a strong positive correlation with fruit weight and showed a significant positive correlation with other fruit and aril parameters like peel weight, aril weight, fresh and dry weights of 100 arils, total no. of arils per fruit, aril length, aril width and seed length. No significant negative correlations were found between fruit volume and other studied traits.

Fresh weight of 100 arils was having strong positive correlation with dry weight of 100 arils and other aril parameters *viz.* peel weight, aril weight, total no. of arils, aril length and aril width however, no significant correlation was found with seed length, seed width and moisture percentage. Howsoever, dry weight of 100 arils was found to be significantly and positively correlated with moisture per cent and seed length, in addition to its strong positive correlation with peel weight, aril weight, total no. of arils, aril length and aril width.

Moisture per cent of the fruit was found to be significantly correlated with seed length as well as the colour parameters of fruit and aril and red coverage of peel. Peel weight was significantly and positively correlated with aril weight total no. of arils per fruit, aril length and width and seed length as well as rind thickness.

Table 15. Component matrix (loadings) in PCA analysis for different quantitative characters in pomegranate genotypes

Parameters	Component		
	1	2	3
Fruit weight (g)	0.94*	-0.21	
Fruit volume cm^3	0.93*	-0.22	
Fruit length (mm)	0.92*	-0.26	
Aril weight (g)	0.92*	-0.22	-0.11
Peel weight (g)	0.91*	-0.18	0.25
Fruit weight (g)	0.91*		
Total number of arils	0.87*		0.17
Dry weight of 100 arils (g)	0.81*	0.22	-0.21
Fresh weight of 100 arils (g)	0.79*	-0.11	-0.15
Aril weight (g)	0.64*	0.13	-0.24
Aril length (mm)	0.61*	0.16	-0.24
Anthocyanin (mg/L)	-0.56*	-0.54*	
Seed length (mm)	0.50	0.33	0.33
Fruit juiciness (%)	0.48		0.15
TSS (°Brix)	0.47	0.11	-0.19
Aril colour L		0.81*	0.12
Total sugar (%)	-0.19	0.73*	0.27
Fruit Colour a		-0.72*	0.21
Moisure per cent (%)	0.45	0.64*	-0.13
Fruit length/width	0.34	-0.62*	
Reducing sugar	-0.23	0.61*	0.23
Fruit colour L	0.10	0.59*	
Red coverage of peel (%)	-0.14	-0.59*	0.40
Aril colour b		0.56*	0.20
Non reducing sugar (%)		0.48	0.18
Crown length (mm)		0.47	-0.31
Fruit colour b	0.15	0.46	0.17
Seed width (mm)		-0.17	
Skin percentage (%)	-0.11		0.72*
Seed percentage (%)	0.11		-0.72*
pH	0.38	-0.23	0.72*
Titratable Acidity (%)	-0.27	-0.48	-0.70*
Ascorbic Acid (mg/100)g	0.15	0.40	-0.68*
Rind thickness (mm)	0.28	0.26	0.54*
Aril colour a			
Extraction Method: Principal Component Analysis.			
3 components extracted.			

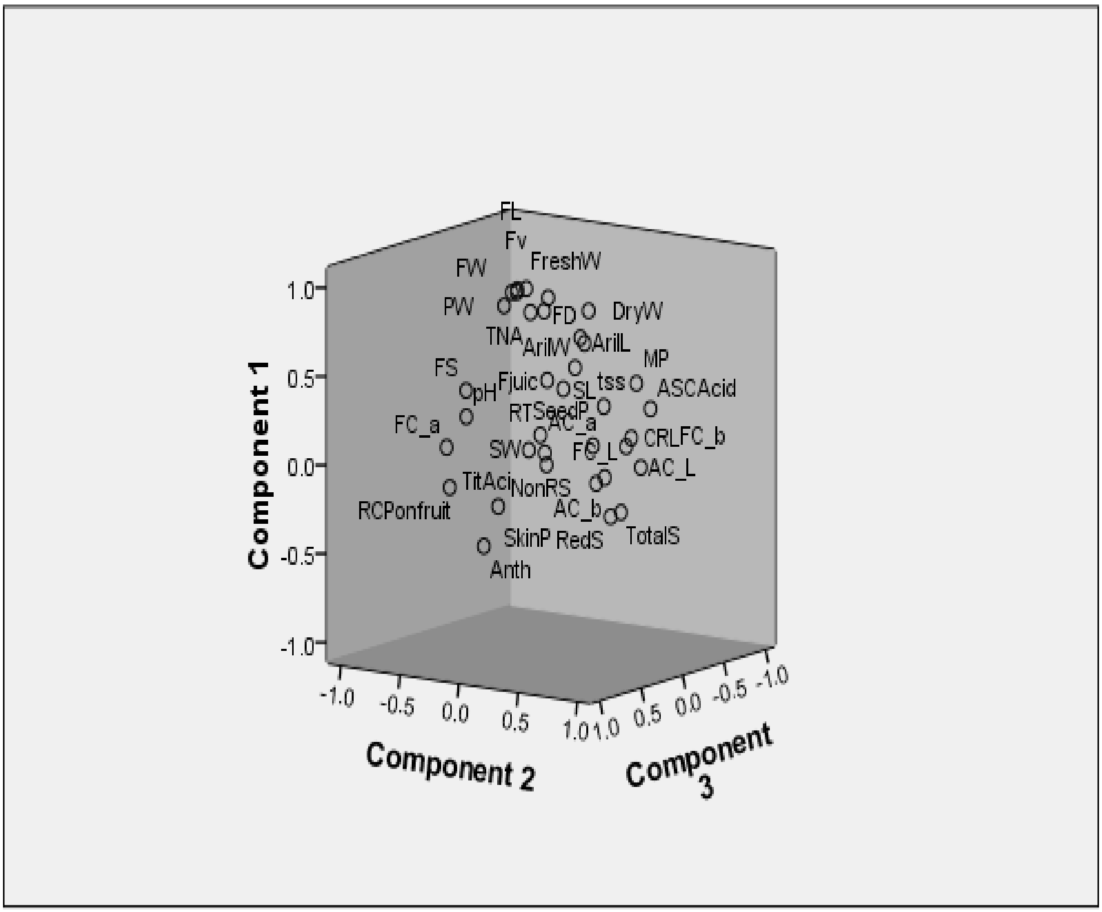


Fig. 4: Component Plot in Rotated Space

Aril weight exhibited a strong positive correlation with total no. of arils per fruit, aril length and width and seed length. In addition, its strong positive correlation was observed with seed percentage while a strong negative correlation with skin percentage. Seed percentage was found to have a perfect negative correlation with skin percentage and also a negative correlation with seed width and rind thickness; however, these two traits had a positive correlation with skin percentage. Total no. of arils was also having strong positive correlation with aril length, aril width and seed length but was weakly correlated with rind thickness.

Aril length and width exhibited a strong positive correlation with each other while seed length had a significant positive correlation with rind thickness. Among the colour parameters, red coverage of peel was positively correlated with fruit and aril colours. Fruit weight was found to have significant positive correlation with most of the traits studied *viz.* fruit length, fruit diameter, fruit volume, fresh and dry weight of 100 arils, peel weight, aril weight, total no. of arils per fruit, aril length and aril width. While a weak negative correlation with skin percentage and crown length was observed.

Among the biochemical parameters, anthocyanin content showed a significant positive correlation with titratable acidity and a strong negative correlation with fruit juiciness percentage and ascorbic acid content. On the other hand, ascorbic acid was found to be positively correlated with titratable acidity while a strong negative correlation existed between this parameter and pH of the juice. Titratable acidity was significantly and negatively correlated with total sugars, pH and reducing sugars. pH of the juice was positively correlated with fruit juiciness while with TSS it showed a negative correlation. No significant correlation could be deduced between fruit juiciness percentage and the sugars of the juice, however, total sugar was found to exhibit a significant positive correlation with non-reducing and reducing sugars. Although, no significant correlation was observed between reducing and non-reducing sugars, a positive correlation existed between reducing sugar and TSS while non-reducing sugars exhibited a negative trend.

4.1.8 Path coefficient analysis

The 35 traits selected for correlation studies were also analysed for phenotypic path coefficient analyses the results of which are presented in the Table 17 (a & b).

Table 16 (a). Pearson's correlation coefficient among quantitative morphological traits in different genotypes of pomegranate

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15
X1	**1.00**														
X2	0.897***	**1.00**													
X3	0.554***	0.143	**1.00**												
X4	0.92***	0.823***	0.494**	**1.00**											
X5	0.67***	0.668***	0.224	0.717**	**1.00**										
X6	0.633***	0.677***	0.113	0.631***	0.865**	**1.00**									
X7	0.276*	0.397***	0.168	0.203	0.225	0.666**	**1.00**								
X8	-0.045	0.155	-0.388***	-0.057	0.01	0.15	0.265*	**1.00**							
X9	0.888***	0.822***	0.427***	0.925***	0.699***	0.637***	0.252*	-0.062	**1.00**						
X10	0.931***	0.854***	0.462***	0.955***	0.683***	0.626***	0.256*	-0.075	0.862**	**1.00**					
X11	0.135	0.143	0.034	0.14	0.052	0.065	0.07	0.055	-0.157	0.319**	**1.00**				
X12	-0.135	-0.143	-0.034	-0.14	-0.052	-0.065	-0.07	-0.55	0.157	-0.319**	0	**1.00**			
X13	0.841***	0.822***	0.307*	0.826***	0.497***	0.520***	0.347**	0.072	0.868***	0.835***	0.012	-0.012	**1.00**		
X14	0.428***	0.484***	0.046	0.473***	0.631***	0.596***	0.244*	0.269*	0.421***	0.415***	0.098	-0.098	0.399**	**1.00**	
X15	0.468***	0.475***	0.17	.510***	0.693***	0.668***	0.270*	0.207	0.437***	0.446***	0.125	-0.125	0.352**	0.841**	**1.00**
X16	0.384**	0.428***	0.017	0.409***	0.191	0.337**	0.420***	0.296*	0.464***	0.344**	0.149	0.149	0.528***	0.237*	0.2
X17	0.053	-0.03	0.226	-0.008	0.138	0.154	0.059	-0.128	0.038	-0.037	-0.269*	0.269*	-0.127	0.198	0.153
X18	0.212	0.285*	-0.074	0.239*	0.1	0.112	0.147	0.095	0.363**	0.147	-0.282*	0.282*	0.276*	0.076	0.096
X19	0.024	-0.134	0.338**	-0.03	-0.128	-0.342	0.438***	-0.412***	0.05	-0.039	-0.261*	0.261*	0.014	0.237*	-0.216
X20	-0.007	0.055	-0.105	0.057	0.019	0.172	0.328**	0.013	-0.002	0.034	0.087	-0.087	0.038	0.194	0.241*
X21	0.194	0.013	0.416***	0.173	0.116	-0.124	-0.447***	-0.328**	0.19	0.137	-0.13	0.13	-0.008	-0.155	-0.075
X22	0.079	0.115	-0.038	0.157	-0.103	0.028	0.259*	-0.059	0.157	0.152	0.035	-0.135	0.174	0.036	0.094
X23	-0.132	0.01	-0.359**	-0.047	-0.215	0.061	0.500***	0.244*	-0.044	-0.165	0.067	-0.067	0.092	0.046	0.03
X24	-0.008	0.023	-0.06	-0.016	0.037	0.075	0.066	0.042	0.042	-0.036	-0.147	0.147	0.007	0.074	0.118
X25	-0.08	0.001	-0.2	-0.012	-0.381	-0.181	0.284	0.146	0	-0.02	0.047	-0.047	0.155	-0.123	-0.153
X26	0.946	0.871	0.464	0.976	0.711	0.651	0.263	0.072	0.941	0.932	0.154	-0.154	0.875	0.431	0.458

Traits	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26
X16	**1.00**										
X17	0.055	**1.00**									
X18	0.417	-0.062	**1.00**								
X19	-0.006	0.208	-0.053	**1.00**							
X20	0.121	0.042	0.146	0.420**	**1.00**						
X21	-0.07	0.163	-0.039	0.521***	0.577**	**1.00**					
X22	0.089	-0.094	0.272*	-0.161	0.648**	0.265*	**1.00**				
X23	0.311	-0.245	0.304*	0.398***	0.524**	0.456***	0.676**	**1.00**			
X24	-0.004	0.166	-0.034	0.017	-0.191	0.167	0.089	-0.017	**1.00**		
X25	0.236	0.276*	0.3001 *	-0.133	0.411***	0.2855 *	0.7373 **	0.8975 ***	0.045	**1.00**	
X26	0.4	-0.011	0.232	-0.007	0.022	0.161	0.159	-0.06	0.009	-0.014	**1.00**

*-Significance at 5% Probability, **-Significance at 1% Probability, ***-Significance at 0.1% Probability

Table 16(b). Pearson's correlation coefficient among quantitative biochemical traits in different genotypes of pomegranate

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9
X1	1.000								
X2	-0.315**	1.000							
X3	0.405***	0.350**	1.000						
X4	-0.070	-0.647***	-0.510***	1.000					
X5	-0.399***	-0.068	-0.325**	0.353**	1.000				
X6	-0.265*	-0.026	-0.540***	-0.012	0.117	1.000			
X7	-0.115	0.064	-0.354**	-0.119	0.169	0.613***	1.000		
X8	-0.252*	-0.080	-0.426***	0.071	0.026	0.819***	0.051	1.000	
X9	-0.261	0.190	0.029	-0.044	0.298	0.116	0.247	-0.032	1.000

*-Significance at 5% Probability, **-Significance at 1% Probability,

***-Significance at 0.1% Probability

X1-Anthocyanin (mg/L)

X2- Ascorbic acid (mg/100g)

X3-Titratable Acidity (%)

X4- pH of the juice

X5- Fruit juiciness (%)

X6- Total sugars (%)

X7- Reducing sugars (%)

X8- Non- Reducing sugars (%)

X9- TSS (° Brix)

For the 26 morphological traits studied, fruit weight was selected as the dependent character to find out the direct and indirect effects of other 25 characters taken as independent characters. Direct effects (positive or negative) on fruit weight was found to be exerted by only a few traits included in study, namely, peel weight, aril weight, seed and skin percentage. Out of these, peel weight and aril weight had a positive direct effect on fruit weight with aril weight having higher direct effect among the two. On the other hand, seed and skin percentage exerted negative direct effect on fruit weight, each of equal magnitude.

Similar trends were observed for indirect effect also where, only the aforestated four traits were found to have indirect effect on fruit weight through various others traits under investigation. Among these, peel weight was observed to exert positive indirect effect on fruit weight through characters like fruit volume, fruit length, total no. of arils per fruit, aril weight and fruit diameter, in order of their decreasing magnitude. A negative indirect effect of peel weight, although of lesser magnitude, was also found, on fruit weight through seed percentage.

Aril weight also exerted direct positive effect on fruit weight through fruit length, fruit diameter, fruit volume, peel weight and total no. of arils, with its highest indirect effect through fruit volume followed by fruit length. Unlike peel weight, aril weight exerted a negative indirect effect on fruit weight through skin percentage. Although, seed percentage was found to have direct negative effect on fruit weight, it exhibited an indirect positive effect on the fruit weight through skin percentage, while an exact opposite trend was observed for indirect effect of skin percentage on fruit weight through seed percentage.

Moreover, for the nine biochemical parameters analysed, TSS was selected as a dependent character to find out the direct and indirect effects of other eight selected as independent characters. Except for anthocyanin and total sugars, all other six traits under study, had a direct positive effect on TSS, among which, non-reducing sugars exhibited the highest positive effect on TSS followed by reducing sugars while total sugars exhibited the highest negative direct effect on TSS followed by titratable acidity.

Positive and negative indirect effects also existed of the independent traits on TSS. Titratable acidity was found to exert a negative indirect influence on TSS through

Table 17 (a). Path coefficient analysis of quantitative morphological traits on fruit weight among different pomegranate genotypes

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X9	0.33	0.30	0.16	0.34	0.26	0.24	0.09	-0.02	0.37	0.32	-0.06	0.06	0.06
X10	0.62	0.57	0.31	0.64	0.45	0.42	0.17	-0.05	0.57	0.66	0.21	-0.21	-0.21
X11	-0.10	-0.10	-0.03	-0.10	-0.04	-0.05	-0.05	-0.04	0.11	-0.23	-0.73	0.73	0.73
X12	0.99	0.10	0.03	0.10	0.04	0.05	0.05	0.04	-0.11	0.23	0.73	-0.73	-0.73
X13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Contd…

Traits	X14	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X25	r
X1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95
X2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87
X3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46
X4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98
X5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.71
X6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65
X7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26
X8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.07
X9	0.32	0.16	0.16	0.17	0.01	0.13	0.02	0.00	0.07	0.06	-0.02	0.02	0.00	0.94
X10	0.56	0.28	0.30	0.23	-0.03	0.10	-0.03	0.02	0.09	0.10	-0.04	-0.02	-0.01	0.98
X11	-0.01	-0.07	-0.09	0.11	0.20	0.21	0.19	-0.06	0.09	-0.03	-0.05	0.11	-0.03	0.15
X12	0.01	0.07	0.09	-0.11	-0.20	-0.21	-0.19	0.06	-0.09	0.03	0.05	-0.11	0.03	-0.15
X13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88
X14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.43
X15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46
X16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40
X17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01
X18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23
X19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01
X20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
X21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16
X22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16
X23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.06
X24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01
X25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01

Table 17(b). Path coefficient analysis of quantitative biochemical traits on TSS among different pomegranate genotypes

Traits	X1	X2	X3	X4	X5	X6	X7	X8	TSS (°Brix)
X1	-0.268	0.084	-0.108	0.018	0.107	0.071	0.030	0.067	-0.261
X2	-0.006	0.019	0.006	-0.012	-0.001	-0.000	0.001	-0.001	0.190
X3	0.162	0.140	0.401	-0.204	-0.130	-0.216	-0.142	-0.171	0.029
X4	-0.009	-0.086	-0.068	0.133	0.047	-0.001	-0.016	0.009	-0.044
X5	-0.092	-0.015	-0.075	0.082	0.232	0.027	0.039	0.006	0.298
X6	83.1500	8.313	169.34	3.81	-36.84	-313.33	-192.35	-256.89	0.116
X7	-20.709	11.58	-63.78	-21.563	30.439	110.479	179.967	9.240	0.247
X8	-62.488	-19.84	-105.68	17.689	6.440	203.090	12.718	247.710	-0.032

Residual value=0.8692

X1-Anthocyanin (mg/L)
X2- Ascorbic acid (mg/100g)
X3-Titratable Acidity (%)
X4- pH of the juice
X5- Fruit juiciness (%)
X6- Total sugars (%)
X7- Reducing sugars (%)
X8- Non- Reducing sugars (%)
X9- TSS (° Brix)

traits like total sugars and pH while pH was found to have a negative indirect effect on TSS through ascorbic acid. Total sugars, which were found to have the highest negative direct effect on TSS, exhibited the highest positive indirect effect through titratable acidity and anthocyanin content.

Reducing sugars, in addition to having positive direct effect, also had a positive indirect effect on TSS through total Sugars while it exerted negative indirect effect through traits like titratable acidity and pH. Non-reducing sugars, like reducing sugars, too exhibited a positive indirect effect on TSS through total sugars while it was found to have a negative indirect effect on TSS through traits like titratable acidity and anthocyanin and ascorbic acid (Table 17b).

4.1.9 Mahalanobi's Diversity (D^2) Analysis

Pomegranate is mainly cultivated for its arils and the aril parameters like total number of arils per fruit, weight of aril, 100 arils fresh and dry weight are among the major yield attributing trait along with fruit weight, peel weight *etc*. In the present study a total of 35 quantitative phenotypic traits including 26 morphological and 9 biochemical parameters were subjected to diversity analysis.

In general, the contribution of biochemical characters was more to the diversity, however the highest contribution was recorded for peel weight (79%) followed by seed width (54.94%). Fruit weight showed a contribution of 3.56%. Among the biochemical trait, the highest contribution towards diversity was observed for non-reducing sugars (7.91%) followed by anthocyanin content (6.72 %) and titratable acidity (5.93%) (Table 18).

Twenty three genotypes were classified into four clusters on the basis of D^2 value (Table 19). Among these four clusters, the maximum number of genotypes (14) was comprised in cluster I, which included Bhagwa, Super Bhagwa, Early Bhagwa, Phule Arakta, Kabul Yellow, CO-1, P-26, Wonderful, P-23, Tobesto, Kaladagi Local, G-137, Dholka, Mridula. Seven genotypes *viz*. UHSP 23, UHSP 81, UHSP 125, Ruby, UHSP 57, KRS, Yearcaud formed the second cluster while cluster III and IV consisted of single genotypes namely Ganesh and Amlidana respectively representing the solitary clusters (Table 19).

Table 18. Per cent contribution to diversity from quantitative traits (morphological and biochemical) in D^2 analysis for different pomegranate genotypes

Sl. No.	Characters	Times ranked 1st	% Contribution
1	Fruit length (mm)	-	-
2	Fruit diameter (mm)	-	-
3	Fruit shape	-	-
4	Fruit volume (cm3)	-	-
5	Fresh wt. of 100 arils	-	-
6	Dry wt. of 100 arils	4	1.58%
7	Moisture %	-	-
8	Crown length (mm)	-	-
9	Peel weight	2	79%
10	Aril wt	-	-
11	Seed%	-	-
12	Skin%	-	-
13	Total No. of Arils/fruit	-	-
14	aril length (mm)	-	-
15	aril width (mm)	-	-
16	seed length (mm)	-	-
17	seed width (mm)	139	54.94%
18	rind thickness (mm)	-	-
19	Red coverage%	-	-
20	FC(L)	-	-
21	FC(a)	-	-
22	FC(b)	6	2.37%
23	AC(L)	19	7.51%
24	AC(a)	-	-
25	AC(b)	-	-
26	fruit weight (g)	9	3.56%
27	Anthocyanin estimation (mg/L)	17	6.72
28	Ascorbic Acid (mg/100gm)	2	0.79
29	Titratable Acidity (%)	15	5.93%
30	pH of the Juice	3	1.19%
31	Fruit Juiciness % (per 100gm aril wt.)	2	0.79%
32	Total Sugars (%)	6	2.37%
33	Reducing Sugars (%)	8	3.16%
34	Non Reducing Sugars (%)	20	7.91%
35	TSS (°Brix)	1	0.40%

Table 19. Cluster composition of different pomegranate genotypes based on quantitative (morphological and biochemical) traits in D^2 analysis

Sl. No.	Cluster No.	No. of genotypes	Name of genotypes
1	Cluster I	14	Bhagwa, Super Bhagwa, Early Bhagwa, PhuleArakta, Kabul Yellow, CO-1, P-26, Wonderful, P-23, Tobesto, Kaladagi Local, G-137, Dholka, Mridula
2	Cluster II	7	UHSP 23, UHSP 81, UHSP 125, Ruby, UHSP 57, KRS, Yearcaud
3	Cluster III	1	Ganesh
4	Cluster IV	1	Amlidana

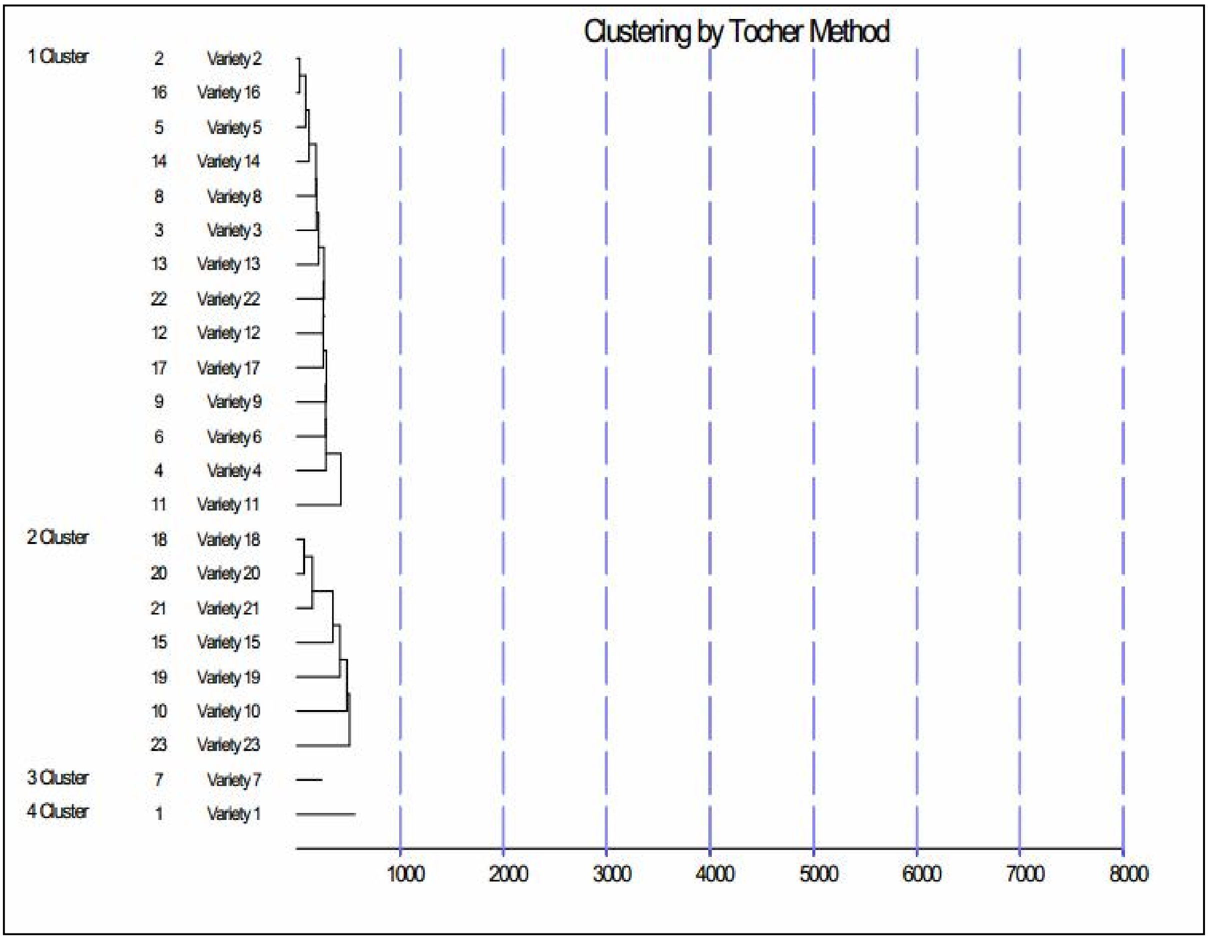


Fig. 5: Cluster diagram by Tocher method involving 4 clusters from 35 quantitative traits in analysis for 23 pomegranate genotypes

The estimation of inter- and intra-cluster distances by D^2 for phenotypic traits revealed that the range of intra-cluster distance varied from a minimum of 0.00 in third and fourth cluster comprising only single genotypes each, to a maximum of 1334.10 in the second cluster. The maximum inter-cluster distance was observed between cluster II and III (8169.86) while the minimum inter-cluster distance of 1904.87 was found between cluster I and IV (Table 20).

Cluster means obtained from D^2 analysis demonstrated wide variation among the clusters for morphological traits, cluster III performed better than other clusters in context of fruit length (112.35 mm), fruit diameter (95.87mm), fruit volume (527.78cm^3) and fruit length by width ratio (1.17) followed by cluster I for fruit length (72.43 mm) and volume (215.94 cm^3) and cluster IV for fruit diameter (72.80 mm). Fresh weight of 100 arils, dry weight of 100 arils, peel weight, aril weight, total no. of arils per fruit, aril length, aril width, rind thickness and fruit weight were also highest in cluster VI *viz.* 46. 89 g, 9.62 g, 192.44 g, 312.55 g, 1077.89 , 11.48 mm, 8.63 mm, 4.19 mm and 505.00 g respectively. While lowest fruit weight and fruit volume was recorded in cluster II being 120.72 g and 123.54 cm^3 respectively (Table 21).

With regard to biochemical parameters (Table 21) anthocyanin content was highest in cluster II (31.18 mg/L) while it was found to be lowest in cluster III (5.20 mg/L). Cluster IV performed best in terms of ascorbic acid (50 mg/100gm) followed by cluster I (27.40 mg/100gm). Highest titratable acidity (1.09%) along with lowest pH (2.53) was recorded for cluster IV, while pH was found to be the highest in cluster III (3.99). Furthermore, fruit juiciness was found to be highest in cluster I, 33.93 % respectively while, cluster IV exhibited highest TSS (15.56 °Brix) closely followed by cluster III (15.53 °Brix).

4.2.1 Molecular Marker Diversity

The molecular diversity of the 25 genotypes was studied by the banding patterns of 215 alleles using Jaccard’s dissimilarity coefficient followed by analysis for cluster distance with 1000 bootstrap values. The result of analysis has been presented in the dendogram (Fig. 7).

Table 20. Inter- and intra-cluster distance for quantitative (morphological and biochemical) traits in D^2 analysis for different pomegranate genotypes

Clusters	**Cluster I**	**Cluster II**	**Cluster III**	**Cluster IV**
Cluster I	805.0	3781.77	2266.86	1904.87
Cluster II		1334.10	8169.86	6042.51
Cluster III			0.00	3420.50
Cluster IV				0.00

Table 21. Cluster means for quantitative (morphological and biochemical) traits in D^2analysis for different pomegranate genotypes

Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
1	Fruit length (mm)	72.43	56.43	112.35	70.57
2	Fruit diameter (mm)	72.28	56.87	95.87	72.8
3	Fruit shape	1.00	1.00	1.17	0.97
4	fruit volume (cm^3)	215.94	123.54	527.78	189.44
5	Fresh wt. of 100 arils	27.48	17.58	46.89	32.55
6	Dry wt. of 100 arils	5.86	2.51	9.62	8.03
7	Moisture %	21.65	14.51	20.53	24.66
8	Crown length (mm)	14.3	13.71	12.83	20.05
9	Peel weight	76.03	40.87	192.44	34.00
10	Aril wt	136	79.84	312.55	153.56
11	Seed%	63.52	64.06	61.89	81.4
12	skin%	36.48	35.94	38.11	18.6
13	Total No. of Arils/fruit	494.18	303.06	1077.89	317.44
14	aril length (mm)	8.86	7.79	11.48	9.63
15	aril width (mm)	5.91	4.68	8.63	6.72
16	seed length (mm)	6.9	5.97	7.28	5.89
17	seed width (mm)	2.93	2.88	2.89	3.04
18	rind thickness (mm)	3.55	3.25	4.19	1.44
19	Red coverage%	68.39	70.19	56.67	47.5
20	FC(L)	56.36	53.61	57.02	48.96
21	FC(a)	26.45	27.45	38.29	21.17
22	FC(b)	32.04	30.36	36.52	17.88
23	AC(L)	42.22	34	43.54	29.02
24	AC(a)	40.48	22.91	17.29	20.19
25	AC(b)	17.12	14.98	16.96	8.14
26	fruit weight (g)	212.03	120.72	505	187.56
27	Anthocyanin estimation (mg/L)	13.97	31.18	5.2	7.31
28	Ascorbic Acid (mg/100gm)	27.4	24.24	25.6	50.00
29	Titratable Acidity (%)	0.11	0.33	0.19	1.09
30	pH of the Juice	3.49	3.41	3.99	2.53
31	Fruit Juiciness % (/100gm aril wt.)	60.72	53.12	53.12	54.89
32	Total Sugars (%)	33.93	22.25	12.46	14.2
33	Reducing Sugars (%)	17.04	15.51	9.95	6.29
34	Non Reducing Sugars (%)	16.89	6.74	2.51	7.91
35	TSS (°Brix)	13.87	11.8	15.53	15.56

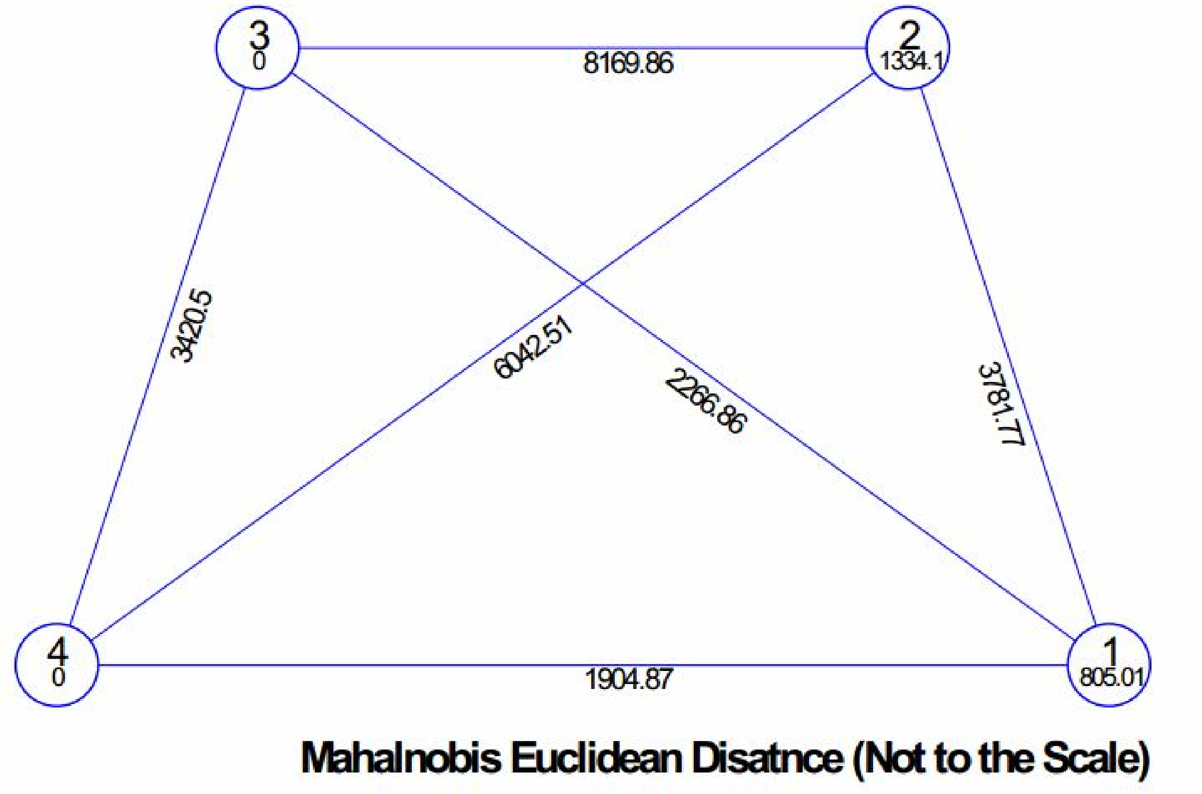


Fig. 6: Cluster diagram for 35 phenotypic traits

The 25 genotypes separated into three main clusters, cluster I, II and III, further forming sub and sub-sub clusters (Fig. 7).

First cluster comprised of a four genotypes *viz.* Yercaud, Tobesto, Phule Arakta and Wonderful. Within the first cluster, two sub clusters were formed with first sub-cluster comprising only Yercaud.

Dholka, KRS, Kabul Yellow, Kaladagi Local, Nana, Bhagwa, CO-1, Ruby and Super Bhagwa clustered together in cluster II, with Kabul yellow forming a solitary sub-cluster, Bhagwa being in a separate sub-sub cluster with Amlidana and Kaladagi Local with Nana. In cluster III, Mridula formed a solitary sub-cluster, while all the four mutants, UHSP 23, UHSP 57, UHSP 81 and UHSP 125 clustered together in the same sub-cluster. Daru also formed a separate sub-cluster whereas P-23 and P-26 were present in the same sub-cluster. The genotypic composition of each cluster is represented in the cluster diagram (Fig. 7).

4.2.2 Microsatellite based allelic diversity

From the 24 polymorphic makers screened across 25 genotypes of pomegranate in the present study, a total of 215 alleles were obtained. In pursuance of studying the informative potential of molecular markers and genetic diversity among genotypes including the effective number of alleles, number of alleles per marker (Na), number of effective alleles (Ne), Shannon Index (I), Observed heterozygosity (Ho) and Expected Heterozygosity (He) were calculated based on frequency of alleles of each locus, information pertaining to which have been presented in Table 22. The number of alleles per marker ranged from 2 (PGKVR031) to 16 (PGKVR139 and PGKVR152). However, the total number of effective alleles obtained ranged from 1.09 for PGKVR101 having only 3 alleles to 9.98 for PGKVR119 having 14 alleles (Table 22).

Shannon Index (I), was found to be highest for the marker PGKVR152 (2.51) followed by PGKVR038 (2.47) and PGKVR119 (2.45) while it was lowest for PGKVR101 (0.21) followed by PGKVR031 (0.23).

Furthermore, observed heterozygosity (Ho) was found to be highest for marker PGKVR100 (0.96) with expected heterozygosity of 0.54, followed by PGKVR115 (0.83) with an expected frequency of 0.74, while it was as low as 0.04 for the markers PGKVR031 and PGKVR153 with an expected heterozygosity of 0.11 and 0.80 respectively.

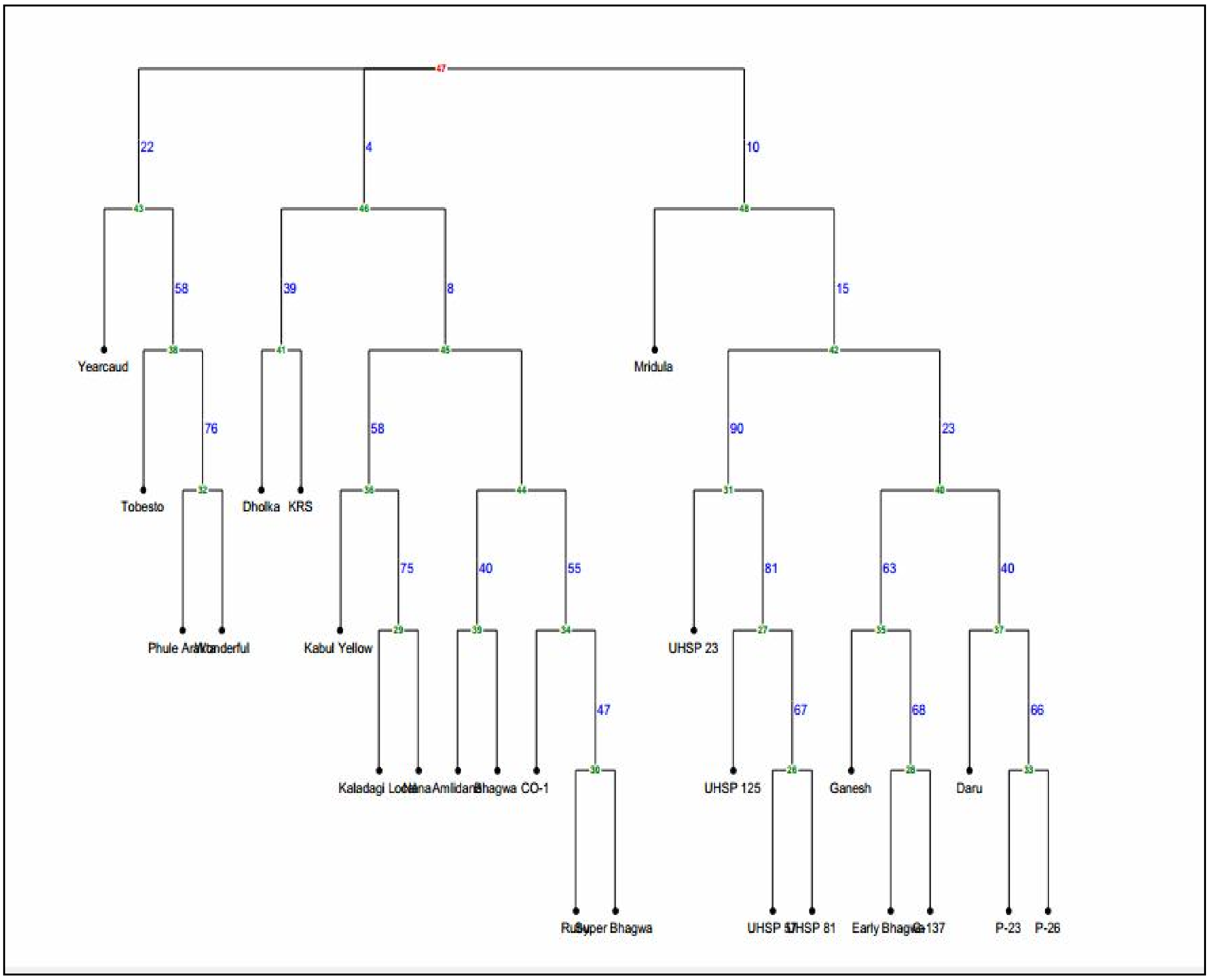


Fig. 7: Jaccard's dissimilarity coefficient for the genetic relationship among 25 pomegranate genotypes obtained from 24 molecular markers (215 alleles)

Table 22. Molecular marker analysis for the polymorphic microsatellite markers in different genotypes of pomegranate

Sl. No.	Molecular Marker	Na	Ne	I	Ho	He
1	ABRII-MP26	13	9.53	2.37	0.57	0.90
2	PGKVR031	2	1.13	0.23	0.04	0.11
3	PGKVR038	15	9.48	2.47	0.67	0.89
4	PGKVR092	11	6.04	2.07	0.29	0.83
5	PGKVR094	8	4.06	1.61	0.08	0.75
6	PGKVR100	3	2.17	0.84	0.96	0.54
7	PGKVR101	3	1.09	0.21	0.09	0.08
8	PGKVR105	4	2.80	1.16	0.24	0.64
9	PGKVR112	5	1.67	0.84	0.16	0.40
10	PGKVR113	10	6.07	2.01	0.52	0.84
11	PGKVR114	6	1.59	0.82	0.35	0.37
12	PGKVR115	5	3.87	1.42	0.83	0.74
13	PGKVR119	14	9.98	2.45	0.61	0.90
14	PGKVR122	7	2.75	1.40	0.52	0.64
15	PGKVR126	11	7.16	2.16	0.08	0.86
16	PGKVR127	12	5.96	2.15	0.71	0.83
17	PGKVR132	10	4.82	1.90	0.27	0.79
18	PGKVR137	10	2.89	1.55	0.32	0.65
19	PGKVR139	16	6.35	2.24	0.32	0.84
20	PGKVR149	9	4.13	1.74	0.13	0.76
21	PGKVR152	16	9.71	2.51	0.57	0.90
22	PGKVR153	7	4.92	1.74	0.04	0.80
23	PGKVR154	11	7.86	2.20	0.08	0.87
24	PGKVR165	6	3.00	1.37	0.08	0.67

Na-Number of alleles, Ne-No of effective alleles, I -Marker information index, Ho- Observed heterozygosity and He-Expected heterozygosity

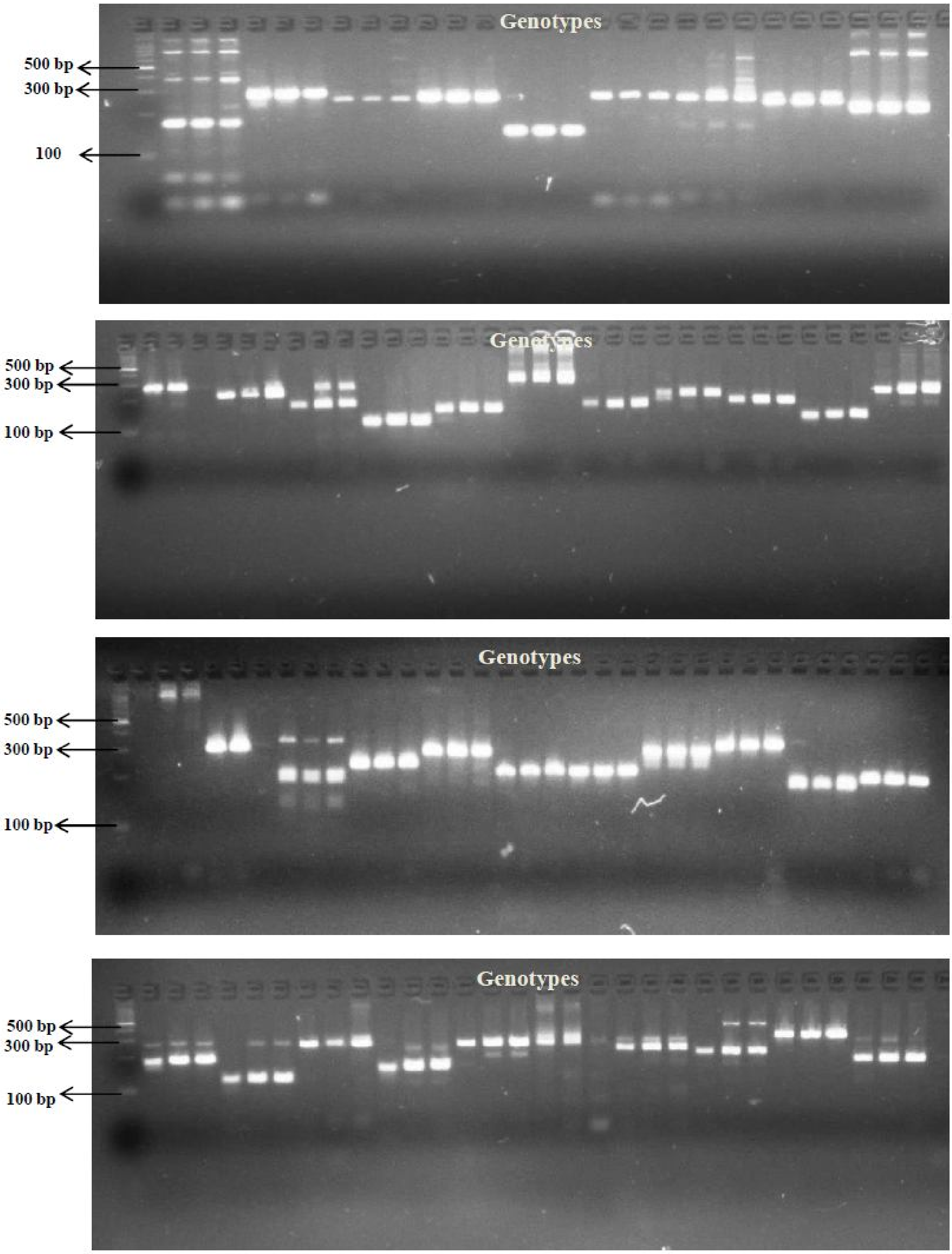


Plate 6. Screening of microsatellite markers among different pomegranate genotypes

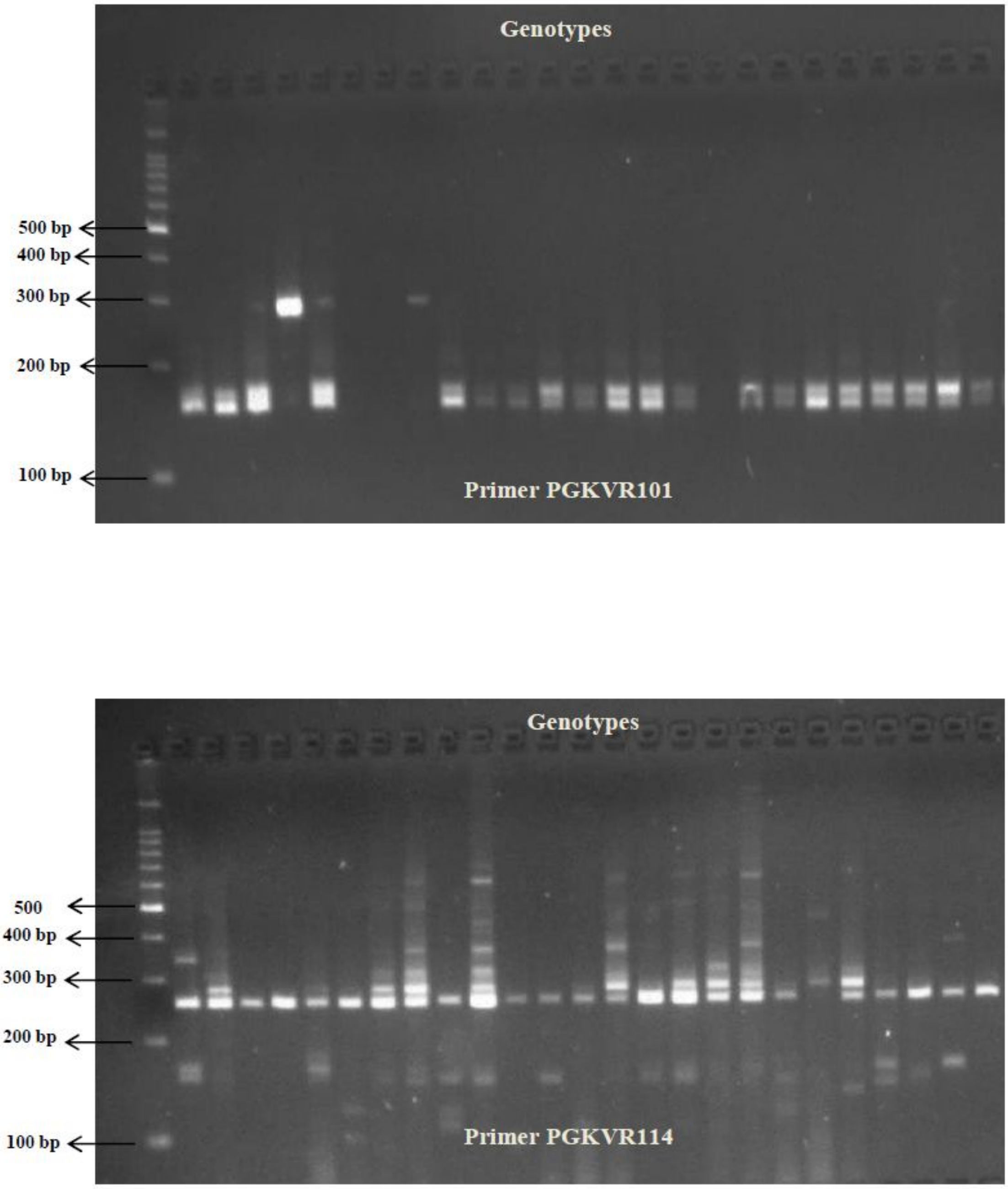
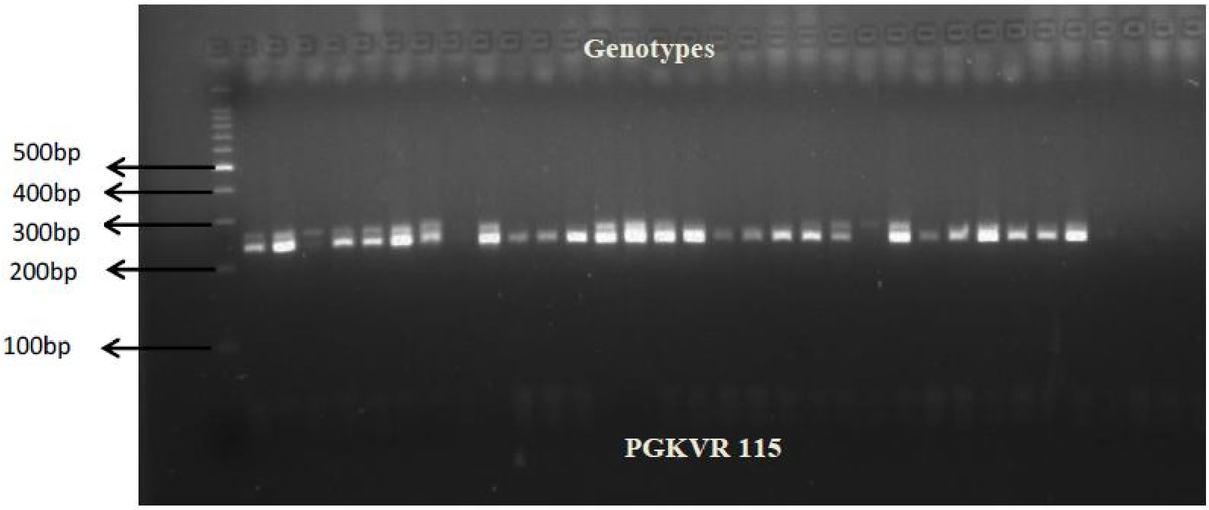


Plate 7. Molecular characterization in pomegranate genotypes using microsatellite marker



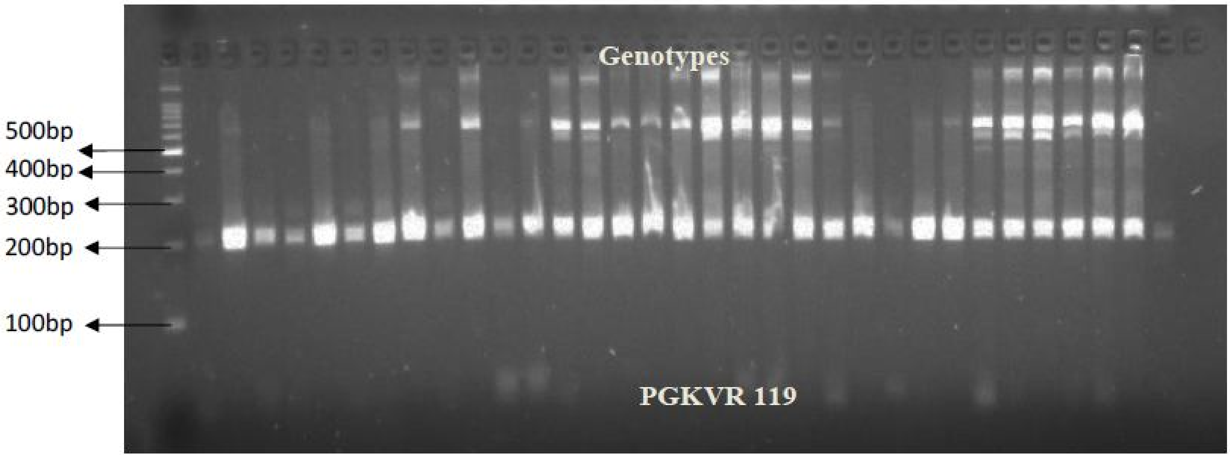


Plate 7. contd…

4.3 Marker- Trait association

All the 24 markers with their total of 215 alleles were subjected to marker trait association with a total of 10 phenotypic traits, including 9 important economic traits and bacterial blight resistance, results of which have been presented in Table 23. Step wise linear regression method was followed to study the marker-trait association and only the markers showing significant association in terms of phenotypic variance (R^2) were taken into consideration. The markers having significant association with the economic traits varied from one (Bacterial blight, fresh weight of 100 arils, total aril weight and total number of arils) to three (fruit weight and titratable acidity).

Cumulative regression value (R^2) for fruit weight was 0.486, which was explained by three alleles. The highest phenotypic variance was obtained for titratable acidity (0.869) which was explained together by the three alleles, with highest contribution by marker PGKVR 119. The same marker (PGKVR 119) was found to be associated with ascorbic acid, explaining a phenotypic variance of 0.494. However, no marker associated with TSS could be obtained with the set of markers used in this study.

Marker PGKVR 112 was found to be associated with all the aril parameters like fresh weight of 100 arils, total aril weight and total number of arils per fruit with R^2 value of 0.294, 0.368, and 0.375 respectively.

With respect to the fruit traits, primer PGKVR 132 and 149 showed a significant association with fruit weight and fruit volume. Furthermore, primer PGKVR 122 was found to be associated with bacterial blight and interestingly was also found to be associated with fruit weight.

Table 23. Comparison of linear regression (cumulative R^2 values) analysis for marker-trait association for selected economic traits from polymorphic microsatellite markers in different pomegranate genotypes

Sl. No.	Traits	Markers	R^2 Value	Sig. F Change
1	Fruit weight	PGKVR 149	0.173	0.048
		PGKVR 132	0.163	0.039
		PGKVR 122	0.150	0.029
2	Fruit Volume	PGKVR 132	0.207	0.029
		PGKVR 149	0.150	0.012
3	Rind thickness	PGKVR 119	0.298	0.007
		PGKVR 139	O.220	0.006
4	Fresh weight of 100 arils (g)	PGKVER 112	0.294	0.008
5	Total aril weight	PGKVR 112	0.368	0.002
6	Total no. of arils/fruit	PGKVR 112	0.375	0.002
7	TSS (°Brix)	-	-	-
8	Titratable acidity (%)	PGKVR 119	0.602	0.000
		PGKVR 149	0.214	0.000
		PGKVR 105	0.053	0.008
9	Ascorbic acid (mg/100g)	PGKVR 119	0.494	0.000
		PGKVR 113	0.205	0.001
10	Bacterial blight	PGKVR 122	0.316	0.003

5. DISCUSSION

Pomegranate, a beloved ancient fruit crop, highly prized for its juicy arils has been deeply embedded in human history and was lauded in the old testament of the Bible, Koran, the Jewish Torah, and the Babylonian Talmud as a sacred fruit which confers the power of fertility, abundance, and good luck. The edible part of fruit contains acids, sugars, vitamins, polysaccharides, polyphenols and minerals, howbeit, several factors including cultivar, environmental conditions; ripening *etc.* can exert an influence on the chemical compositions of the fruit, affecting fruit quality and health beneficial components.

Different genotypes show significant diversity in pomological traits related to fruit properties, such as fruit fresh weight, skin thickness, along with juice biochemical characteristics, such as anthocyanin content, vitamin C content, sugars, acidity, that could be attributed to several factors including differences in geographical origin, ecological adaptation to sites and nature. The variability at genotypic level may due mainly to recombination (resulting from outcrossing) combined with sexual propagation for long-term and uncontrolled spread of plant material (Mars 1996) in the past. Pomegranate is known to be at least partially cross-pollinated (Jalikop and Sampath-Kumar, 1990). Several attempts at characterization of pomegranate has been made in the recent past, however the complete information pertaining to the popular Indian cultivars' germplasm diversity at the phenotypic and/or molecular level is meagre till date. Hence, the present study was undertaken to explore the genetic variability and diversity existing in the 23 popular cultivars grown in India using 40 phenotypic characters consisting 37 traits, (28 morphological and 9 biochemical traits) quantitatively analysed and three traits qualitatively analyzed. For genotyping 2 wild forms, Daru and Nana were included along with 23 aforesaid genotypes using 24 microsatellite markers. Details of the present investigation have been discussed in this chapter under the headings phenotypic characterization, molecular characterization and marker-trait association.

5.1 Phenotypic characterization

The phenotypic variability of the 23 popular genotypes have been comprehensively studied using 40 characters out of which 37 traits (28 morphological and 9 biochemical traits) were quantitatively analysed and subjected to various statistical analysis such as ANOVA, mean, range and genetic variability components (GCV, PCV, h^2, GA and GAM) while the qualitatively analysed three traits were subjected to frequency distribution analysis. All the quantitative traits were analysed further for principal component analysis, Pearson's correlation coefficient analyses, path coefficient analyses and Mahalanobis D^2 for diversity analyses.

Analyses of Variance (ANOVA) revealed significant variation for almost all the traits under study, suggesting the existence of sufficient genetic variation among the pomegranate genotypes selected for the investigation. Hence, all the traits were further subjected to the above mentioned statistical analysis which have been discussed below in separate sub-headings

5.1.1 Mean, Range and genetic variability estimates

Variations in the population are the pre-requisite for the improvement of any trait. Among the variations, the amount of phenotypic variation which is heritable to the next generation mainly accounts when a breeder makes decisions regarding selections. This genetic variation is often studied based on range and genetic variability estimates such as genotypic variance (GV) and phenotypic variance (PV). However, the unit less measurements corresponding to these variability estimates, *viz.* GCV and PCV which are expressed in percentage, are more suitable while dealing with the study of traits with different units, as in the present study, in order to compare these components across the traits.

Furthermore, for identifying the characters exhibiting high variability, the absolute values of variability may not be of much help, and hence, the coefficients of variability are used which indicates only the extent of variability present for a particular character without demarcating it into heritable and non-heritable components.

In this study a wide range of variation was observed for almost all the traits under investigation indicating that there is an abundant scope for further improvement by breeding using suitable parents superior for various traits. Wide range of variability has also been reported in pomegranate for various traits by Manohar *et al.* (1981); and Pandey and Bist (1998). Mean values of all characters showed considerable variations between genotypes. Significant differences were recorded between genotypes for all morpho-pomological characteristics based on analysis of variance. Due to the high diversity in the investigated traits, it's obvious that these can be used in future breeding programs to obtain superior varieties

Variation in fruit weight, a major yield attributing trait, was huge ranging from 56.01-505.00 g with very high h^2 value of 97.23 % suggesting the amenability of this trait for improvement. This was in accordance with earlier reports regarding fruit weight by Tehranifar *et al.* (2010). Zamani *et al.* (2013) and Khadivi-Khub *et al.* (2015) also observed almost same trend for fruit weight ranging from 45.56–374.12 g and 69.77 to 341.91 g respectively, while Sarkhosh *et al.* (2009) reported fruit weight 165–376 g for cultivated pomegranates. Fruit size is considered to be an important trait for fresh market trade and the variation of fruit weight is mainly influenced by the genotype but pedo-climatic conditions may also have an important role to play (Tehranifar *et al.*, 2010).

Fruit volume for the different genotypes ranged from 62.00-527.78 cm^3 with a mean of 200.23 cm^3 while Mir *et al.* (2010) reported the range of 100.28-237.62 cm^3 with a mean value of 174.30 cm^3 for pomegranate cultivars under the Karewa belts of Kashmir. The total no. of arils per fruit varied in the present study ranged from 150.67-1077.89 which is wider than that reported by Mir *et al.* (2010) *i.e.* 275.88-546.94, indicating the suitability of the varieties of the present investigation for improvement of this trait

Pomegranate arils contain juice, pulp, and the woody part rich in raw fibres and different other compounds, but from taste point of view, one of the most important traits is TSS of the arils. In this study, the highest TSS value was found to be 17.95 °Brix and lowest was 9.62 °Brix with an average of 13.39 °Brix which is almost similar to what reported for Spanish cultivars (Martinez *et al.* 2006). In the previous studies, TSS values for different pomegranate accessions were in the range 14.40–17.00 °Brix in Greece (Drogoudi *et al.* 2005), 14.00–16.80 °Brix in Spain (Melgarejo *et al.,* 2000), 11.40–16.20 °Brix in Iran (Tehranifar *et al.* 2010) and 13.56-15.77 °Brix in India (Mir *et al*., 2010). These differences can be attributed not only to the different accessions but also to environmental conditions and harvesting times (Tehranifar *et al.,* 2010).

Ascorbic acid is another important compound in pomegranate fruit juice which contributes to its health benefits. The range of ascorbic acid in the present investigation was found to be from 20.24-50.00 mg/100 g with an average of 27.34 mg/100 g which is higher than some of the earlier reports by Mir *et al.* (2010), 9.40-13.36 mg/ 100 ml, and Tehranifar *et al.* (2010), 9.91mg 100 g^{-1} to 20.92mg 100 g^{-1} which may be due to differences in genotypes, growing conditions as well as harvesting times.

Pomegranate juice is reported to have greater antioxidant capacity than other fruit juices and beverages (Seeram *et al.*, 2008) which can be attributed to the high level of phenolic compounds (Gil *et al.*, 2000). Pomegranate is known to contain considerable amount of phenolic compounds, including anthocyanins mainly 3-glucosides and 3, 5-diglucosides of delphinidin, cyanidin and pelargonidin, which contributes to the red colour of pomegranate juice apart from their anti-oxidant activity. There were significant differences in the total anthocyanin content among the studied cultivars ranging from 4.35-61.44 mg/L with an average of 18.53 mg/L. Wide variations for anthocyanin has also been reported for different cultivars of turkey, with values ranging from 8.1 and 36.9 mg per 100g of juice (Cam *et al.*, 2009) and 9–115 mg per litre of juice for Tunisian accessions of pomegranate (Hasnaoui *et al.,* 2011a). These results indicated that the levels of various biochemical as well as morphological traits varied among different cultivars of pomegranate and that a high genetic heterogeneity exists within the studied cultivars.

With respect to genetic variability components of the quantitative phenotypic data all the 35 characters revealed moderate to high Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) along with heritability components such as heritability (h^2 in broad sense) and Genetic advance (GA).

Most of the traits observed exhibited a heritability of more than 60% depicting higher heritability of these traits. Among the traits analysed the highest heritability

99.13% was recorded in dry wt. of 100 arils followed by titratable acidity with 98.28% while the lowest was observed for seed width (32.28%).

In this study, it was observed that the phenotypic coefficient of variation (PCV) was higher than its corresponding genotypic coefficient of variation (GCV) for all the traits. Also a high magnitude of coefficient of variability (phenotypic and genotypic) was observed for fruit and aril traits like fruit weight, fruit volume, fresh weight of 100 arils, dry weight of 100 arils, total aril weight and biochemical traits like titratable acidity (%), anthocyanin content and reducing and non-reducing sugars while low to moderate level of variation was observed in TSS, pH and seed parameters like seed length and width. These results indicate that higher magnitude of genotypic coefficient of variations for the aforementioned traits offer a better opportunity for improvement through selection.

Wide range of variability in tree and fruit characters have also been reported by Meena *et al.* (2003), Singh *et al.* (2010) and Mir *et al.* (2010) in pomegranate. Estimates of heritability in broad sense were higher (>90 per cent) for traits like fruit weight, fruit volume, fresh wt. of 100 arils, dry wt. of 100 arils, peel weight, total no. of arils/fruit and titratable acidity. Similar reports for fruit weight, fruit volume, number of seeds per fruit, aril weight have been presented by Mir *et al.* (2010).

Mir *et al.* (2006) also found high heritability for plant height, fruit volume, fruit set percentage, acidity, gross fruit yield, rind weight and number of fruits/plant. The results obtained are also in close agreement with findings of Meena *et al.* (2003); Pandey and Bist (1998). The high estimates of heritability are considered useful to the plant breeders as it enables to base the selection programme on phenotypic performance. Johnson *et al.* (1955) accentuates heritability estimates in conjunction with genetic advance to be usually more helpful in predicting its resultant effect from selecting the best individuals.

The genetic advance expressed in percentage of mean (GAM) ranged from 6.83 g for seed width to 119.05 g for peel weight among the morphological traits while for biochemical traits it varied between 17.94 for pH of the juice to 223.56 for titratable acidity. In the present study, high heritability was found to be associated with high genotypic advance for most of the characters. High heritability coupled with high genetic advances have also been reported by Navjot *et al.* (2009) in ber in traits like fruit weight, pulp stone ratio, total soluble solids and fruit yield per plant. Panse and Sukhatme (1967) emphasised 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 that dominance or epistatic effects are of considerable value for these characters and hence little improvement in these characters is possible through selection.

5.1.2 Frequency distribution

In the present study, frequency distribution graph for the three qualitative traits, seed hardiness, fruit shape and juice colour showed that most of the genotypes studied were soft seeded type. Soft seediness is a highly desirable trait in pomegranate as far as the consumer acceptance and suitability for processing is concerned. Also, soft-seeded pomegranates were recommended by Jalikop and Kumar (1998) as parents for developing high-juice cultivars due to their significantly higher content of juice. Khadivi-Khub *et al.* (2014) in their study, recommended soft seeded pomegranates as parents for developing high-juice cultivars owing to their significantly higher juice content. Further, Khadivi-Khub *et al.* (2015) also emphasized that soft seediness is an important trait to be used for cultivation and in breeding programs for decreasing hard-seediness in commercial pomegranate cultivars.

Fruit shape (on the basis of fruit length/width) was found to be either round or ovate for all the cultivars studied with highest frequency of round fruited type. Fruit shape is an important trait which could be considered while selecting varieties suited for different types of packaging.

Juice colour of the cultivars varied from whitish-pink to dark red where highest frequency was observed for red colour juice. Juice colour of Bhagwa was found to be dark red in colour which adds to its suitability as processed products like juice.

5.1.3 Scoring for bacterial blight

The screening of 25 genotypes (including two wild forms previously discussed) for bacterial blight and foliar and fruit spots, under field conditions, revealed that almost all the popular cultivated forms used in this study are slightly to moderately susceptible, with Bhagwa being the most susceptible one. The severity percentage for Daru and Nana cultivars was very low as compared to other cultivars supporting the earlier evidences of these varieties being important donor source for bacterial blight (Jalikop *et al.*, 2006). However, since in the present investigation, scoring was done under the field conditions and no artificial inoculation was made, the results need to be further validated.

5.1.4 Principal component analysis

In the characterization of germplasm, 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 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 components analysis (PCA) is one of the most efficient approaches to find out the variations being explained by different traits thus reducing the variables under study.

PCA is a data analysis tool generally used to reduce the dimensionality (number of variables) of a large number of inter-related variables, at the same time retaining as much of the information (variation) as possible. PCA calculates an un-correlated set of variables (factors or principal components). These factors are ordered such that the initial few retain most of the variation present in all of the original variables. Thus helping in identification of most relevant characters that can be employed as descriptors by explaining as much of total variation present in the original set of variables as possible with as few components as possible (Ramesh, 2015).

In the present study, a total of 35 quantitative traits, including both morphological as well as biochemical parameters were subjected to principal component analysis (PCA). The results of PCA indicated that the total variability was being explained by 35 principal components, where first three principal components with Eigen values greater than 3 explained highest variation with 55.37% of the total variability observed. Variations in most of the fruit and aril characters like fruit weight, fruit volume, fruit length, fruit diameter, peel weight, aril weight, total number of arils, aril weight, fresh weight of 100 arils, dry weight of 100 arils, aril length, aril width was explained by principal component 1 (PC1) which accounted for 27.77% of the total variation.

The results were up to a considerable extent, in agreement with the results obtained in previous studies (Mars and Marrakchi, 1999; Zamani *et al.,* 2007; Drogoudi *et al.,* 2005; Durgac *et al.,* 2008; Sarkhosh *et al.,* 2009). Mars and Marrakchi (1999) studied 30 pomegranate accessions of Tunisia and reported that the discriminating characters for their germplasm were fruit size, colour and juice characteristics. The same traits were found to be important in the study conducted by Drogoudi *et al.* (2005) where they determined several characteristics of 20 Greek pomegranate cultivars. Moreover, according to Zamani *et al.* (2007), fruit characteristics in pomegranate had the highest loading values for the first component in component analysis. The results obtained in this study along with the earlier reports indicate that fruit traits could be successfully used for study of pomegranate genotypes as they exhibit highest variation.

5.1.5 Correlation studies among the quantitative traits

Correlation coefficient measures the mutual relationships between the various traits, which is helpful for understanding the association among various traits. This eventually enables a breeder to devise efficient strategies for indirect selection using component character along with simultaneous selection of multiple traits. Fruit weight is an important economic trait for pomegranate which has the most important bearing on the total yield of the crop. Thus, in order to increase the yield and make efficient selection for higher yield, it is highly important that the associations of different quantitative traits with fruit weight should be known.

Only quantitative traits showing continuous distribution can be used for correlation studies and hence 35 quantitative traits, including 26 morphological and 9 biochemical traits used for the present study were subjected to Pearson’s correlation analysis.

As presented in the result part of this dissertation, fruit weight was found to have significant positive correlation with most of the traits studied *viz.* fruit length, fruit diameter, fruit volume, fresh and dry weight of 100 arils, peel weight, aril weight, total no. of arils per fruit, aril length and aril width. Although no significant negative correlation with any of the studied characters could be deduced. This result is in harmony with earlier report by Mir *et al.* (2006), where they found a significant positive correlation between fruit weight and fruit diameter, fruit volume, juice content, fruit set and number of fruits/plant and gross fruit yield. Verma *et at.* (2002) has reported positive correlations of fruit weight with fruit diameter and fruit volume in case of strawberry.

Peel weight was found to be significantly and positively correlated with Fruit weight, fruit length, aril weight, total no. of arils per fruit, aril length and width and seed length as well as rind thickness. This finding is in agreement with the results of Zamani *et al.* (2013) and Karimi and Mirdehghan (2013).

Zamani *et al.* (2013) found peel weight to be significantly correlated with fruit weight (r = 0.86), aril weight (r = 0.73) and aril length (r = 0.51) and Karimi and Mirdehghan (2013) reported a significant correlation between peel weight and fruit diameters (r = +0.94), and fruit length (r = +0.93) however, in contrast to the findings of this study, they observed a negative correlation of peel weight with number of arils in fruit (r = –0.78). Moreover, they reported a positive correlation between number of arils in fruit and fruit length (r = +0.74), and a similar trend has been observed in this study for the aforementioned trait. Khadivi-Khub *et al.* (2015) also found that fruit weight, fruit diameter and fruit length were positively correlated with each other and asserted that these characters could be used to predict each other.

The extremely high correlation observed between fruit volume and weight (r = 0.976) indicates that weight can interchangeably be used to indicate size. Also, aril number per fruit exhibited a strong linear relationship with fruit size. Thus, any of the characteristics loading high on the factor analysis fruit index (fruit volume, fruit weight, aril weight per fruit, skin and pericarp weight, and aril number) could serve as a measure for grading pomegranate fruit for different purposes (*e.g.* fresh fruit versus extracted arils) where fruit weight, diameter, or volume could be used as the index of size. Understanding that, number of arils dictates final fruit size can have important implications on cultural practices. Since, each aril is the product of fertilization, obtaining large fruit and high yields will depend upon careful crop management during the early stages of pollination and fertilization and subsequent fruit set and development. An interest in understanding fruit attributes and how fruit size relates to the per cent of edible aril weight is pertinent, especially with the onset of mechanical extraction methods (Blasco *et al.*, 2009) for marketing of pomegranate arils in a ready-to-eat form.

Among the colour parameters, red coverage of peel was positively correlated to fruit and aril colours, and moisture percentage of the fruit was found to be significantly correlated with seed length as well as the colour parameters of fruit and aril and red coverage of peel. Thus the fruit colour can be an indication of aril colour and since the moisture percentage of arils which is influenced by aril weight and seed length, the latter two traits can be useful for indirect selection of varieties with attractive fruit colour, which is one of the major factor influencing consumer acceptances.

Among the biochemical traits studied anthocyanin content showed a significant positive correlation with titratable acidity and a strong negative correlation with fruit juiciness percentage and ascorbic acid content, suggesting that fruits with more juiciness are likely to have less anthocyanin and hence less colour development. However, since both traits are important for a cultivar, a breeder should try to strike a balance between these two traits if developing a variety with more juice and attractive colour is the objective.

Titratable acidity (TA) was found to be significantly and negatively correlated with pH, which in turn was showing negative correlation with TSS which is in consonance with the findings of Khadivi-Khub *et al.* (2015). Zamani *et al.* (2013) reported titratable acidity (TA) to be negatively correlated with TSS (r = -0.56) and pH (r = -0.86) and similar reports are available from the investigations of Zamani *et al.* (2007) and Mir *et al.* (2009).

Correlation coefficient provides information about the traits that are most important in assessing the germplasm (Norman *et al.*, 2011). Furthermore, these variables can also be used to predict other ones, and thus becomes important for the characterization of accessions. Nevertheless, a close relationship between traits could facilitate or hinder gene introgression since selection for a desirable trait, may favour the presence of another desirable trait from germplasm (Khadivi-Khub, 2014).

5.1.6 Path coefficient analysis

Path coefficient analysis provides a comprehensive insight into the inter-relationship between various characters. In pomegranate, fruit yield is influenced by a number of inter-dependent traits. This inter-dependence between the component traits influences the direct relationships of traits with each other, making the correlation coefficient derived information less steadfast.

In the present investigation, a total of 35 phenotypic traits, were subjected to path coefficient analysis, with separate analysis for morphological (26) and biochemical (9) traits. The results of path coefficient for morphological traits showed that direct effects (positive or negative) on fruit weight was found to be exerted only by peel weight, aril weight, seed and skin percentage, with aril weight having the highest direct effect, indicating that aril weight is an important yield attributing component in pomegranate.

Peel weight was observed to exert positive indirect effect on fruit weight through characters like fruit volume, fruit length, total no. of arils per fruit, aril weight and fruit diameter, pointing that these traits could be used for indirect selection of genotypes for higher yield.

Lower values of residual effects for morphological traits (0.0005) indicated the wide variability for the independent characters selected for the study which was also supported by the PCA analysis, suggesting that above traits would have a decisive role in breeding programme for improving yield in pomegranate.

Based on the results of path coefficient analysis for biochemical traits, it could be deduced that TSS, which is one of the most important traits determining the flavour of pomegranate is influenced positively by all the independent traits under study except anthocyanin and that reducing sugars exert a high positive direct influence, emphasizing the importance of reducing sugars for development of quality attributes of pomegranate.

Titratable acidity was found to exert a negative indirect influence on TSS through traits like total sugars and pH while pH was shown to have a negative indirect effect on TSS through ascorbic acid.

Total sugars, which were found to have the highest negative direct effect on TSS, exhibited the highest positive indirect effect through titratable acidity and anthocyanin content. Since, pomegranate is used both in its fresh and processed forms, the understanding of inter-dependence of biochemical traits would be highly desirable to develop cultivars pertaining to the destined markets.

In this study, a high residual effect of 0.869 was found for the path analysis of biochemical traits as compared to a lower value of 0.0005 for the morphological traits, indicating that, the variations present in morphological traits are considerably higher than that in biochemical traits and that the other parameters could also be responsible for TSS (which was a dependent trait among the biochemical parameters) than the biochemical parameters analysed in the present study. In contrast, for the morphological characters, the lower residual value indicates that the morphological characters selected in the present study as independent variables for the dependent trait (fruit weight) are sufficient to explain the direct and indirect effects.

5.1.7 Morphological diversity analysis

For the study of genetic diversity, Mahalanobi's D^2 analysis is reputed as one of the most powerful approaches, especially when diversity in morphological traits are to be used as a criterion for selection of diverse parents for breeding. It provides detailed information about the number of characters contributing to the diversity by considering the genetic variation of the respective traits in the population, irrespective of the number of traits included in the study. Here, the genotypes are grouped into different clusters based on the diversity among the genotypes and the information like number of genotypes in each cluster, intra- and inter- cluster distances as well as the mean values for each trait under study for the corresponding cluster could be obtained that is helpful for selection of diverse genotypes from the diverse cluster as per the breeding objectives.

In the present study 23 pomegranate cultivars were analysed for different phenotypic traits including morphological and biochemical parameters and all these traits were subjected to diversity analysis.

Very interestingly, the fruit parameter, peel weight (79 %) followed by seed width (54.94 %). contributed maximum to the diversity. However, the contribution of biochemical traits such as non-reducing sugars (7.91 %), anthocyanin content (6.72 %) and titratable acidity (5.93 %) indicates the effectiveness of the plant material for diversity analysis for both productivity as well as quality traits. The diverse parents identified in the present study would be utilized for selection of superior progenies for both yield and nutritional quality in the breeding program.

The 23 genotypes studied clustered in 4 different clusters for all the phenotypic traits. Among these four clusters, the maximum number of cultivars (14) was comprised in cluster I, which included Bhagwa, Super Bhagwa, Early Bhagwa, PhuleArakta, Kabul Yellow, CO-1, P-26, Wonderful, P-23, Tobesto, Kaladagi Local, G-137, Dholka, Mridula. The grouping of the Bhagwa and its superior clones and other cultivars in a single cluster indicates that, these cultivars would be the result of clonal selections from the single ancestor which could be further confirmed by molecular phylogeny. Seven genotypes *viz.* UHSP 23, UHSP 81, UHSP 125, Ruby, UHSP 57, KRS, Yearcaud formed the second cluster again indicates the different ancestor. The presence of all the mutants of Bhagwa in to a single cluster but the differentiation from its parent indicates that the mutation created greater variation among these mutants from its parent. Remaining two clusters (III and IV) consisted of single cultivars each namely Ganesh and Amlidana respectively representing the solitary clusters indicting their nature of diversity form other two clusters. The formation of different clusters thus indicates the presence of diversity among different genotypes.

The perusal of data pertaining to intra- and inter cluster distances divulges that cluster III and IV had minimum intra-cluster distance while for cluster II it was recorded to be maximum. This emphasizes that cluster II has genotypes which are relatively distant from each other as compared to the cluster I which has lower D^2 distance. Although lesser number of cultivars are present in the II cluster than cluster I, their higher intra-cluster distance necessitates further evaluation by molecular markers to clearly understand the diversity between the cultivars present within this cluster.

Further, the maximum inter-cluster distance was observed between cluster II and III suggesting large genetic differences among the genotypes comprised in these clusters whilst, the minimum inter-cluster distance was noted between cluster I and IV attesting significantly lesser genetic divergence among the genotypes of these clusters. Larger inter-cluster distance is a denotative that the genotypes comprised in these clusters are genetically diverse and can be employed in hybridization programme for getting better recombinants in the segregating generations. Correspondingly, lower intra-cluster distances demonstrated the narrow genetic base of the clusters and hence, selection of parents from these clusters should be avoided. Different intra- and inter-cluster distances have previously been recorded in various fruit crops like pomegranate, walnut, almond, and pecan cultivars (Akbarpour *et al.*, 2010).

With regard to morphological traits, cluster III performed better than other clusters in context of fruit weight, fruit length, fruit diameter, fruit volume and fruit length by width ratio. Also, fresh weight of 100 arils, dry weight of 100 arils, peel weight, aril weight, total no. of arils per fruit, aril length, aril width, rind thickness and fruit weight were also highest in cluster III which was a solitary cluster comprising only the cultivar Ganesh. This indicates that for the improvement in fruit morphological traits especially the most important one with bearing on total yeild like fruit weight, aril weight, peel weight, total no. of arils *etc.* Ganesh would be the most suitable parent.

For biochemical parameters, cluster IV comprising single cultivar Amlidana, showed the highest titratable acidity and ascorbic acid values while the lowest pH. The highest mean TSS was observed for cluster IV however, it might be due to presence of only single cultivar in this cluster. Mean values for reducing sugars were highest for cluster I comprising most of the popularly grown cultivars of India like Bhagwa, Super Bhagwa, Early Bhagwa, Phule Arakta and Mridula. The wide variations for different traits among the clusters suggest that, depending upon the breeding objectives, diverse parents can be selected from these cluster. For example, Amlidana being acidic can be used for developing the cultivars suitable for preparation of the condiment, Anardana.

The present study is anticipated to provide comprehensive information about the variability present among the popular Indian genotypes of pomegranate with regard to morphological traits including some very important economic traits like fruit weight, volume, length, width as well as aril weight, aril number per fruit *etc.* that have a direct bearing on total fruit yield. Also, regarding biochemical traits like TSS, titratable acidity, ascorbic acid, anthocyanin, pH, reducing sugars *etc.* which play an important role in determining the flavour of pomegranate and its suitability for use in fresh market trade, processing or pharmaceutics.

The data obtained in the study were extensively analysed using various statistical analyses such genetic variability estimates, principal component analysis, frequency distribution, correlation and path coefficient analysis and diversity analysis to draw the conclusions of the study in the light of best of our understanding. Although, the genotypes selected for phenotypic characterization were only 23, they included diverse cultivars which are commercially grown in different pomegranate growing parts of India.

Hence, the present investigation on the phenotypic characterization of pomegranate for fruit parameters, would positively be useful for the people (students and scientists alike), interested in understanding the pomegranate diversity in India and/or undertaking a pomegranate research.

5.2 Molecular characterization

5.2.1 Molecular Marker Diversity

The 25 pomegranate genotypes used in the study, formed three main clusters, cluster I, II and III, where the first cluster comprised of a 4 genotypes *viz.* Yercaud, Tobesto, Phule Arakta,and Wonderful. Furthermore, Dholka, KRS, Kabul Yellow, Kaladagi Local, Nana, Bhagwa, CO-1, Ruby and Super Bhagwa clustered together in cluster II, with Kabul yellow forming a solitary sub-cluster, Bhagwa being in a separate sub-sub cluster with Amlidana and Kaladagi Local with Nana. All the four mutants, UHSP 23, UHSP 57, UHSP 81 and UHSP 125 clustered together in cluster II, revealing their genetic relationship. Daru formed a separate sub cluster whereas Nana and a local cultivar of Karnataka, Kaladagi local clustered together indicating that it might be possessing the traits similar to Nana and may have some resistance to bacterial blight as that of Nana.

5.2.2 Microsatellite based allelic diversity

From the 24 polymorphic makers screened across 25 genotypes of pomegranate in the present study a total of 215 alleles were obtained. The no of alleles per marker ranged from 2 (PGKVR031) to 16 (PGKVR139 and PGKVR152). However, the total number of effective alleles obtained ranged from 1.09 for PGKVR101 having only 3 alleles to 9.98 for PGKVR119 having 14 alleles. High number of alleles obtained in markers like PGKVR139, PGKVR 152 and PGKVR119, suggests their suitability for screening large number of genotypes.

Furthermore, observed heterozygosity (Ho) was found to be highest for marker PGKVR100 (0.96) with expected heterozygosity of 0.54, showing high heterozygosity of this locus. High heterozygosity in pomegranate is expected due to its clonally propagated as well as cross pollinated nature.

5.3 Marker- Trait association

Marker-trait association through step-wise linear regression analysis, revealed some associations of significant magnitude. The association of marker PGKVR112 with all the aril parameters supports the finding of their high correlation presented in this study. Furthermore, primer PGKVR 122 was found to be associated with bacterial blight and interestingly was also found to be associated with fruit weight. This result, to some extent support the findings of Singh *et al.* (2015) where they reported same marker PGCT001 associated with both fruit weight and bacterial blight, suggesting that these two traits may be strongly associated.

However, since the number of genotypes used in this study as well as the number of polymorphic markers used for screening was less, further validation of these markers and their association with traits mentioned herein is needed in larger germplasm or a mapping population. Nevertheless, this information could be used as basic information about the association of these markers and trait for further research by students or scientists who are interested in carrying out research in this aspect.

6. SUMMARY AND CONCLUSION

Being a perennial and vegetatively propagated crop, clonal selection is the preferred breeding method of crop improvement in pomegranate although few hybrids have been developed. Due to its highly heterozygous nature, easy fruit set, high percentage of seed germination and relatively short juvenile phase, creation of variation for economic traits through hybridization would be a meaningful approach for its improvement. In order to select superior and diverse parents for hybridization, complete characterization of existing cultivars adapted to Indian conditions would be a paramount. Since most of the fruit and nutritional quality parameters are polygenic and highly influenced by environment, there is a need for evaluation of these cultivars for molecular diversity for effective selection of parents in breeding program. A detail study of the genotypes from a morphological, biochemical and molecular point of view will help in identifying the best variety among the popular varieties for different characters. Identification of genetic diversity with respect to various desirable traits will provide an opportunity to select best genotype(s) for cultivation and will help breeders to select and breed genotypes with higher levels of desired properties.

Analysis of variance for morphological characters revealed significant variation for all the selected traits in the present study across the genotypes indicating sufficient amount of variation and hence, an abundant scope for further improvement by breeding using suitable parent superior for various traits.

Variation in fruit weight, a major yield attributing trait was huge ranging from 56.01-505.00 g with a very high h^2 value of 97.23% suggesting the amenability of this trait for improvement. Fruit size, fruit volume, total number of arils showed very wide range of variation among the genotypes indicating the scope for improvement with these genotypes in breeding program for the economic traits.

Pomegranate arils contain juice, pulp, and the woody part rich in raw fibres and different other compounds, but from taste point of view, one of the most important traits is TSS of the arils. In this study, the highest TSS value was found to be 17.95 $^{\circ}$Brix and lowest was 9.62 $^{\circ}$Brix with an average of 13.39 °Brix which is also supported by previous evidences. Ascorbic acid, an important compound in pomegranate fruit juice contributing to its health benefits, showed a wider range of variation as well.

With respect to genetic variability component of the quantitative phenotypic data all the 35 characters revealed moderate to high Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) along with heritability components such as heritability (h^2 in broad sense) and Genetic advance (GA).

Most of the traits observed, exhibited a heritability of more than 60% depicting higher heritability of these traits. Among the traits analysed highest heritability 99.13% was recorded in dry wt. of 100 arils followed by titratable acidity with 98.28% while lowest was observed for seed width (32.28%). Also a high magnitude of coefficient of variability (phenotypic and genotypic) was observed for fruit and aril traits like fruit weight, fruit volume, fresh weight of 100 arils, dry weight of 100 arils, total aril weight and biochemical traits like titratable acidity (%), anthocyanin content and reducing and non-reducing sugars while low to moderate level of variation was observed in TSS, pH and seed parameters like seed length and width. These results indicate that higher magnitude of genotypic coefficient of variations for the aforementioned traits offer a better opportunity for improvement through selection.

Among the qualitative traits, soft seediness, red or dark red juice colour and shape of the fruit are important parameters for consumer acceptance, suitability for processing packaging *etc.* In the present investigation, many of the cultivars exhibited desirable phenotypes like soft seeded, red juice and round shape.

Principal Components Analysis (PCA) is one of the most efficient approaches to find out the variations being explained by different traits thus reducing the variables under study. 35 characters were partitioned in to three principle components with the cumulative variance of 55. 37 %. Variations in most of the fruit and aril characters like fruit weight, fruit volume, fruit length, fruit diameter, peel weight, total number of arils, aril weight, fresh weight of 100 arils, dry weight of 100 arils, aril length and aril width was explained by principal component 1 (PC1) which accounted for 27.77% of the total variation indicating their suitability for recording observation in the breeding program with large number of germplasm or large segregating loci rather than concentrating on any other traits with less variance.

Correlation and path coefficient analysis are the two important statistical analyses which provide the insight into character association and their magnitude of association respectively. In the present study, among the morphological characters fruit weight was considered to be economic trait and among the biochemical traits, TSS was considered as an economic trait and hence they were selected as dependent variables to understand the characters association as well as the direct and indirect effects of other variables on these dependent variables in path analysis. Fruit weight was found to have significant positive correlation with most of the traits studied *viz.* fruit length, fruit diameter, fruit volume, fresh and dry weight of 100 arils, peel weight, aril weight, total no. of arils per fruit, aril length and aril width. Titratable acidity (TA) was found to be significantly negatively correlated with pH, which in turn was showing negative correlation with TSS.

The results of path coefficient analyses for morphological traits, showed that direct effects (positive or negative) on fruit weight was being exerted only by peel weight, aril weight, seed and skin percentage, with aril weight having highest direct effect, indicating that aril weight is an important yield attributing component in pomegranate. Based on the results of path coefficient analysis for biochemical traits, it could be deduced that TSS which is one of the most important traits determining the flavour of pomegranate is influenced positively by all the independent traits under study except anthocyanin and that reducing sugars exert a high positive direct influence, emphasizing the importance of reducing sugars for development of quality attributes of pomegranate. Since pomegranate is used both in its fresh and processed forms, the understanding of interdependence of biochemical traits would be highly desirable to develop cultivars pertaining to the destined markets.

In the present study 23 pomegranate cultivars were analysed for different phenotypic traits including morphological and biochemical parameters and all these traits were subjected to diversity analysis. The 23 genotypes studied clustered in 4 different clusters for all the phenotypic traits. With regard to morphological traits, cluster III performed better than other clusters in context of fruit weight, fruit length, fruit diameter, fruit volume and fruit length by width ratio. Also, fresh weight of 100 arils, dry weight of 100 arils, peel weight, aril weight, total no. of arils per fruit, aril length, aril width, rind thickness and fruit weight were also highest in cluster III which was a solitary cluster comprising only the cultivar Ganesh. This indicates that for the improvement in fruit morphological traits especially the most important one with bearing on total yeild like fruit weight, aril weight, peel weight, total no. of arils *etc.* Ganesh would be the most suitable parent.

For biochemical parameters, cluster IV comprising single cultivar Amlidana, showed highest titratable acidity and ascorbic acid values while lowest pH. Mean values for reducing sugars were highest for cluster I comprising most of the popularly grown cultivars of India like Bhagwa, Super Bhagwa, Early Bhagwa, Phule Arakta and Mridula. The wide variations for different traits among the clusters suggest that depending upon the breeding objectives; diverse parents can be selected from these clusters. For example, Amlidana being acidic can be used for developing the cultivars suitable for preparation of the condiment, Anardana.

Daru and Nana was found to have very less bacterial blight severity as compared to the popular cultivars of India like Bhagwa, Early Bhagwa and Ganesh. A more comprehensive research for bacterial blight resistance in the studied genotypes is anticipated to give more discreet results regarding the resistance or susceptibility levels of these genotypes under Indian conditions.

Marker PGKVR 122 was found to be associated with bacterial blight and also with fruit weight, Marker PGKVR 112 was found to be associated with all the aril parameters like fresh weight of 100 arils, total aril weight and total number of arils per fruit, primer PGKVR 132 and 149 showed a significant association with fruit weight and fruit volume, suggesting that these markers could be used for selection of genotypes superior for these traits. However, further validation is recommended before these are used in Marker Assisted Selection (MAS).

From the present investigation, elaborative information has been generated with respect to detailed phenotypic characterization. The data generated, along with the detailed statistical analysis like variability and heritability components, PCA, morphological and molecular diversity, provided comprehensive information about the existence of variance among these popular cultivars. Correlation and path coefficient analysis and PCA provided the information about the importance of characters to be considered for improvement in the economic traits of pomegranate. The marker-trait association identified for the economic traits in the present study is anticipated to be useful for MAS but, only after validation and confirmation of these markers in the larger genetic background. Thereupon, the present investigation would pave the way for following future research areas of pomegranate.

1. Selection of superior cultivars as parents in hybridization program based on diversity and *per se* performance of each genotypes

2. The molecular markers identified during the fingerprinting of these important Indian cultivars of pomegranate could be further used for identification of parents in the breeding program as well as clonal fidelity testing.

3. Preliminary information on the marker-trait association obtained in the present investigation could be a hope for MAS, once their association is confirmed and validated in mapping populations and diverse genetic background either by bi-parental mapping or by association mapping.

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MOLECULAR MARKER PROFILING AND ITS ASSOCIATION WITH ECONOMIC TRAITS OF POPULAR INDIAN CULTIVARS OF POMEGRANATE (*Punica granatum* L.)

NUSRAT PERVEEN **2017** **SARVAMANGLA CHOLIN**
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ABSTRACT

The present investigation was carried out with 25 pomegranate genotypes at University of Horticultural Sciences, Bagalkot, Karnataka during 2016-17 using randomized complete block design with three replications. These genotypes included popular cultivars, four mutant lines and two wild forms, which were subjected to extensive phenotypic characterization using morphological and biochemical parameters together with molecular marker profiling using 24 microsatellite markers as well as field screening for resistance to bacterial blight and foliar and fruit spots.

Variability studies demonstrated higher PCV, GCV, heritability and GAM implying the existence of sufficient variation among the studied genotypes. High heritability with high genetic advance was recorded for most of the characters *viz.* fruit weight, fruit volume, peel weight, aril weight, total number of arils and titratable acidity indicating effective selection for these traits

Many of the cultivars studied, exhibited desirable phenotypes like soft seediness, red juice and round shape. Daru and Nana were found to have very less bacterial blight severity as compared to the popular cultivars like Bhagwa, Early Bhagwa and Ganesh. In the principle component analysis, 35 characters analysed were partitioned in to three PCs with the cumulative variance of 55.37%. Correlation studies revealed significant positive correlation between fruit weight and fruit volume, fresh and dry weight of 100 arils, peel weight, aril weight and total number of arils per fruit while path coefficient analyses affirmed a significant contribution of peel and aril weight on fruit weight.

D^2analysis grouped 23 genotypes into four distinct clusters and high genetic divergence was found for genotypes falling in clusters II and III. Based on marker trait association, markers like PGKVR112, PGKVR 132 and PGKVR 149 exhibited significant R^2 value for aril parameters, fruit weight and fruit volume while, marker PGKVR 122 was found to be associated with bacterial blight and also with fruit weight.

Consequently, the present investigation illustrated existence of wide range of variations for most of the characters among the pomegranate genotypes, providing opportunities for genetic gain through selection or hybridization in future breeding programme.

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