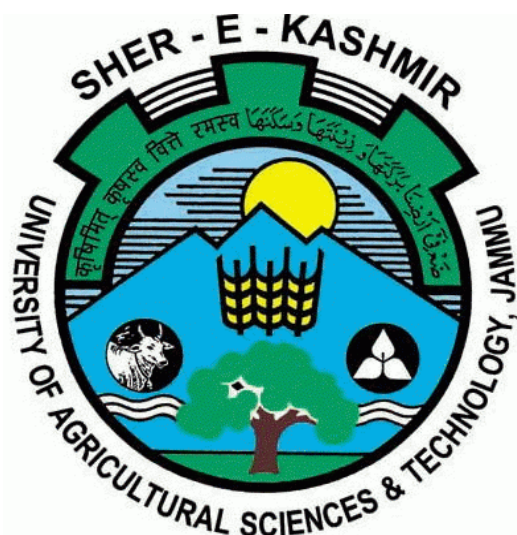


**MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR
CHARACTERIZATION OF WILD POMEGRANATE
(*Punica granatum* L.) GENOTYPES OF JAMMU REGION**

**by
Safina Kosser
(J-19-D-370-A)**

Thesis Submitted to
Faculty of Agriculture
in partial fulfillment of the requirements
for the degree of

**DOCTOR OF PHILOSOPHY
IN
HORTICULTURE (FRUIT SCIENCE)**

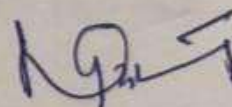


Division of Fruit Science
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
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2024

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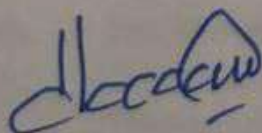
The work has been carried out by **Ms. Safina Kosser** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.



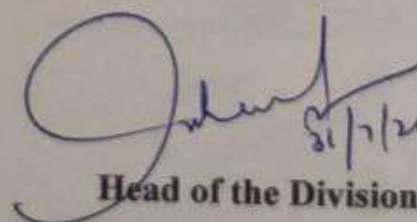
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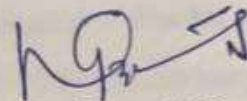
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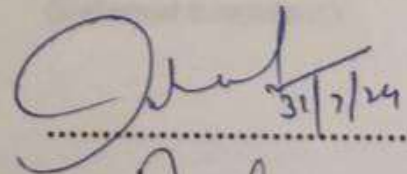
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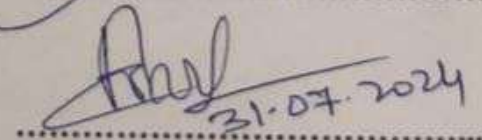
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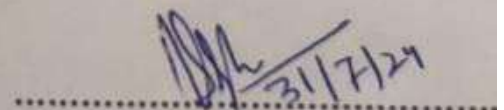
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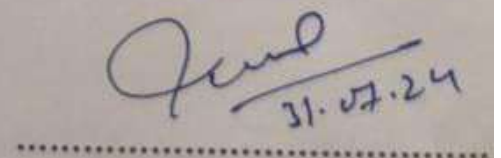
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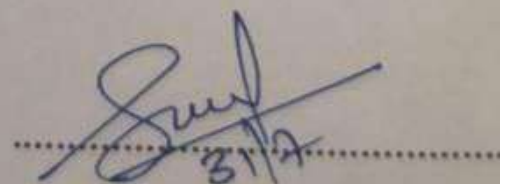
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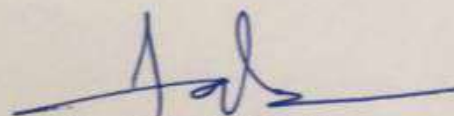
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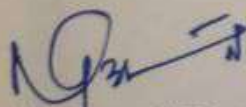


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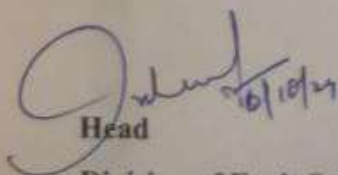
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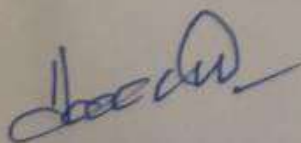
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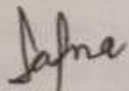
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Date: 06/12/24

Place: Chatha, Jammu


Safina Kosser

ABSTRACT

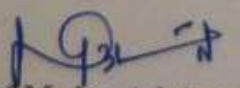
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The present investigation entitled "Morphological, Biochemical and Molecular characterization of wild pomegranate (*Punica granatum* L.) genotypes of Jammu region" was undertaken during 2020-21 and 2021-22 in different districts of Jammu region (Rajouri, Poonch and Udhampur) to assess the morphological, biochemical and variability of wild pomegranate genotypes. On the basis of study conducted, the morphological and biochemical traits were recorded as per UPOV descriptor 2011. Results of the study showed that there was a marked variation in seventy wild pomegranate genotypes where JWPR (Sel-5 and Sel-36) showed the best result among all the collected genotypes. Maximum fruit weight (110.01 and 111.51g), fruit length (56.53 and 57.54mm), fruit width (55.30 and 55.91mm), no of arils (394 and 452), 100 aril fresh weight (15.13 and 16.18g), aril width (6.44 and 7.27mm), aril length (8.75 and 9.14mm) and highest fruit yield (7.02 and 6.27 kg/bush) were recorded in wild pomegranate genotypes JWPR (Sel-5) and JWPP (Sel-36) respectively. Similarly biochemical characteristics were also recorded maximum in JWPR (Sel-5 and Sel-36). Maximum TSS (17.90 and 17.80°Brix, titratable acidity (6.84 and 6.91%) total sugars (14.27 and 14.16%), reducing sugar (9.66 and 9.72%), non reducing sugar (4.50 and 4.55%) and ascorbic acid (23.19 and 23.44mg/100g) respectively.

The molecular characterization of seventy wild pomegranate genotypes was carried out with 25 microsatellite markers. Major allele frequency ranged from HvSSRT_823 (0.606) and HvSSRT_45 (0.393) with an average of 0.479, polymorphic information content ranged from PGCT093A (0.595) and HvSSRT_104 (0.14) with an average of 0.482. Dendrogram was generated using SSR data to determine pairwise distance between all possible pairs of wild genotypes, two main clusters were obtained with sub-clusters. However, morphological and biochemical traits alone cannot be regarded as critical indicators to know genetic diversity and need to be backed with DNA markers for efficient and reliable genetic diversity studies. In this study JWPR (Sel 5 and 36) that showed the excellent traits of interest and high genetic dissimilarity can be used for variety release or can be further used in breeding programmes to get segregates.

Keywords: Wild pomegranate, variability, morphological, biochemical, SSR markers and cluster analysis


Signature of Major Advisor

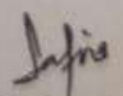

Signature of the Student

TABLE OF CONTENTS

Chapter No.	Particular	Page No.
1	INTRODUCTION	1-5
2	REVIEW OF LITERATURE	6-21
3	MATERIALS AND METHODS	22-43
4	RESULTS	44-87
5	DISCUSSION	88-98
6	SUMMARY AND CONCLUSIONS	99-106
	REFERENCES	107-118

LIST OF TABLES

Table No.	Particular	Page No.
3.1	Name of the farmer and geographical locations of wild Pomegranate accessions taken into study	23
3.2	List of selected SSR primers along with their primer sequence	40
3.3	Thermal profiles used for DNA amplification	41
		After Page No.
4.1	Tree characteristics/Variation in Tree height, tree growth habit and trunk type of wild pomegranate genotypes of Jammu region	45
4.2	Variation in leaf blade shape, leaf blade length, leaf blade width and petiole length of wild pomegranate genotypes of Jammu region	48
4.3	Frequency percentages of tree growth habit, trunk type and leaf shape of wild pomegranate genotypes of Jammu region	51
4.4	Variation in start of flowering, end of flowering, duration of flowering and calyx color of wild pomegranate genotypes of Jammu region	53
4.5	Variation in petal length, petal width, calyx length and calyx width of wild pomegranate genotypes of Jammu region	55
4.6	Variation in fruit shape, fruit color, crown length and rind thickness of wild pomegranate genotypes of Jammu region	59
4.7	Variation in fruit weight, fruit length, fruit width and juice percent of wild pomegranate genotypes of Jammu region	61
4.8	Variations in aril color, seed texture, number of arils, weight of 100 arils, aril length and aril width of wild pomegranate genotypes of Jammu region	65
4.9	Frequency distribution of fruit shape, fruit color, aril color and seed texture of wild pomegranate genotypes of Jammu region	67
4.10	Variation in number of fruits and yield (kg/tree) of wild pomegranate genotypes of Jammu region	69
4.11	Variation in TSS, total sugars, reducing sugar and non reducing sugar of wild pomegranate genotypes of Jammu region	72
4.12	Variation in titratable acidity and ascorbic acid of wild pomegranate genotypes of Jammu region	74

4.13	Descriptive statistical analysis of different characters of wild pomegranate genotypes of Jammu region	77
4.14	Estimates of variability parameters for various characters in wild pomegranate genotypes of Jammu region	79
4.15	Phenotypic (P) and genotypic (G) correlation coefficients among ten characters of 70 wild pomegranate genotypes of Jammu region	81
4.16	Principal component analysis for different character of wild pomegranate genotypes of Jammu region	83
4.17	Principle component analysis of various biochemical components of wild pomegranate genotypes of Jammu region	84
4.18	List of amplified SSR primers along with major allele frequency, no of alleles scored and polymorphic information content	86

LIST OF FIGURES

Figure No.	Particular	Page No.
3.1	Growth habit in pomegranate Tree	26
3.2	Leaf shape in pomegranate	26
3.3	Calyx width in pomegranate	28
3.4	Petal length and petal width in pomegranate	28
3.5	Fruit shape in pomegranate	29
3.6	Crown length in pomegranate	29
3.7	Fruit length and fruit width in Pomegranate	30
3.8	Aril length and aril width in pomegranate	31
		After Page No.
4.1	Principal component analysis of different characters of wild pomegranate genotypes of Jammu region	83
4.2	Principal component of wild pomegranate genotypes based on biochemical characters	84
4.3	Cluster dendrogram of seventy wild pomegranate genotypes on the basis of morphological and biochemical characters	85
4.4	Cluster analysis pattern of seventy wild pomegranate genotypes based on SSR data	87

LIST OF PLATES

Plate No.	Particulars	After Page No.
1	Variations in tree characters of wild pomegranate genotypes collected during the study	47
2	Variations in leaf characters of wild pomegranate genotypes	51
3	Variations in fruit characters of wild pomegranate genotypes	63
4	Quality check of DNA isolates from wild pomegranate genotypes	67
5	SSR based DNA based amplification pattern of wild pomegranate genotypes with Primer -HvSSRT_45, M depicts 100bpladder	87
6	SSR based DNA amplification pattern of wild pomegranate genotypes with primer HvSSRT_104, M depicts 100bp Ladder	87
7	SSR based DNA amplification pattern of wild pomegranate genotypes with primer HvSSRT_639, M depicts 100bp Ladder	87
8	SSR based DNA amplification pattern of wild pomegranate genotypes with primerPom006, M depicts 100bp ladder	87
9	SSR based DNA amplification pattern of wild pomegranate genotypes with primer HvSSRT_823, M depicts 100bp Ladder	87
10	SSR based DNA amplification pattern of wild pomegranate genotypes with primer PGCT093A,M depicts 100bp Ladder	87
11	SSR based DNA amplification pattern of wild pomegranate genotypes with primer PGCT088, Mdepicts 100bp ladder	87
12	Best wild Pomegranate genotypes JWPR (Sel-5) and JWPP (Sel-36)	87
13	Best wild pomegranate accessssions planted at KVK Rajouri	87

LIST OF ABBREVIATIONS

cd	critical difference
µg	micrograms
bp	base pair
cm	centimeter
CTAB	cetyl trimethyl ammonium bromide
dNTP	deoxy ribo nucleotide triphosphate
ddH₂O	double distilled water
EDTA	ethylene diamine tetracetic acid
g	gram
HCl	hydrochloric acid
h	hour
kg	kilograms
l	litres
m	meter
M	molar
mg	milligrams
MgCl₂	magnesium chloride
min	minutes
ml	milliliters
mm	millimeter
N	normal
NaCl	sodium chloride
nm	nanometer
pH	potential of hydrogen ion
ppm	parts per million
r.p.m	revolutions per minute
TBE Buffer	tris borate EDTA buffer

TE Buffer	tris EDTA buffer
Tris	tris amino methane
Tris-Cl	tris hydrochloride
v	volts
v/v	volume/volume
°C	degree celsius
SSR	simple sequence repeats
DNA	deoxyribonucleic acid
%	percent

INTRODUCTION

The pomegranate is a large thorny shrub member of the Punicaceae family (Mercure, 2007). Its chromosome number is $2n=18$, and it is classified into a single genus called "*Punica*" which has three species: *Punica granatum* is a commonly cultivated and domesticated plant; *Punica protopunica* is an ancestral species indigenous to the Socotra islands of the Arabian Peninsula, also known as "Yamen" (Shilikina, 1973); and *Punica nana* is an ornamental species that is prized for its diverse range of shapes and colors in its single and double flowers (Melgarejo *et al.*, 2009). Based on recent molecular studies for taxonomic classification, the family "Lythraceae" includes the genus "*Punica*" (Graham *et al.*, 2005). The name "*Punica*" is derived from Phoenicia (Carthage) due to the mistaken belief about its origin, and "pomegranate" translates to grainy apple. The primary centre of origin of pomegranate is Iran Transcaucasia-Caspian region. The region from Iran through Turkmenistan to Western Himalayas in northern India is considered as a secondary origin for pomegranate. There is significant diversity in pomegranates, with approximately 500 known varieties, around 50 of which are globally marketable (IPGRI, 2001). The ability of pomegranate trees to adapt to various agro-climatic conditions has enabled their distribution from Eurasia to the Himalayas (Levin, 2006; Holland *et al.*, 2009; Still, 2006). The semi-tropical regions of Persia are considered as the primary origin of the pomegranate (Levin, 1994). From there, it spread to India, China, Afghanistan, and Pakistan, with the Himalayan range being considered a secondary origin (Diganta *et al.*, 2009). There is a strong cultural connection between the Mediterranean region and the domestication of pomegranates, where it was an important dietary component and valued for its medicinal properties. Pomegranate is considered one of the first five domesticated edible fruits, along with fig, date palm, grape, and olive. The domestication of pomegranates likely began in the Neolithic age (Still, 2006; Levin, 2006), with early cultivation in the Transcaucasia-Caspian region and northern Turkey. By the 1st century CE, pomegranate cultivation had spread from Iran to the Indian peninsula. It reached Indonesia by 1416, was distributed throughout Europe by the Greeks, and was introduced to the New World by Spanish sailors (Holland *et al.*, 2009). Pomegranate is extensively cultivated in various regions including India, Iran,

China, Turkey, USA, Spain, Azerbaijan, Armenia, Afghanistan, Uzbekistan, the Middle East, Pakistan, Tunisia, Israel, dry regions of Southeast Asia, Peninsular Malaysia, the East Indies and tropical Africa. India is the world leader in pomegranate acreage and production with 278 thousand ha area and 3188 thousand metric tones of annual production (Anonymous, 2022). In India, pomegranate is primarily commercially cultivated in Maharashtra, Karnataka and Rajasthan due to its better adaptability to arid climate and to lesser extent grown in Uttar Pradesh, Himachal Pradesh, Punjab, Haryana, Tamil Nadu and Andhra Pradesh.

Wild pomegranate, locally known as "Daru" and referred as 'Dhruni', is a important fruit crop that grown in the hilly terrains and forests of the Jammu and Kashmir union territory. This tree has been an integral part of the traditions of the local inhabitants in the Himalayan region for centuries. The fruits are harvested for their fleshy seeds, which are sundried to produce "anardana," a commercial product used in both medicinal and culinary applications. Notably, wild pomegranate fruits are characterized by much smaller arils, thicker rinds, and higher acidity compared to their cultivated counterparts (Kher, 1999). These wild varieties also exhibit numerous suckers, thorny twigs with irregular patterns, and small leaves (Singh & Gupta, 2018; Thakur *et al.*, 2011). Locally, the fruit from the wild pomegranate is utilized to prepare 'chutneys'-a blend with mint, green chillies, and sugar-known to cool the body during summer and aid in treating stomachaches and constipation in humans. Additionally, it finds its use in cooking, as well as in the preparation of juice blends, smoothies, and alcoholic beverages (Singh and Sethi, 2003)

The fruit of wild pomegranate is recognized as a rich source of natural antioxidants and health-promoting compounds such as anthocyanins, phenolics, and flavonoids, exhibiting potential anti-tumoral effects (Sineh Sepehr *et al.*, 2012). This recognition has led to an increased demand for its value-added products, attributed to its abundance in natural antioxidants and health-enhancing constituents like organic acids, anthocyanins, phenolics, vitamins, and minerals (Thakur *et al.*, 2010). These bioactive components are primarily concentrated in the juicy arils of the fruit, with sufficient amounts of organic acids, vitamins, and minerals, thereby categorizing it as a functional food with nutraceutical properties. Characterization of wild germplasm is essential for efficiently utilization plant genetic resources. Although numerous studies have been conducted on the morphological and physiological descriptors of wild

pomegranate germplasm (Singh *et al.*, 2015; Parashuram *et al.*, 2018; Khadivi-Khub *et al.*, 2020), they are best with several limitations (Patil *et al.*, 2020), mainly being influenced by the environment. Therefore, considering both morphological and molecular characters provide an opportunity to unambiguously characterize genetic variability. The most crucial step in any breeding program is the screening and identification of superior genotypes, a process that is both costly and time-consuming. Genetic diversity is fundamental to all selection and crop improvement efforts. As the genetic diversity increases, the selection scope also broadens. To incorporate the desirable characteristics in a variety, the knowledge about the genetic diversity of crop is paramount. The extensive genetic diversity presents breeders with an opportunity to introduce new cultivars featuring desirable traits (Patil *et al.*, 2013). Therefore, the assessment of genetic diversity serves as the initial step in fruit-breeding programs. Many individuals rely on the high levels of crucial phytochemicals found in fruits for both nutritional and therapeutic purposes. However, human exploitation of natural resources has led to environmental degradation, thereby posing a threat to wild pomegranate populations in their natural habitats. Consequently, studies focusing on wild pomegranate diversity are invaluable for identifying superior genotypes suitable for breeding programs and establishing core collections for germplasm conservation and further detailed investigations (Arlotta *et al.*, 2022).

The absence of a germplasm conservation program for wild pomegranates has resulted in a lack of cultivar improvement initiatives. Despite the extensive morphological and biochemical diversity found within wild pomegranate accessions, which could be utilized to develop improved cultivars in the region. Germplasm improvement programs in pomegranates have traditionally relied on morpho-chemical and molecular characterization. Morphological and biochemical analysis offer basic information for genotype comparison in breeding programs, aiding in the evaluation of growth performance under varying climatic conditions.

Numerous studies have explored the morphological diversity of fruits and the biochemical composition of both cultivated and wild pomegranates across various regions around the world. A significant level of variation in fruit weight, length, seed weight, rind weight, acidity, and tss has been documented among wild pomegranate genotypes (Ercisli *et al.*, 2007; Bakshi *et al.*, 2014; Singh & Gupta, 2018). Similar variations were observed by Khadivi *et al.* (2020) regarding fruit weight, fruit peel

color, aril color, fresh arils weight, and total soluble solids in Iran, and by Aziz *et al.* (2020) and Nafees *et al.* (2015) in Pakistan. The discovery and utilization of diverse pomegranate genotypes are prime concerns in pomegranate breeding, aimed at identifying their favorable fruit and yield characteristics. These morphological traits serve as primary markers utilized in germplasm management (Karimi *et al.*, 2008). In addition to morphological and biochemical studies, molecular analysis is used for characterization and cultivar improvement by examining qualitative trait-linked traits. This approach is necessitated by the inconsistency in the expression of these characteristics, rendering morphological information alone insufficient for evaluating genetic diversity. DNA markers, being independent of environmental conditions and limitless in number, exhibit a high level of polymorphism and offer promising avenues for comprehensive genetic analysis. The development of SSRs (Simple Sequence Repeats) has been introduced as a rapid tool for identifying pomegranate cultivars, particularly in the study of nutraceutical and genetic diversity among new pomegranate genotypes compared to leading commercial varieties (Arlotta *et al.*, 2022). Recognizing and preserving genetic diversity is crucial for any crop improvement program. Classifying or grouping genotypes using an appropriate scale is essential for understanding the available variability. Morphological characterization of native germplasm is the first step for their rational introduction in modern and efficient production chains and a standardized description of phenotypic traits based on the adoption of specific descriptors is a prerequisite for this purpose (Patil *et al.*, 2013). Given the nutritional and nutraceutical properties of wild pomegranates, industrializing various commercially processed plant products becomes crucial in addressing nutrient deficiencies, particularly in remote regions of developing countries like the Himalayas, where cultivated bioactive components can serve as a cost-effective source. Despite the wide morphological and biochemical diversity present in wild pomegranate germplasm, which could be harnessed to develop genetically superior cultivars, there has been limited research focusing on characterizing this neglected and underutilized crop in the Himalayas. While some studies have explored wild pomegranate in the region, its potential in plant breeding remains largely untapped. Therefore, there is an need to identify elite genotypes having desirable traits within the extensive germplasm of wild pomegranate in this

area. Keeping in view of the above facts the present study was planned with the following objectives:

1. To characterize the wild pomegranate genotypes using morphological and biochemical traits
2. To study the genetic diversity among wild pomegranate genotypes using molecular markers

REVIEW OF LITERATURE

The global trade of pomegranate fruit and its by products has witnessed a significant surge due to their recognized nutritional, medicinal, and economic value (Sturgeon and Ronnenberg, 2010). Pomegranate, deeply ingrained in human culture since ancient times, has been utilized for both culinary and medicinal purposes, a fact supported by scientific research. Phytochemicals present in different parts of the pomegranate plant—such as leaves, bark, flowers, and fruit provide various health benefits, including blood pressure regulation, antimicrobial effects, and anti-aging properties. Moreover, these phytochemicals have shown promise in combating chronic ailments such as diabetes, male infertility, and cancer (Holland *et al.*, 2009). Understanding the genetic diversity of a crop is essential for its improvement, as it provides a basis for choosing optimal parental combinations. Evaluating genetic diversity helps in the effective conservation, management, and utilization of germplasm resources. Consequently, DNA-based polymorphisms have emerged as the preferred method for estimating genetic diversity. Molecular techniques offer the most accurate estimate of genetic diversity, overcoming limitations posed by environmental influences. Some research work done in India and abroad for characterization of pomegranate with respect to morphological, biochemical and molecular aspects has been reviewed under following heads;

2.1 Morphological characterization**2.2 Bio-chemical characterization****2.3 Correlation****2.4 Molecular characterization****2.1 Morphological Characterization**

Morphological changes primarily involve variations in observable plant characteristics, such as plant height, leaf shape, fruit size, fruit color, seed size, and other visual traits. Both qualitative and quantitative features are provided by morphological descriptors (Mars and Marrakchi, 1999) and biochemical analyses (Hasnaoui *et al.*, 2011) to compare genotypes for breeding and selection programmes or to assess growth under various climatic circumstances (Martinez *et al.*, 2006,

Drogoudi *et al.*, 2005 and Dafny-Yalin *et al.*, 2010). Pomegranate accessions from both domesticated and wild sources exhibit high levels of variation in a number of fruit characteristics, including fruit size, skin and aril colour, seed hardness, juice content, acidity, sweetness, and fruit maturity (Ercisli *et al.*, 2007; Ozguven *et al.*, 1997). Based on the hardness of the seeds, pomegranate fruits are typically divided into four categories: hard, semi-hard, soft and semi-soft (Zamani, 2007). Fruit size did not significantly correlate with an individual's typical aril weight (Holland *et al.*, 2009). Wetzstein *et al.* (2011) found that fruit volume, fruit weight, and total aril weight of pomegranate were found to be closely correlated, and any of these parameters might be used to determine fruit size. Pollination causes the ovary's ovules to grow into varying numbers of arils. This is a 50% of the fruit of the pomegranate that may be eaten, and it is made up of 22% seeds and 78% juicy portion (El-Nemr *et al.*, 1990). Depending on the cultivar and its agro-climatic conditions, arils vary in size, shape, and juiciness. The fruit colour varies from yellow (Ganesh) to red (Bhagwa) and average fruit weight ranged from 250-300g (Patil *et al.*, 2013). Huge diversity has been observed with related to aril and shape in pomegranate. Aril taste varies from sweet (Bhagwa) to sour insipid (Daru, Nana) (Saxena *et al.*, 1987). Patil *et al.*, 2005) studied different of pomegranate and found that TSS in Ganesh, Arakta and kesar was 14.5⁰B, 13⁰B, 14⁰B with 0.57, 0.59 and 0.59% acidity; 10.4, 10.2 and 9.65 % total sugars; 8.40, 8.00 and 7.3 % reducing sugars; 2.00, 2.20 and 2.35 % non-reducing sugars respectively.

Thakur *et al.* (2010) observed the significant difference in biochemical traits and antioxidant activity in cultivars imported from Iran. Consequently, many researchers have concentrated on local cultivars, examining morphological fruit characteristics such as fresh weight, skin thickness, and physicochemical properties.

Singh *et al.* (2004) assessed the performance of nine pomegranate cultivars (Ganesh, P-23, P-26, G-137, Dholka, Jodhpur Red, Basein Seedless, Jalore Seedless, GKVK-1) in Haryana, India. They observed that the plant height was highest in Jodhpur Red (2.38 m) and lowest in cultivar Bassein Seedless (1.84 m).

Sharma and Bist in 2005 evaluated eight cultivars of pomegranate in Himachal Pradesh. "Chawla" had the tallest and widest plants while "PS-75-K-5" had the smallest. "Chawla" was the most vigorous while "PS-75-K-5" was the least.

"Ganesh", "Mridula", and "G-137" had spreading growth habits, while the others were erect. In a study conducted by Varasteh and colleagues in 2006, an assessment was carried out on significant fruit traits across five commercial pomegranate varieties in Iran. The findings revealed that the 'Malas-e-Yazdi' variety exhibited the largest fruit size, with dimensions reaching 103.55 mm in length and 109.50 mm in diameter. Conversely, the 'Malas-e-Torsh-e-Saveh' variety was identified as having the smallest fruit, with measurements of 83.60 mm in length and 91.76 mm in diameter.

Singh *et al.* (2011) collected 17 germplasm accessions of pomegranate from the foot hills of Himachal Pradesh and evaluated them at CIAH, Bikaner for growth, flowering, fruiting, yield, and fruit quality. Among these accessions, 14 of them were found to be well-suited to hot arid conditions. The maximum plant height of 2.70 m was recorded in Collection No.-17, while Amlidana had the minimum height of 1.45 m. Collection No.-17 had the widest plant spread in both N-S and E-W directions, measuring 2.50 m and 2.60 m, respectively. On the other hand, Amlidana had the smallest N-S and E-W plant spread, measuring 1.20 m and 1.30 m, respectively.

Samadi (2011) studied pomegranate cultivation in Afghanistan and found that the country is home to forty-eight known cultivars. These include 20 "sweet," 17 "medium sweet" (sub-acid), and 11 "sour" varieties. Additionally, these cultivars can be classified into three groups based on seed quality: hard-seeded, intermediate-seeded, and soft-seeded.

Wetzsteim *et al.* (2011) found that the fruit parameters such as fruit volume and weight, aril weight and number, pericarp weight, seed weight, juice/pulp content and fruit volume were correlated with each other. Any one of these characteristics can be considered as an indicator of fruit size. Among them, number of arils per fruit was highly correlated with fruit size. Orhan *et al.* (2013) used ten thousand seedlings for morphological characterization and found enormous morphological variation between all genotypes, in terms of individual fruit weight, fruit length and breadth, fruit colour, aril colour and fruit firmness. The genotypes differed from 147-769 g for fruit weight, 65.19-107.08 mm for fruit width, 56.84-96.93 mm for fruit length, 2.69-6.05 mm peel thickness and 14.8-49.8 kg/cm² fruit firmness.

Patil *et al.* (2013) conducted a study to analyze the physico-chemical characteristics of two pomegranate varieties, Ganesh and Arakta. The study found that

the morphological characteristics differed between the two: the Ganesh variety had a yellowish color with pink patches, while the Arakta variety was dark red. The arils of the Ganesh cultivar were pinkish, whereas those of the Arakta cultivar were blood red. The weight of the arils was 167.50 g in Arakta and 140.35 g in Ganesh, while the peel weight was 70.55 g in Ganesh and 86.70 g in Arakta. The average fruit weight was 210.90 g for Ganesh and 252.40 g for Arakta.

Nafees *et al.* (2015) studied 42 pomegranate accessions using thirteen morphological fruit traits and discovered that principal component analysis of fruit length (mm), fruit diameter (mm), crown length (mm), aril, and seed dimensions (mm) accounted for 93.9% of the total morphological diversity. They also found that fruit weight and its dimensions exhibited high diversity and strong correlation across all accessions.

Nadia *et al.* (2015) conducted a comprehensive characterization of cultivated and wild pomegranates based on various morphological traits. The parameters studied included fruit weight (36-215 g), fruit length (30-66.9 mm), fruit width (8.45-64.61 mm), peel thickness (0.2-4.2 mm), number of arils (180-490), and leaf area (1.4-9.5 cm²). Through Cluster Analysis (UPGMA) and Principal Component Analysis (PCA), the study identified significant morphological differences between cultivated and wild pomegranates.

Zarei (2017) reported both positive and negative correlations between various morphological traits in pomegranates. Seed hardness showed a positive correlation with the percentage of 100 arils, while fruit color was positively correlated with aril color, anthocyanin content, seed hardness, and vitamin C. However, fruit color was negatively correlated with aril length. In a separate study, Basaki *et al.* (2017) characterized 38 sour pomegranate genotypes based on 15 pomological traits, observing significant variability across all morphological parameters. Genotype "6-3-S" stood out for its large fruit size, high aril yield, red color, juicy arils, and soft seeds, making it suitable for food processing and marketing.

.Dandachi *et al.* (2017) studied the assessment of 78 local pomegranate varieties grown across the country revealed the substantial diversity. Using 28 quantitative and 10 qualitative descriptors, the study found that male and hermaphrodite flowers, petal

width, fruit weight, size, juice pH, and sugar/acid ratio were the most distinguishing traits among these varieties.

Parashuram *et al.* (2018) reported the average performance of 25 genotypes, with measurements ranging from 80.25 to 207.10 g for fruit weight, 4.81 to 6.61 cm for fruit length, and 4.94 to 7.08 cm for fruit diameter. Additionally, 100 arils weight ranged from 18.77 to 45.23 g, total arils weight from 26.65 to 122.05 g, and rind weight from 34.93 to 82.90 g. Rind thickness varied from 1.83 to 3.69 mm, while aril length ranged from 8.58 to 11.4 mm and aril width from 5.4 to 7.76 mm. Tss content ranged from 15.86 to 19.20 °Brix, acidity from 1.51 to 3.42%, and fruit juiciness from 26.01 to 52.02%. Among these morphological traits, total arils weight exhibited the highest coefficient of variation (43.55%), followed by fruit weight (33.75%), rind weight (31.53%), and 100 arils weight (29.77%).

Beghe *et al.* (2019) conducted the first-ever characterization of ancient pomegranate varieties cultivated in Emilia Romagna, Italy. This characterization involved the evaluation of 38 quantitative morphometric characteristics associated with leaves, flowers, fruits, and seeds, alongside the utilization of 42 Random Amplified Polymorphic DNA (RAPD) markers and 12 Simple Sequence Repeat (SSR) markers. The morphological assessments unveiled significant variability among the accessions, with descriptors related to fruits and seeds displaying the highest discriminatory potential.

Khadivi *et al.* (2020) conducted a study to assess the diversity of wild pomegranates in the Northern regions of Iran, focusing on their morphological and pomological characteristics. The study revealed that the fruit weight ranged from 19.20 to 185.00 g, with an average of 59.89 g. Additionally, the color of the fruit peel exhibited considerable variability, ranging from yellow to brown-red. The weight of 100 arils showed wide variation across accessions, ranging from 5.75 to 27.55 g, with an average of 11.77 g. Furthermore, the accessions displayed diverse aril colors, including glassy, pale milk, pink, white-red, red, and dark red. Seed hardness was predominantly categorized as hard, although some accessions exhibited soft and semi-soft seeds (14 and 48 accessions, respectively). The authors suggest that most of the studied accessions could serve as suitable parents in breeding programs aimed at

enhancing juice quality, seed softness, and the visual appearance of both arils and fruit.

Peng *et al.* (2020) assessed thirty-seven pomegranate varieties from seven provinces in China, examining twelve phenotypic traits and twelve biochemical indicators (seeds and juices). They found that fruit and aril fresh weight ranged from 210.5 to 576.5 g and 121.0 to 327.5 g, respectively. The edible rate varied between 42.58% and 64.80%, while seed weight ranged from 1.80 to 3.41 g, seed number from 249.1 to 838.9, fruit height from 10.51 to 15.48 mm, fruit diameter from 11.46 to 17.50 mm, skin thickness from 2.14 to 6.98 mm, and shape index from 0.82 to 0.96 across different genotypes.

Mehta and Gupta (2021) conducted correlation and factor analysis of quantitative traits across four wild pomegranate provenances: Narag, Waknaghat, Jonaji, and Nauni, each consisting of three genotypes. Sixteen quantitative traits related to leaves, flowers, and fruits of wild pomegranate were subjected to statistical analysis. Results indicated significant variations among different provenances for all quantitative traits except leaf length, leaf breadth, and leaf petiole length. Notably, the Narag provenance exhibited the highest values for most traits, including leaf area (5.46 cm²), flower petal length (20.16 mm), fruit length (5.61 cm), fruit breadth (5.48 cm), fruit rind thickness (3.32 mm), fruit fresh weight (78.61 g), fruit rind weight (35.45 g), fruit aril weight (43.16 g), and the number of arils per fruit (294.82).

Khadivi and Arab (2021) conducted an assessment of the morphological and pomological diversity among 70 native pomegranate genotypes. The study revealed significant variations in key characteristics. Fruit weight displayed a broad range, spanning from 103.28 to 407.59 g indicating diverse fruit sizes. Total aril weight per fruit ranged from 51.55 to 238.97 grams, reflecting differences in the quantity of edible arils within the fruits. Fruit peel color exhibited considerable variability, encompassing shades of yellow, yellow-red, red, and red-brown. Aril color showcased significant variation, offering options like light milky, pink, white-red, red, and red-black, representing diverse visual and taste qualities. Seed hardness also varied, with some genotypes featuring soft seeds, others semi-soft, and some hard seeds. Additionally, positive correlations were observed between total aril weight and

various fruit characteristics, including length, diameter, weight, stalk diameter, peel weight, and aril shape.

Ashraifi *et al.* (2022) conducted the morphological diversity of 103 wild pomegranate (*Punica granatum* L. var. *spinosa*) specimens in northeastern Iran, evaluating 46 traits related to trees, flowers, and fruits. Their study found a broad range of fruit weights from 17.93 to 99.9 g, with an average weight of 48.92 g. The total aril weight varied significantly, ranging from 0.54 g to 64.78 g, with an average of 24.25 g, while the weight of 100 arils ranged from 4.89 to 46.21 g, with an average of 13.79 g.

2.2 Biochemical Characterization

Poyrazoglu *et al.* (2002) analyzed thirteen pomegranate varieties from four regions in Turkey for their organic acids and phenolic compounds. The total titratable acidity ranged from 4.58 to 17.30 g/L, while total sugars ranged between 139.6 and 160.6 g/L in the juices of the cultivars. Drogoudi and Tsipouridis (2005) evaluated twenty pomegranate accessions from various regions in Northern Greece for their physical and chemical characteristics. The total phenolics varied between 22.5 and 69.7 mg/100ml, and anthocyanin levels ranged from 42.7 to 72.4 mmol/100 ml.

Sharma and Bist (2005) collected four wild germplasm accessions of pomegranate from Gharsa and Nagar valleys of Kullu Lahual Spiti district of H.P. The total soluble solids of the wild pomegranate ranged from 10.30 to 12.80 °Brix, and the acidity ranged from 3.36 to 4.24 per cent. Martinez *et al.* (2006) evaluated five new pomegranate varieties (ME14, ME15, PTO2, PTO7 and CRO1) for their morphological and biochemical traits and found that PTO7 had higher acidity content.

Ozgen *et al.* (2008) evaluated arils from six pomegranate cultivars obtained from various sites in the Mediterranean region of Turkey for their chemical and antioxidant properties. The cultivars exhibited varying taste and acidity levels. 'Kan,' 'Dikenli incekabuk,' and 'Katirbasi' had high total soluble solids (TSS) content above 17%. 'Serife' had the highest acidity while 'Tatli' had the lowest. 'Kan' had dark red color, high TSS, and relatively high acidity, making it a suitable choice for both fresh fruit and juice markets. All selected cultivars had high levels of total phenolics (TP) and anthocyanins. The TP content varied between 1245 and 2076 mg gallic acid

equivalent (GAE)/l of fruit juice. The Total monomeric anthocyanins (TMA) varied between 6.12 and 219 mg cyanidin 3-glucoside equivalents/l of fruit juice, with 'Kan' having the highest TMA.

Akbarpour *et al.* (2009) examined twelve pomegranate cultivars for various chemical characteristics. The reducing sugar content of the cultivars ranged from 13.89 to 29.83 g/100 ml, the total soluble solids (TSS) ranged from 15.17% to 22.03%, and the acidity ranged from 0.35% to 3.30%. Additionally, the total antioxidant capacity varied from 225.17 to 705.50 mmol/100g among the cultivars.

Tehraniifar *et al.* (2010) studied twenty different Iranian pomegranate (*Punica granatum* L.) cultivars to assess their chemical properties and antioxidant potential. Their findings revealed that the juice yield of these cultivars varied between 26.95% and 46.55%. The total soluble solids ranged from 11.37 °B to 15.07 °B, titratable acidity was between 0.33 g/100 g and 2.44 g/100 g, and total sugars ranged from 13.23 g/100 g to 21.72 g/100 g. Additionally, the total anthocyanin content varied from 5.56 mg/100 g to 30.11 mg/100 g, while the total phenolic content ranged from 295.79 mg/100 g to 985.37 mg/100 g across the different cultivars.

Wani *et al.* (2012) studied the genetic variability among various pomegranate selections from the Kashmir valley (SKAU-Pg-Sr-001 to SKAU-Pg-Sr-017) to identify those with superior yield and quality traits. They found that the total soluble solids (TSS) ranged from 11.50% in SKAU-Pg-Sr-013 to 16% in SKAU-Pg-Sr-005. The highest juice content was 62.37% in SKAU-Pg-Sr-017, followed by 56.85% in SKAU-Pg-Sr-015. Acidity levels varied between 0.30% in SKAU-Pg-Sr-017 and 0.57% in SKAU-Pg-Sr-013. SKAU-Pg-Sr-017 also had the highest TSS/acidity ratio of 53.34. Reducing sugars ranged from 6% in SKAU-Pg-Sr-013 to 10.12% in SKAU-Pg-Sr-007, while non-reducing sugars ranged from 0.66% in SKAU-Pg-Sr-004 to 3.06% in SKAU-Pg-Sr-017. The highest total sugar content was found in SKAU-Pg-Sr-012 at 12.92%, and the lowest in SKAU-Pg-Sr-013 at 7.24%. Anthocyanin content varied from 9.14 mg/100g of fruit in SKAU-Pg-Sr-016 to 19.30 mg/100g in SKAU-Pg-Sr-017.

Rawat *et al.* (2012) characterized wild pomegranate genotypes from the Himalayan region and recorded various parameters, including 2.43% total sugar, juice content of 71.35%, Total Soluble Solids (TSS) of 12.00%, and malic acid

concentration of 4.17%. Ferrarai *et al.* (2014) conducted a study to evaluate 13 pomegranate genotypes, both sweet and sour, of Israeli origin. Significant differences were observed for several parameters, particularly fruit and aril size, maturity index of fruits, titrable acidity, total polyphenol content, antioxidant activity of juices, and oil content of seeds.

Ismail *et al.* (2014) characterized six Egyptian pomegranate (*Punica granatum* L.) cultivars (Araby, Assuity, Hegazy, Manfalouty, Nab El Gamal and Wardi). The juice volume ranged from 62.41 to 71.81 ml/100g arils for Wardi and Nab El Gamal cultivars. The SSC content ranged between 16.01 (Hegazy) and 12.55 per cent in Assuity. The anthocyanins content ranged from 1.47 to 10.03 mg/100g for Araby and Hegazy.

Bakshi *et al.* (2014) studied the variability in wild pomegranates based on various physical and chemical characteristics. They found that individual fruit weight ranged between 85.64 and 131.60 grams, with lengths from 5.52 to 7.42 cm and breadths from 5.17 to 5.99 cm. Seed weight per fruit varied from 34.60 to 56.0 grams, and peel weight ranged from 49.04 to 68.0 grams. In terms of chemical properties, total soluble solids ranged from 11.0 to 15.5°Brix, titratable acidity varied between 5.26 and 6.80%, total sugars were from 9.32 to 13.86%, and tannins ranged from 121.12 to 149.70 mg/100 ml.

Nafees *et al.* (2016) conducted a study to screen elite pomegranate genotypes by examining biochemical diversity in both wild and cultivated varieties. The results indicated significant morphological diversity in wild pomegranate fruits compared to cultivated ones. The first six principal components accounted for 80.75% and 75.49% of the diversity in 53 wild and 62 cultivated pomegranate genotypes, respectively. The study recorded high coefficients of variance (10.78–18.62%) and a wide range of values for total soluble solids, titratable acidity, ascorbic acid content, total soluble sugars, and total phenolic content, with ranges of 0.10–1.25, 5.88–29.96, 9.69–19.85 mg, and 175.05–595.42, respectively. Additionally, Marmol *et al.* (2017) found that total sugar content ranged from 15.5 to 15.9 g/100 ml in twenty Spanish pomegranate genotypes.

Khadivi *et al.* (2020) studied biochemical parameters and revealed that the range of total soluble solids was 9.30-27.00 % with an average of 17.09 and fruit taste was sour-sweet in 12 accessions and also sweet in 11 accessions.

Faraj (2023) conducted a study to assess the morpho-phytochemical properties of pomegranate functional foods to identify the best genotypes for genetic enhancement. The study revealed significant morphometric differences ($P \leq 0.05$) among 24 genotypes, with the following mean values for various parameters: fruit weight (299.21 g), peel thickness (3.47 mm), weight of 100 arils (38.59 g), total flavonoid content (TFC) (54.50 $\mu\text{g/ml}$), total phenolic content (TPC) (47.97 $\mu\text{g/ml}$), antioxidant activity (21.08 $\mu\text{g/ml}$), and total soluble solid content (TSS) (14.59 Brix).

2.3 Correlation

Karale and Desai (2000) explored the relationship between sugar content (measured as total soluble solids or TSS) and acidity in pomegranate juice. They found that as the sugar content increased, so did the acidity, indicating a clear positive correlation between the two factors. Singh and Gupta (2018) investigated the genetic diversity of wild pomegranates in different areas of Himachal Pradesh. They looked at various traits like fruit size, weight, sugar content, and vitamin C levels in 15 different wild pomegranate varieties. They discovered that these traits were strongly inherited, meaning they could be passed down from parent to offspring reliably. Additionally, Singh and Gupta found that all these traits were interconnected. Particularly, fresh seed weight with aril was highly correlated with fruit width, fruit length, total sugar, and reducing sugar.

Khadivi *et al.* (2018) observed that 100 arils fresh weight was significantly and positively correlated with fruit length ($r=0.52$), fruit diameter ($r=0.54$), fruit shape ($r=0.21$), sepal length ($r=0.43$), fruit weight ($r=0.60$), aril color ($r=0.31$), aril length ($r=0.66$), aril width ($r=0.60$), and seed length.

Kumar *et al.* (2019) investigated the correlation between various morphological traits of pomegranate and found significant differences ($P < 0.05$) among all the genotypes studied. The highest critical differences at 5% significance level were observed as 13.21 for fruit weight and 11.98 for aril percentage. Additionally, differences of 2.55 in thorn number per shoot length (m) were noted.

Conversely, the lowest critical differences were observed in leaf blade width (0.143), leaf blade length (0.145), fruit length (0.456), and seed hardness (0.48), respectively.

Khadivi *et al.* (2020) reported significant and positive correlations between fruit taste and various fruit characteristics. Fruit taste was found to be correlated with fruit length ($r = 0.28$), fruit diameter ($r = 0.29$), fruit weight ($r = 0.34$), aril color ($r = 0.16$), 100 arils fresh weight ($r = 0.30$), and fruit juice color ($r = 0.21$). Similarly, fruit quality exhibited positive and significant correlations with several parameters, including fruit length ($r = 0.33$), fruit diameter ($r = 0.38$), fruit weight ($r = 0.38$), aril color ($r = 0.60$), 100 arils fresh weight ($r = 0.53$), aril length ($r = 0.36$), aril width ($r = 0.22$), fruit juice color ($r = 0.65$), fruit taste ($r = 0.25$), and total soluble solids (TSS) ($r = 0.37$). Furthermore, TSS demonstrated positive and significant correlations with aril color ($r = 0.42$) and fruit juice color ($r = 0.43$).

Mehta and Gupta (2021) studied correlation in wild pomegranate genotypes). It was found that leaf length was in positive correlation with several fruit characteristics viz., fruit length ($r = +0.826$), Fruit breadth ($r = +0.745$), fruit fresh weight ($r = +0.735$), fruit rind weight ($r = +0.650$), fruit aril weight ($r = +0.725$) and no. of arils per fruit ($r = +0.623$).

Dhiman *et al.* (2022) studied variability parameters in pomegranates and found that the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all traits, with a narrow difference between the two indicating minimal environmental influence. The highest GCV and PCV values were recorded for the number of branches (136.708% and 136.78%, respectively). The number of branches also exhibited the highest heritability and genetic advance as a percentage of the mean. Additionally, fruit length showed a highly significant and positive correlation with fruit diameter ($r_g = 0.998$, $r_p = 0.988$).

2.4 Molecular markers

A basis for the maintenance of biology and genetic advancement is provided by plant germplasm with the greatest genetic diversity. For parental selection in breeding and genetic improvement, it is crucial to characterize genetic diversity. Between the 1960s and 1990s, isozyme detection technologies and molecular biology were used to explore the genetic variety of plants at all levels, from morphology to genes. Basic genetic pathways are understood using molecular markers, and advanced

breeding techniques are successfully supported by gene sequencing. Biochemicals (secondary metabolites), macromolecules, proteins, and deoxyribonucleic acids make up markers. Zamani (1990) noted that pomegranate morphological attributes are helpful in genotype identification; nevertheless, Kumar (1999) shown that morphological characters may not be entirely reliable in genetic studies due to their variability with environmental and culture circumstances. Detailed breeding work demonstrated that distinct pomegranate cultivars differed significantly in the expression and function of the genes governing rind and aril color (Holland *et al.*, 2009); however, morphological differences in a few selected cultivars' traits were not reflected in molecular marker polymorphism.

Curr *et al.* (2010) recognized a new set of 9 SSR markers for assessing the pomegranate accessions. This set of microsatellites showed high levels of polymorphism and heterozygosity. Hasnaoui *et al.* (2010) developed eleven new microsatellite markers (SSR) for *Punica granatum* and assessed a set of 27 pomegranate accessions from Tunisia. They revealed 25 alleles and number of alleles per locus ranged between 1 and 4, and heterozygosity from 0.037 and 0.592. This new set of SSR markers could be very useful for genetic diversity studies.

Zeinalabedini *et al.* (2012) studied genetic diversity within Iranian pomegranate cultivars using twelve simple sequence repeat (SSR) markers. Their analysis detected forty-three alleles, averaging 3.59 alleles per locus. The locus ABRII-MP26 exhibited the highest levels of polymorphism. The mean values of expected heterozygosity and polymorphic information content were 0.521 and 0.458, respectively. These findings underscore the broad genetic base of the Iranian National Pomegranate Collection, ensuring future progress in breeding programs. The effectiveness of SSR markers highlights their utility in genetic diversity studies and the development of germplasm management strategies for pomegranate cultivation.

According to Ferrarai *et al.* (2014) genetic investigation of the pomegranate collection, 53 SSR loci were found to be capable of differentiating between genotypes and estimating genetic distances, indicating that SSR markers could be effective to detect the genetic diversity of pomegranate.

Ravishankar *et al.* (2014) designed 171 loci of primers pair of which 167 produced reproducible polymorphic band with twelve genotypes of pomegranate.

Overall 951 alleles were identified, which varied from 1 to 14/locus with average 5.56. PIC range varies from 0.00 to 0.910 (average of 0.5427).

Dalal (2015) studied the genetic diversity in pomegranate (*Punica granatum* L.) using 12 simple sequence repeat (SSR) markers. Out of the developed primers, five showed monomorphic alleles across all pomegranate populations, while seven loci displayed polymorphic banding patterns. A total of 25 alleles were identified, with an average of 2.08 alleles per locus. The mean expected heterozygosity (H_e) was 0.3315, and the average polymorphic information content was 0.2572. The genetic distance among the 11 varieties ranged from 0.0606 to 0.268.

Pirseiyedi *et al.* (2016) developed 12 polymorphic microsatellite markers from genomic library of *Punica granatum* L. Alleles per locus varied from two to five with average 2.9. The PIC ranged from 0.26 to 0.65 and heterozygosity ranged varies from 0.15 to 0.87. Noormohammadi *et al.* (2012) used three molecular markers RAPD, IISR and SSR for analysis of genetic diversity among thirty six Iranian pomegranate genotypes. Based on molecular data, Nei genetic diversity (H), Shanon index (I) and polymorphic information content (PIC) were calculated. UPGMA tree classified all thirty six genotypes of pomegranate into three major groups.

Calizakan *et al.* (2017) studied genetic diversity using new microsatellite markers to characterize 78 pomegranate accessions from Turkey. Utilizing six SSR primers, they identified a total of 41 alleles, averaging 4.6 alleles per locus, with a mean probability of identity (PI) value of 0.366. These results indicated a high level of polymorphism in the pomegranate germplasm. Among the 30 accessions, five synonymous groups were detected. This microsatellite-based key is a preliminary step toward establishing a database for marker-assisted identification of pomegranate accessions in Turkey.

Zarei and Sahraroo (2018) selected 16 simple sequence repeats (SSRs) markers for genetic diversity analysis in 50 pomegranate (*Punica granatum* L.) accessions from five regions of Iran. All SSR markers were polymorphic and produced 48 alleles. The average 15 heterozygosity of the 16 SSR primers was 0.33 and 0.48, respectively. The observed average polymorphic information content (PIC) was 0.41. Cluster analysis grouped all genotypes based on their geographical

origins. Analysis of molecular variance (AMOVA) showed that most of the genetic variation was among populations.

Kaur *et al.* (2018) conducted a studied phytochemical analysis and assessed the antioxidant potential of various parts of the wild pomegranate, including red and green fruits, leaves, and flowers. They found that red fruits had the highest amounts of carbohydrates and proteins, followed by green fruits, while flowers and leaves had lower amounts. However, leaves contained higher levels of phenolics and tannins compared to the other tissues and fruit extracts. The red fruit exhibited the highest radical scavenging effect, with an EC₅₀ value of 70.33 µg/ml. The ferric reducing potential was also significantly higher in red fruit (310.99 ± 0.98 µmol Fe²⁺/g dry matter) compared to green fruit extracts. The results suggest that methanolic extracts of red fruits are pharmacologically more active and can be utilized for studying the pharmacokinetics of various bioactive compounds present in wild pomegranate.

Mahajan (2018) observed genetic diversity among 20 pomegranate genotypes, which included both cultivated varieties and wild germplasm, using simple sequence repeats (SSR) markers. Genomic DNA from the plants was isolated with a modified CTAB method. Seventeen SSR markers were screened across the selected pomegranate germplasm to analyze their molecular diversity patterns. Out of these markers, twelve were polymorphic, and five were monomorphic. The polymorphic primers produced 29 SSR marker alleles, averaging 1.71 alleles per locus. The maximum number of alleles, two per marker, was observed for twelve markers. Polymorphic information content (PIC) values ranged from 0.12 to 0.38, with an average of 0.29 per marker. The observed heterozygosity values ranged from 0.12 to 0.50, with an average of 0.36. Markers PgSSR33, PgSSR16, and PgSSR25 were found to be highly polymorphic and can be effectively utilized in future pomegranate breeding programs.

Patil *et al.* (2018) studied the genetic diversity and population structure among 42 diverse pomegranate genotypes using a set of twenty-one class I hypervariable SSR markers (> 24 bp) previously reported from the analysis of the cv. Dabenzi genome. The study included 16 indigenous cultivars, 13 exotic cultivars, and 13 wild accessions. A total of 66 alleles were detected, averaging 3.14 alleles per marker. The average polymorphic information content (PIC) was 0.44, observed heterozygosity

(Ho) was 0.21, and Shannon's gene diversity index (I) was 0.95, indicating moderate genetic diversity. Pairwise genetic distance ranged from 0.07 to 0.80, with a mean value of 0.53. Population structure analysis divided the genotypes into four subpopulations (SP1, SP2, SP3, and SP4). The analysis revealed that 25% of the variation was due to differences among populations, while 75% was within subpopulations, indicating significant genetic differentiation and low gene flow.

Nafees *et al.* (2020) studied genetic diversity using highly polymorphic microsatellite (SSR) markers to estimate the gene flow and molecular diversity in 95 wild and domesticated pomegranate accessions collected from 14 districts of Pakistan, where the plants are considered to be a minor and neglected fruit crop for research and crop production. Resolution of the DNA fragments showed allelic lengths that ranged from 100 to 430 bp, and a total of 58 loci were amplified using 29 polymorphic primers with a mean of two loci per primer. An allelic frequency range of 0.4088 to 0.8579 was identified in all 95 genotypes using the primers PGCT091b and ABRII-MP30. However, the range of genetic diversity in the selected pomegranate germplasm was 0.2489 to 0.6328, which was detected using selected primers. The lowest heterozygosity (0.0842) was measured using the POM_AAC3 marker, whereas the maximum level of 0.7737 was estimated using PGCT089b in the selected wild and domesticated pomegranate accessions. The genetic base of the pomegranate germplasm was broad, with a maximum PIC value of 0.5598. The wild pomegranate accessions exhibited more genetic diversity than the domesticated accessions.

Aziz *et al.* (2020) studied genetic diversity using simple sequence repeat markers, identifying 303 alleles with an average of 7.39 alleles per marker. The polymorphic information content (PIC) ranged from 0.12 (PGCT093B) to 0.88 (Pom006), with an average of 0.54. The average genetic distance (GD) across all genotypes was 0.52. The lowest GD was between the Chattar Class and Thorar genotypes (GD = 0.27), while the highest was between the Khun Bandway and Akhor Ban genotypes (GD = 0.74). The study revealed significant genetic diversity among wild pomegranate accessions in the Azad Jammu and Kashmir region of Pakistan.

Gunnaih *et al.* (2021) evaluated genetic diversity and relatedness among 61 cultivars from six countries and 35 wild accessions from the Himalayas (India) using

60 SSR markers. Seventeen of these markers were polymorphic and identified 55 polymorphic alleles, averaging 3.24 alleles per locus and 1.69 effective alleles. The study found moderate polymorphic information content (PIC) of 0.33 and moderate gene diversity of 0.37.

Parasharaum *et al.* (2022) assessed 40 pomegranate varieties in an Indian collection using morphological traits, biochemical parameters, and SSR markers. Fruit volume, weight, and color were crucial for classification. The accessions formed eight clusters, partially aligning with their origins, with foreign cultivars in distinct groups. SSR markers identified 66 alleles, with 'IC-3187200' and 'Gul-e-Shah Red' being the most genetically dissimilar.

Zuriaga *et al.* (2022) analysed the genetic diversity of 94 accession in Spain using 19 microsatellite markers and identified 85 different pomegranate genotypes. The findings of the study open the door for future genetic investigations, enabling the identification of suitable parental combinations to create diverse populations. Dar *et al.* (2022) assessed the genetic variability and relationship among and between the different populations of wild pomegranate showed significant differences among the studied population.

MATERIALS AND METHODS

The present investigation entitled “**Morphological, biochemical and molecular characterization of wild pomegranate (*Punica granatum* L.) genotypes of Jammu region**” was carried out during the year 2021 to 2022 at different selected wild pomegranate growing areas of Jammu province, the research work was carried out at School of Biotechnology and Molecular Laboratory and Division of Fruit Science, SKUAST-J, Chatha, Jammu.

3.1. Geographic features of site of study

The study area falls in Rajouri, Poonch and Udhampur districts of Jammu region. Rajouri district of Union Territory of Jammu and Kashmir is located at 33.38°N 74.31°E with an average elevation of 915m (3001 feet). It has three main seasons: summer (April to June) with warm to hot temperatures, monsoon (July to September) bringing heavy rainfall and lower temperatures, and winter (October to March) featuring cold weather and occasional snowfall, especially in higher altitudes. Poonch is situated between 33°25' to 34° 01' North latitude and between 73°58' to 74°35' east longitude. It has an average elevation of 981 meters (3218 feet). The Climate of Poonch is somewhat cooler and summers are short and summer temperature generally does not cross 31 degrees. Winters are cool and hilly characterized with rainfall due to western disturbances. Snowfall is uncommon but may occur in cool months. Rainfall is 669 millimeters (26.3 in) in the wettest months.

Udhampur district is situated in the southeastern part of the Jammu and Kashmir state, with its central city, Udhampur, positioned at 32.93°N 75.13°E. This city is located in a relatively flat terrain within the district, with an elevation of approximately 756 meters (2480 feet), and it seldom witnesses snowfall. Specifically focusing on the climate of Udhampur city, it can be classified as sub-tropical. During the summer season, temperatures typically do not exceed 40 degrees Celsius, while in the winter months, the mercury generally drops to around 2 degrees Celsius or occasionally even reaches zero. The region receives an annual rainfall of about 130 cm, primarily during the monsoon season and winters, owing to Western Disturbances. Snowfall in Udhampur is a rare.

Table 3.1: Name of the farmer and geographical location of wild Pomegranate accessionstaken into study.

S. No.	Name of Farmer/Habitat	Total Number of Plants Selected	Code	Geographical location of Selected Plants		
				Latitude	Longitude	Elevation
1	Mr. Nisar Ahmed Fatehpur Rajouri	4	JWPR(SEL-1) JWPR(SEL-2) JWPR(SEL-3) JWPR(SEL-4)	33° 25.624N 33° 25.623N 33° 25.786N 33° 25.808N	74° 18.376E 74° 18.371E 74° 18.372E 74° 18.374E	1184 1184 1183 1182
2	Mr. Shamshir Fatehpur Dara Rajouri	3	JWPR(SEL-5) JWPR(SEL-6) JWPR(SEL-7)	33° 43.293N 33° 43.324N 33° 43.323N	74° 29.443E 74° 29.443E 74° 29.402E	1290 1281 1281
3	Wild Dadassan Rajouri	1	JWPR(SEL-8)	33° 43.208N	74° 29.441E	1201
4	Dhanore Rajouri Wild	2	JWPR(SEL-9) JWPR(SEL-10)	33° 22.271N 33° 22.271N	74° 46.446E 74° 21.157E	1671 1671
5	Mr Layquit husssain Choudharynar Rajouri	1	JWPR(SEL-11)	33° 25.277N	74° .46.448E	812
6	Mr Naseer Ahmed Chaudarynar Rajouri	3	JWPR(SEL-12) JWPR(SEL-13) JWPR(SEL-14)	33° 24.647N 33° 24.647N 33° 24.644N	74° 20.929E 33° 24.647N 33° 24.647N	1172 1172 1172
7	Wild Nadian darhal Rajouri	3	JWPR(SEL-15) JWPR(SEL-16) JWPR(SEL-17)	33° 48.059N 33° 48.274N 33° 48.274N	74° 40.217E 74° 40.207E 74° 40.207E	1657 1651 1651
8	Wild Gurdana bala Rajouri	4	JWPR(SEL-18) JWPR(SEL-19) JWPR(SEL-20) JWPR(SEL-21)	33° 23.567N 33° 23.570N 33° 23.886N 33° 23.898N	74° 17.157E 74° 17.164E 74° 16.911E 74° 16.928E	1074 1074 1078 1077
9	Gambhir Moglan, Rajouri	3	JWPR(SEL-22) JWPR(SEL-23) JWPR(SEL-24)	33° 40.685N 33° 40.685N 33° 40.681N	74° 25.228E 74° 25.228E 74° 25.221E	1224 1224 1226
10	Kalakot wild Rajouri	3	JWPR(SEL-25) JWPR(SEL-26) JWPR(SEL-27)	33° 37.128N 33° 37.128N 33° 37.130N	74° 40.378E 74° 40.378E 74° 40.376E	702 702 704
11	Manjakote wild Rajouri	3	JWPR(SEL-28) JWPR(SEL-29) JWPR(SEL-30)	33° 47.095N 33° 47.095N 33° 48.102N	74° 25.065E 74° 25.065E 74° 25.089E	1480 1480 1225
12	Zahoor Khan Chanayal Mandhar Poonch	4	JWPP(SEL-31) JWPP(SEL-32) JWPP(SEL-33) JWPP(SEL-34)	33° 56.760N 33° 56.760N 33° 56.765N 33° 52.883N	74° 22.024E 74° 22.024E 74° 22.065E 74° 21.157E	1586 1586 1551 1587

13	Mr. Mohd Amin KhanDargaloon Poonch	3	JWPP(SEL-35) JWPP(SEL-36) JWPP(SEL-37)	33 ⁰ 52.884N 33 ⁰ 52.861N 33 ⁰ 52.801N	74 ⁰ 22.157E 74 ⁰ 22.156E 74 ⁰ 22.155E	1458 1458 1453
14	Irshad Ahmed Garah Mendhar Poonch	3	JWPP(SEL-38) JWPP(SEL-39) JWPP(SEL-40)	33 ⁰ 56.717N 33 ⁰ 56.717N 33 ⁰ 56.717N	74 ⁰ 19.021E 74 ⁰ 19.024E 74 ⁰ 19.022E	1447 1447 1448
15	Mr. Zahid Nambal Poonch	3	JWPP(SEL-41) JWPP(SEL-42) JWPP(SEL-43)	33 ⁰ 56.765N 33 ⁰ 56.768N 33 ⁰ 56.762N	74 ⁰ 19.031E 74 ⁰ 19.036E 74 ⁰ 19.044E	1419 1420 1420
16	Mr. Ishfaq Ahmed Nambal Poonch	3	JWPP(SEL-44) JWPP(SEL-45) JWPP(SEL-46)	33 ⁰ 56.754N 33 ⁰ 56.754N 33 ⁰ 56.756N	74 ⁰ 19.026E 74 ⁰ 19.026E 74 ⁰ 19.025E	1415 1415 1415
17	Kandi Gulata Poonch	4	JWPP(SEL-47) JWPP(SEL-48) JWPP(SEL-49) JWPP(SEL-50)	33 ⁰ 56.851N 33 ⁰ 56.851N 33 ⁰ 56.855N 33 ⁰ 56.861N	74 ⁰ 19.058E 74 ⁰ 19.058E 74 ⁰ 19.056E 74 ⁰ 19.059E	1510 1510 1512 1512
18	Wild Dogra Kud, Udampur	4	JWPP(SEL-51) JWPP(SEL-52) JWPP(SEL-53) JWPP(SEL-54)	33 ⁰ 05.260N 33 ⁰ 05.260N 33 ⁰ 05.110N 3 ⁰ 04.270N	75 ⁰ 29.093E 75 ⁰ 29.093E 75 ⁰ 29.071E 75 ⁰ 28.321E	1435 1435 1425 1679
20	Wild Tamatar morh, Udampur	3	JWPP (Sel-55) JWPP (Sel-56) JWPP (Sel-57)	33 ⁰ 07.379N 33 ⁰ 07.379N 33 ⁰ 08.256N	75 ⁰ 29.641E 75 ⁰ 29.641E 75 ⁰ 17.047E	1518 1518 1423
21	Chenani, Udhampur	3	JWPP(Sel-58) JWPP(Sel-59) JWPP (Sel-60)	33 ⁰ 03.491N 33 ⁰ 03.493N 33 ⁰ 03.496N	75 ⁰ 26.783E 75 ⁰ 26.785E 75 ⁰ 26.782E	1079 1079 1077
22	Ramban Peerah	4	JWPP(Sel-61) JWPP(Sel-62) JWPP(Sel-63) JWPP(Sel-64)	33 ⁰ 16.955N 33 ⁰ 16.655N 33 ⁰ 16.852N 33 ⁰ 16.791N	75 ⁰ 30.238E 75 ⁰ 31.031E 75 ⁰ 31.288E 75 ⁰ 31.036E	921 932 936 968
24	Chanunta Udhampur	3	JWPU(Sel-65) JWPU(Sel-66) JWPU(Sel-67)	32°84.695N 32°84.691N 32°84.694N	75°19.548E 75°19.544E 75°19.546"E	710 710 710
25	Mr. Lal chand Panchari Udhampur	3	JWPU(Sel-68) JWPU(Sel-69) JWPU(Sel-70)	32 ⁰ 54.580N 33 ⁰ 54.581N 33 ⁰ 54.580N	75 ⁰ 16.753E 75 ⁰ 32.812E 75 ⁰ 32.810E	770 770 770

3.2 Technical Programme

The plant material used in present study was collected from three districts of Jammu Division i.e. Rajouri, Poonch and Udhampur. The study was completed in three experiments viz; morphological, biochemical and molecular characterization. In the first stage, a survey was conducted and information was collected regarding the wild pomegranate trees in the three districts. Accordingly, 100 genotypes were initially identified from selected districts. Out of 100 genotypes, 30 genotypes were discarded on the basis of poor pomological traits such as fruit quality, low yield efficiency and damaged fruit due to insect attack and cracking. Remaining 70 wild pomegranate genotypes (Table 3.1) were studied to assess diversity and to select elite wild pomegranate genotypes possessing superior attributes of quality traits by using International Union for the Protection of New Varieties of Plants (UPOV, 2011). In the third stage, the trees having a good quality fruits and high yield efficiency were used for morphological characters. In the fourth stage, based on the UPOV 2011 descriptor the wild pomegranate fruits from identified genotypes were used for biochemical analysis and in the last stage, identified genotypes were selected for molecular characterization.

3.2.1 Experiment 1

Characterization of wild pomegranate genotypes on the basis of morphological traits of fruits

Fruit samples of wild pomegranate accessions, free from defects, decay, mechanical damages and sunburn were collected and studied in the laboratory. Fruit qualitative parameters were recorded by following pomegranate plant descriptor guidelines (UPOV, 2011) detailed as follows:

3.2.1.1 Tree characteristics

3.2.1.1.1 Tree height

The height of the tree was measured from ground level to the tip of the highest shoot with the help of Ravi Altimeter and expressed in meter.

3.2.1.1.2 Tree Growth habit

Tree growth habit was recorded by taking the orientation of growing branches

on visual basis as specified by descriptor for pomegranate by UPOV (2011) and growth was categorized as following:

Upright	:	1
Spreading	:	2
Drooping	:	3

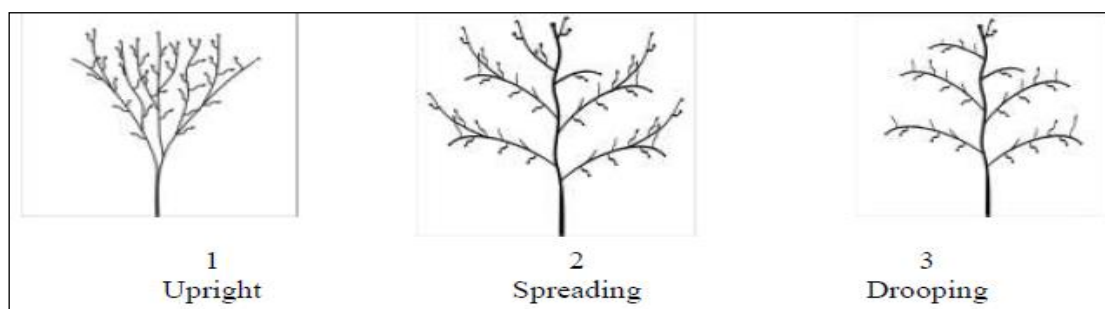


Fig. 3.1: Growth habit in pomegranate tree

3.2.1.1.3 Trunk Type

The observation for trunk type was observed visually and categorized as multi trunk and single trunk.

3.2.1.2 Leaf characteristics

3.2.1.2.1 Leaf blade shape

Leaf shape was recorded according to shapes given in the descriptors for pomegranate for each selected tree and was scored as

Elliptic lanceolate	:	3
Lanceolate	:	5
Broad elliptic	:	7

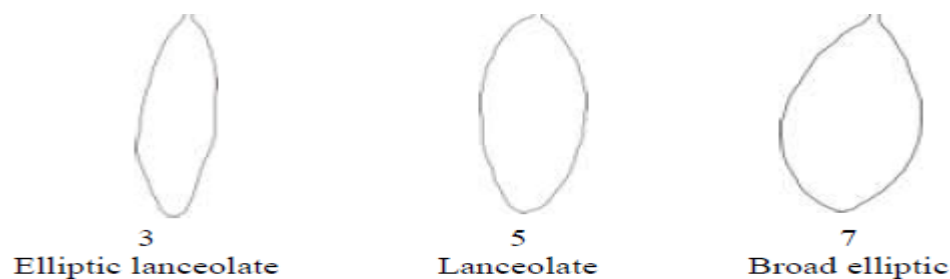


Fig. 3.2: Leaf shape in pomegranate

3.2.1.2.2 Leaf length (cm)

Average length of ten leaves apart from petiole was taken from 3rd and 4th positions from tip of the bearing shoot was measured as leaf length and expressed in centimeter (cm). Scale ruler (30cm/12") was used to calculate the leaf length.

3.2.1.2.3 Leaf width (cm)

Average width of ten leaves chosen from 3rd and 4th positions from tip of the bearing shoot was measured as leaf width. The leaf width was recorded in the middle of the leaf and conveyed in centimeter (cm). Scale ruler (30cm/12") was used to calculate the leaf width.

3.2.1.2.4 Petiole length (mm)

Petiole length was recorded with the help of vernier caliper. Average of 10 fully developed leaves taken from each selection.

3.2.1.3 Flowering characteristics

3.2.1.3.1 Start of flowering

Date of start of flowering was recorded when about 5-10 per cent flower had opened.

3.2.1.3.2 End of flowering

End of flowering was taken when 10 per cent flower buds were still to open i.e. 90 per cent flowers had shed.

3.2.1.3.3 Duration of flowering

Duration of flowering was calculated by counting the days taken from start of flowering to end of flowering.

3.2.1.3.4 Calyx color

Calyx color was observed visually and categorized into orange and red.

3.2.1.3.5 Calyx length (mm)

Calyx length was recorded individually on five opened flowers for each selected trees with the help of vernier caliper.

3.2.1.3.6 Calyx width (mm)

Calyx width was recorded individually on five closed flowers for each selected trees with the help of vernier caliper.

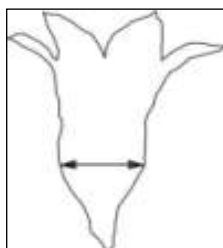


Fig. 3.3: Calyx width in pomegranate

3.2.1.3.7 Petal length (mm)

Petal length was recorded individually on five opened flowers for each selected trees with the help of vernier caliper.

3.2.1.3.8 Petal width (mm)

Petal width was recorded individually on five opened flowers for each selection with the help of vernier caliper.

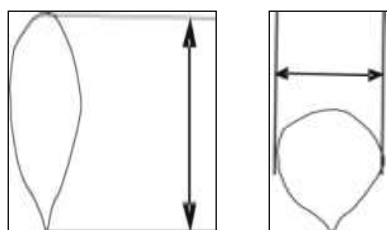


Fig 3.4: Petal length and petal width in pomegranate

3.2.1.4 Fruit characteristics

3.2.1.4.1 Fruit shape

Fruit shape was recorded according to shapes given in the descriptors for pomegranate for each selected tree and was scored as

Round	: 1
Ovate	: 3
Oval	: 5
Elliptical	: 7

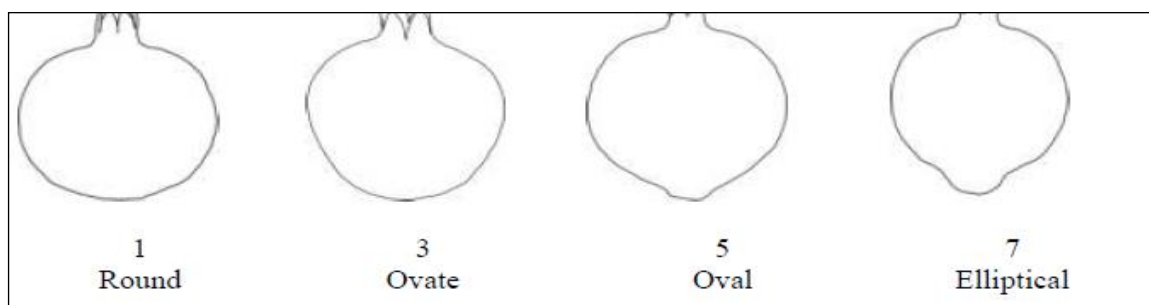


Fig 3.5: Fruit shape in pomegranate

3.2.1.4.2 Fruit color

Fruit color was observed at maturity stage and categorized as

1. Green
2. Reddish green
3. Reddish yellow
4. Greenish yellow

3.2.1.4.3 Rind thickness (mm)

Rind thickness was measured in millimeter (mm) using a digital vernier caliper of model (KBD-MT 0014).

3.2.1.4.4 Crown length (mm)

The crown length of fruits was measured in millimeter (mm) using a digital vernier caliper of model (KBD-MT 0014) following pomegranate plant descriptor (UPOV, 2011) as demonstrated in Figure 3.6

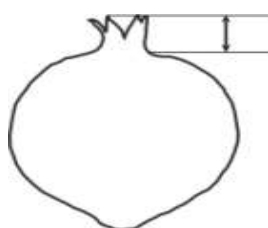


Fig. 3.6 Crown length in pomegranate

3.2.1.4.5 Fruit weight (g)

Ten fruits were randomly selected from each selection plants by moving around the tree at shoulder height. Fruits were of almost uniform size and free of any disease

damage or insect attack. Digital weighing balance (KDC, TH- 500,) was used to weigh fruits in grams.

3.2.1.4.6 Fruit length (mm)

The length of fruits was measured in millimeter (mm) using a digital vernier caliper of model (KBD-MT 0014) following pomegranate plant descriptor (UPOV, 2011) as demonstrated in Figure 3.7.

3.2.1.4.7 Fruit width (mm)

The width of fruits was measured using digital vernier caliper (KBD-MT 0014) in (mm) by following pomegranate plant descriptor (UPOV, 2011) shown in Figure 3.7.

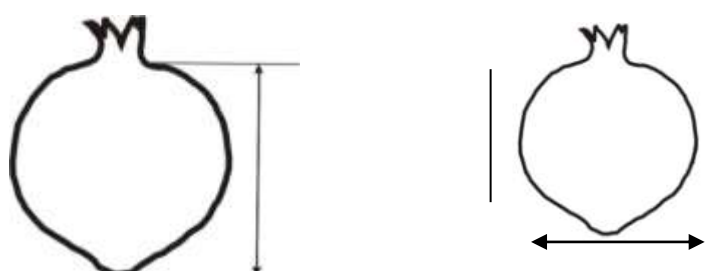


Fig 3.7 Fruit length and fruit width in pomegranate

3.2.1.4.8 Juice (%)

In every direction 5 number of fruits has been collected so total 20 fruits from each selected tree has been taken. Juice content was estimated by extracting juice from 10 fruits by juice extractor. The extracted juice was filtered by muslin cloth and juice percentage was worked out on fresh fruit weight basis. The average juice percentage was obtained from the following formula:

$$\text{Juice (\%)} = \frac{\text{Average juice weight}}{\text{Average fruit weight}} \times 100.$$

3.2.1.5 Aril characteristics

3.2.1.5.1 Aril colour

Individual fruits were observed after cutting in pieces and the colors of fruit aril were scored and recorded. Based on observation it was grouped in following

categories.1. White, 2. Light yellow, 3. Light pink, 4. Pink, 5. Red, 6. Dark red and 7.others

3.2.1.5.2 Seed texture

3.2.1.5.3 Number of aril

The ripe fruits were harvested and the aril, were extracted and counted manually. The average number of aril per fruit was taken from five fruits.

3.2.1.5.4 Aril weight (g)

After peeling out of rind, arils were separated and 100 arils were counted and weighed with a digital weighing balance in grams.

3.2.1.5.5 Aril length and width (mm)

Ten out of 100 weighed arils were randomly selected to measure length and width in millimeter (mm) using digital vernier caliper (KBD-MT 0014) by following pomegranate plant descriptor mentioned in Figure 3.8.



Fig. 3.8: Aril length and aril width in pomegranate

3.2.1.6 Yield characteristics

3.2.1.6.1 Number of fruits

At the time of harvesting all the fruits were counted manually.

3.2.1.6.2 Yield (Kg/bush)

The total fruit yield per plant was calculated by weighing fruit samples collected among each direction of plant and expressed in kg per plant.

3.2.1.7 Biochemical characteristics

3.2.1.7.1 Total soluble solids (TSS, °Brix)

A digital refractometer (ATAGO RX 5000, Japan Development Assistance) was used to measure total soluble solids (TSS) in ripe fruits. Equipment was first standardized with distilled water by attaining reading at $\leq$ zero. A drop of uniformly mixed PAJ sample was placed on tip of lens of refractometer and its lid was covered. The reading was recorded each time by pressing start button. Then washed the lens of refractometer with distilled water and dried with tissue paper before a new PAJ sample was loaded.

3.2.1.7.2 Total sugars (%)

Total sugar was estimated by the Lane and Eynon method, as described by Ranganna (1986).

(a) Sample preparation: Twenty-five grams of arils were macerated and homogenized with distilled water for 5 minutes. The homogenate was transferred to a 250 ml volumetric flask and neutralized with 1.0 N NaOH. To this, 2 ml of 45% lead acetate was added, mixed thoroughly, and allowed to stand undisturbed for 10 minutes. The solution was then de-leaded with 2 ml of 22% potassium oxalate, and the volume was adjusted to 250 ml. The solution was filtered through Whatman No. 4 filter paper and the filtrate was designated as 'Solution A'. For estimating reducing sugars, Solution A was used directly. To estimate total sugars, 50 ml of Solution A was transferred to a 250 ml titration flask, hydrolyzed with 5 ml of concentrated hydrochloric acid (HCl), and left overnight. The solution was then neutralized with 5.0 N NaOH and diluted to 250 ml with distilled water, resulting in 'Solution B'.

(b) Titration: Fehling's solutions (A) and (B) were employed for the titrimetric determination of reducing and total sugars. In a conical flask, 5 ml of each Fehling's solution (A) and (B) were mixed with 25 ml of distilled water. This mixture was titrated with 'Solution A' for reducing sugars and 'Solution B' for total sugars until a brick-red color appeared. A few drops of methylene blue indicator were then added, and titration

continued until a brick-red precipitate was formed. Throughout the process, the flask was kept on a heating plate to maintain boiling. The percentages of reducing and total sugars were calculated using the formula.

$$\text{Total sugar (\%)} = \frac{\text{Fehling factor} \times \text{volume made up}}{\text{Titrate value} \times \text{weight of sample}} \times 100$$

3.2.1.7.3 Non reducing sugars (%)

Non-reducing sugars were obtained by subtracting reducing sugars from total sugars. The calculation was completed following the steps indicated in (A.O.A.C., 1990).

$$\text{Non-reducing sugars (\%)} = (\text{Total sugars} - \text{Reducing sugars})$$

3.2.1.7.5 Titratable acidity (%)

Filtered fresh extracted homogenized PAJ (10 mL) was taken in 100 mL conical flask and after that add distilled water to the volume up to 50 mL. Phenolphthalein (2-3) drops were added in solution as an indicator. Diluted PAJ was titrated against NaOH (1/10 N) drop wise and shaken the flask till pink color end point was achieved (AOAC., 1990) and g/l percent titratable acidity (TA) of acetic acid was measured by following formula:

$$\text{Titratable acidity (\%)} = \frac{0.1\text{N used} \times 0.0064}{\text{PAJ (mL) taken for titration}}$$

Where,

PAJ= Pomegranate aril juice

3.2.1.7.8 Ascorbic acid (mg/100g)

The ascorbic acid was measured by the using the procedure given by A.O.A.C. (1990).

Reagents

Metaphosphoric acid solution (3%)

Metaphosphoric acid (HPO₃) : 15 g

Glacial acetic acid : 40 ml

Volume : 500 ml

2, 6-Dichlorophenol Indophenol dye : 50 mg

Sodium bicarbonate	: 42 mg
Volume	: 200 ml

Preparation of standard ascorbic acid solution

A solution was prepared by dissolving 5 mg of ascorbic acid in a 3% metaphosphoric acid solution, bringing the total volume up to 50 ml with additional metaphosphoric acid. This solution was then utilized to calibrate the dye, with the endpoint being identified by the emergence of a pink color.

For the extraction of ascorbic acid from fruit, 5 g of the sample was blended with 3% metaphosphoric acid, adjusting the final volume to 50 ml. A small amount of activated charcoal was added to remove any red coloration. This mixture was then filtered through coarse filter paper. From this filtrate, a 2 ml sample was taken and placed in a conical flask for titration against the 2,6-Dichlorophenol Indophenol dye. The completion of the titration was indicated by a persistent pink color lasting for one minute. The content of ascorbic acid was determined using a specific formula, and the results were reported as mg of ascorbic acid per 100 g of the fruit sample.

$$\text{Ascorbic acid (mg/100g fresh weight)} = \frac{T \times D \times V}{AE \times WS} \times 100$$

T= Titre value

D= Dye factor

V= Volume made up

AE= Aliquot of extract taken for estimation

WS= Weight of sample taken for estimation

Estimation of variability parameters

Different variability parameters estimated were as under:

3.7.2 Phenotypic coefficient of variation

Phenotypic coefficient of variation (PCV) was expressed as

$$PCV = \frac{\sqrt{\sigma^2 P}}{\bar{x}} \times 100$$

3.7.3 Genotypic coefficient of variation

Genotypic coefficient of variation (GCV) was expressed as

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$$

3.7.4 Components of variance

The variance for each trait comprised variance arising from the cultivars difference (genotypic variance) and that arising from the environmental factor (in this case years). The expected mean sum of squares would be as under:

$$MS \text{ for cultivars} = \sigma^2_e + y \sigma^2_g = MS_2$$

$$MS \text{ for error} = \sigma^2_e = MS_3$$

$$\text{Genotypic variance, } \sigma^2_g = \frac{MS_2 - MS_3}{y}$$

$$\text{Phenotypic variance} = \sigma^2_P = \sigma^2_g + \sigma^2_e$$

3.7.5 Heritability (broad sense)

It was measured as the ratio of genotypic variance to phenotypic variance.

$$h^2(bs) = \sigma^2_g / \sigma^2_P$$

3.7.6 Genetic gain

Genetic gain (per cent of mean) was expressed as

$$\text{Genetic gain} = \frac{h^2(b.s) \times sp \times K}{\bar{X}}$$

Where,

$h^2(b.s)$ = Heritability in broad sense,

sp = Phenotypic standard deviation

K = Selection intensity (the value of k at 5 % was taken as 2.06) and

$\bar{X}$ = Population mean of the trait.

Statistical analysis

The experimental data of all the metric characters studied were subjected to the statistical analysis. Standard method of analysis of variance was used for analysing the data. The statistical analysis was carried out for each observed

characters by using MS Excel, INDOSTAT statistical package (version 7.00, Hyderabad, India), Statistical Tool for Agricultural Research (STAR, version 2.0.1), Origin Pro 2022 (version 9.9.0.225) and R packages.

3.2.2 Experiment No 2: Molecular characterization of wild pomegranate genotypes of Jammu region.

3.2.1 Plant materials

In the present investigation, seventy genotypes of wild pomegranate genotypes selected from three districts i.e. Rajouri, Poonch and Udhampur were tagged and leaf samples were taken from each genotype and subjected to further analysis at School of Biotechnology, SKUAST- Jammu.

3.2.2 Methods

3.2.2.1 DNA isolation

Young leaves were collected from seventy selected genotypes for DNA extraction.

3.2.2.2 Reagents

The solutions required for DNA isolation and purification were prepared as follows:

(A) Preparation of Stock solutions (500 ml) for DNA extraction

a) 0.5 M EDTA	:	93.05 g
b) 1M Tris-Cl	:	60.55 g
c) 5 M NaCl	:	146.1 g

The pH of 0.5 M EDTA and 1 M Tris-Cl was adjusted to 8.0. The solution was autoclaved after making the final volume upto 500 ml.

d) CTAB Buffer

CTAB (2 %)	:	10 g
0.5M EDTA	:	20 ml
1 M Tris-Cl	:	50 ml
5 M NaCl	:	140 ml

ddH₂O : 880 ml
 β -mercaptoethanol : 0.2 %

The final volume of CTAB buffer was made up to 1000 ml using ddH₂O. It was then autoclaved after adjusting the pH to 8.0. β -mercaptoethanol (0.2 %) was added immediately before use.

e) TE Buffer (10 X)

1 M Tris-Cl : 100 ml
 0.5 M EDTA : 20 ml

880 ml ddH₂O was added to make 1L TE Buffer. pH was adjusted to 8.0 and autoclaved.

f) RNase (10mg/ml) – 10ml

5 M NaCl : 30 μ l
 1 M Tris-Cl : 100 μ l
 ddH₂O : 987 μ l
 RNase : 10mg

The solution was boiled for 15 minutes, allowed to cool at room temperature and stored at 4°C.

3.2.2.3 DNA extraction and preparation

Genomic DNA isolation was performed using the method described by Doyle and Doyle (1990), with minor modifications. Leaf tissue was ground into a fine powder with liquid nitrogen in a sterilized mortar and pestle. Then, 2.5 grams of the powdered leaf tissue was mixed with 5 ml of pre-warmed 2X CTAB extraction buffer (65°C) in sterilized 50 ml polypropylene DNA extraction tubes. The mixture was thoroughly combined by inverting the tubes several times and incubated in a water bath at 65°C for one hour. Three-quarters of the tubes were immersed in water, and the contents were mixed occasionally by gentle inversion every 5-10 minutes. After incubation, the samples were cooled to room temperature for 5 minutes, then 5 ml of chloroform: isoamyl alcohol (24:1) solution was added. The samples were mixed thoroughly by gentle inversion. DNA was precipitated by adding an equal volume of

ice-cold isopropanol and spooled out using sterile glass hooks. The precipitated DNA was treated with 1 ml of 70% ethanol and allowed to stand for 10 minutes. The tubes were then centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded, and the DNA pellet was dried at room temperature. After drying the pellet to remove the alcohol, 50 micro litre of TE buffer was added to dissolve the DNA pellet.

3.2.3 DNA purification

As DNA extracted contained very high amount of RNA and polysaccharides, purification of DNA was necessary. DNA sample was treated with RNase (10 mg/ml) and incubated in water bath at 37°C for 1 hour to remove RNA contamination from DNA samples. DNA of the genotypes were purified by adding equal volume of the solution of phenol: chloroform (1:1). Contents were then centrifuged at 15000 rpm for 5 minutes at room temperature (25°C). Supernatant was transferred to fresh sterilized centrifuge tube and extracted with chloroform: isoamyl alcohol (24:1) and again centrifuged at 10000 rpm for 5 min at 25°C. Again supernatant was transferred to fresh sterilized centrifuge tube and equal amount of chloroform: isoamyl alcohol (24:1) was added and again centrifuged at 10000rpm for 5 minutes at 25°C. Again supernatant was transferred to fresh sterilized centrifuge tube. DNA from the aqueous phase was precipitated by adding one-tenth volume of 3M sodium acetate (pH 5.2) and two volumes of ice cold ethanol. DNA was precipitated by centrifugation at 10000 rpm for 5 minutes at 25°C. Supernatant was carefully removed and pellet was washed with 1ml of 70 per cent ethanol for twenty minutes. Again centrifuged at 10000 rpm for 5 minutes at 25°C. Supernatant was discarded and sample tubes were kept open at 37°C until the last trace of ethanol had evaporated. The DNA pellet was dissolved in appropriate volume of 1X TE buffer and stored at -20°C.

3.2.3.1 DNA Quantification

Quality and quantity of genomic DNA was estimated by using Agarose gel electrophoresis and Nanodrop method. Quantity of DNA was checked by spectrophotometer by measuring absorbance from 260 nm to 280 nm to detect other possible contaminants.

3.2.3.2 Agarose Gel Electrophoresis:

Before proceeding for gel electrophoresis, stock solutions to be used for

electrophoresis were prepared as follows:

a) Preparation of Buffers

1. TBE Buffer (10 X) for 1L

Tris Base : 108 g

Boric acid : 55 g

0.5 M EDTA (pH 8) : 40 ml

Double distilled water was used to make the final volume upto 1L, mixed with the help of magnetic stirrer and autoclaved.

2. Tank Buffer (1X TBE)

100 ml 10X TBE was dissolved in 900 ml double distilled water to make 1L of 1 X TBE.

3. DNA Loading Dye (6X)

Glycerol : 30 % (v/v)

Bromophenol Blue : 0.25 % (w/v)

Xylene cyanol : 0.25 % (w/v)

DNA was quantified by loading 2 μ l of DNA mixed with 6 μ l of loading dye (ready to use) into separate wells on 1 % agarose gel. It was prepared by melting 1 g agarose in 100ml 1X TBE (Tris, Borate, EDTA) buffer in a microwave for 2 minutes. It was allowed to cool for a couple of minutes and then stained with ethidium bromide and stirred for some time. It was then poured in the casting tray with combs in it and allowed to solidify for 20-25 minutes at room temperature. DNA concentration standards were also loaded to estimate the concentration of DNA in each sample. The electrophoresis was carried out at 75V for 2 hours. It was then viewed under gel documentation system. The concentration of DNA was determined by comparing the intensity of bands with that of standards.

3.2.4 SSR assay

3.2.4.1 Primers used for DNA amplification

For the present study, a set of 25 arbitrary highly polymorphic random primers were selected from the published literature previously described and used for pomegranate germplasm characterization. The list of SSR primers used for identification and evaluation of genetic diversity of seventy wild pomegranate genotypes is given in Table 3.2.

Table 3.2: List of selected SSR primes along with their primer sequence

Primer name	Forward sequence 5' to 3'	Reverse Sequence 3' to 5'	Annealing temperature (°C)
HvSSRT_45	ATCACAGGTTTCGGAATACC	TCATATAATGCATGGGCTCC	55
HvSSRT_104	CTCTTGGATGAGCTGTAGG	AACTTCTCCTATGCACACC	55
HvSSR_174	ACACAAATGACTCCACACC	CGGATCAGAAGAAAACAAGG	55
HvSSR_269	AGGGTAAACTCAACCACC	GGAGAGATCGAACTTTCCC	55
HvSSRT_317	AAATATCTGAAGTTCGGCG	ACATTGTAGGGAATCGTGG	55
HvSSRT_360	TTGACAAAACGGACAAACC	ATCCATTCCATTCTCCCC	55
HvSSRT_483	TGGATGGTTTTAACGACCG	GGATCTACCGAGGAAGAGG	55
HvSSRT_490	TTTTCCCAATATGCTTGC	AACAAAGGAGATATCCGGC	55
HvSSRT_587	TGAAATGAAGGGTCCTTGC	CTTCCTCTGTGAATTCCCC	55
HvSSRT_639	TAGGTCATCCCCATGAACC	AAAGGGTTGCTTACACTCC	55
HvSSRT_69	TGGGAATCATACTGTTGGC	TCCAAACCAATACCACACC	55
HvSSRT_725	TAATTTTCGTCGTTGAACGC	TGAAGAGAGAGAGAGAGGG	58
HvSSRT_799	CATATGTGTCACGGGAAGG	AGTGACGTAGAAAAACCTCC	55
HvSSRT_823	TCATTTAGCTAAGGCGACC	TGGCTGATTGCAATCTTCC	55
HvSSRT_969	TGCTCCATATAAACTTCACG	TATTTGATGCATAAGCGCC	58
HvSSRT_900	AGACATAGCGTTCAATCGG	GTTGTCTTCCCTTGTAGAGG	55
POM_AAC13	TCTCCCGACAACAAATCAC	CCCGACACAACACATACTTCAG	53
Pom006	TACTAGGTGGAACCGAACTT	CCTTGACAACCTCATCTCAT	55
Pom021	GACTGGAAGAAGCAGAGACT	GAAAAGGAAGTAGCAGAGCA	56
PGCT110	GAGCCATTGTAGAGACAAGA	GACTGCTGACAACTTTCTTT	53
PGCT093A	GTAGCCACTTTAGGGCGAGA	CGTCTAAAAGCGACAGCAAG	58
PGCT066b	CGAGGAGTGGTCCAGGTTAG	AACAGACGACAAGGGGAATG	58
PGCT086b	TGGTGATTCGTGTTGTTTTC	CAACAACCTCCTCTGCTCTC	55
PGCT088	TCTCTCTCTACCCCGACACC	TAGCGTCAAGATTGTGAAAAGG	55
PGCT111	TATCTGTGCGCAGGAAGGATG	GAAGCCAATTCTCAAAGATG	58

3.2.4.2 PCR amplification

3.2.4.3 Components used for PCR Reactions

For performing Polymerase Chain Reaction following reagents were used:

(a) Primers

(b) dNTPs: 100mM stock of each dNTP was diluted to 2.5 mM of Dntp

(i.e., 25µl of each dNTP + 900µl of Milli Q water).

(c) Taq Polymerase

(d) PCR Buffer 10 X with MgCl₂

PCR (polymerase chain reaction) was carried out in a 10 µl reaction volume containing 1.25 µl Mg Cl₂, 1µl dNTPs, 2 µl PCR buffer, 0.5 µl forward primer, 0.5 µl reverse primer, 2 µl DNA template, 0.15 µl DNA Taq polymerase and 2.6 µl sterile water. The PCR reaction was run in thermal cycler machine (Bio Rad) for DNA amplification. The products were then analysed using gel electrophoresis.

3.2.4.4 PCR Amplification Program

PCR tubes containing master mix and DNA template were thoroughly mixed and subjected to the thermal profile given in Table 3.3. The amplification reaction was carried out in gradient mastercycler. An initial denaturation step of 5 minutes was programmed in the thermocycler, followed by a loop of 35 cycles each consisting of denaturation (at 94°C for 1 minute), annealing (at 53-60°C for 1 minute and 40 seconds), elongation (at 72°C for 40 seconds) and the final extension was performed (at 72 °C for 7 minutes).

Table 3.3: Thermal profiles used for DNA amplification

Step	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 minutes	1
Denaturation	94	1minutes	} 35
Annealing	53-58	1 minutes 40 sec.	
Extension	72	90 seconds	
Final Extension	72	7 minutes	1

3.2.4.5 Resolution of PCR products

Amplified DNA fragments were resolved in horizontal electrophoresis using 3 % agarose gels and visualized by staining with ethidium bromide (10 mg/ml). Gel casting plate was washed with distilled water and dried. Plate was wiped with ethanol, air-dried and ends were sealed with tape. 3 % agarose gel solution was prepared by heating it in oven. The solution was cooled to about 50°C by keeping it at room temperature for 5-10 minutes. Agarose gel was then poured into the gel casting plate inserted with an appropriate comb with required well number and size to get a 0.5 cm thick gel. Gel was allowed to solidify for 30 minutes. After that sealing tapes were removed from both the ends. Gel plate was then placed in the electrophoresis chamber and submerged using 1 X TBE (Tris Borate EDTA) buffer. The comb was removed gently. DNA samples were prepared by adding loading dye solution (1µl/mL). The samples were loaded in the wells using micropipette. Cover was placed on electrophoretic unit and proper connections were made using power supply. Electrophoresis was carried out at constant voltage (3 V/cm of gel) until the dye had moved to 3/4 length in the gel. PCR amplification products were viewed by fluorescence under UV light (high UV wavelength 350 nm).

3.2.4.6 Scoring of bands

SSR-PCR bands were detected in the gel using gel documentation system. Bands were scored as size of amplified PCR product. All the scorable bands were considered as single locus per allele. Their size was estimated using 100bp standard marker. The presence or absence of bands in all genotypes for 27 primers were used to generate bi-nomial data using excel sheet. Bands were marked as present only if the DNA amplification produced the fragment of a particular sequence and absent if the DNA amplification lacked that fragment. The banding pattern of all the genotypes against each primer was compared. Bands present in one genotypes and absent in another genotypes were regarded as variables and used to score polymorphism.

3.2.5 Data analysis

In order to check the informativeness and discriminatory power of SSR markers utilized in the present study, certain parameters alleles per locus and polymorphic information content were calculated.

(a) Alleles per locus

The variation in the number of alleles across multiple loci within a population can be expressed as the average number of alleles per locus:

$$\text{Number of alleles per locus} = \sum \frac{\text{Number of alleles}}{\text{Number of loci}}$$

(b) Polymorphic information content (PIC)

The markers with more alleles have more polymorphism information content. Average PIC indicates the ability of utilized markers to differentiate genotypes. It was calculated according to following formula.

$$\text{PIC Value} = 2f_i(1-f_i)$$

Where, f_i = frequency of bands present and

$1-f_i$ = frequency of bands absent

Bi-nomial data matrix of all the genotypes generated from SSR primers were subjected to the UPGMA (Unweighted pair group method with arithmetic averages) analysis and a dendrogram was constructed using DARwin version 6.0 software. Genotypes were divided in various clusters and sub-clusters based on genetic diversity among them and linkage distance was calculated.

RESULTS

The present investigation entitled “**Morphological, biochemical and molecular characterization of wild pomegranate (*Punica granatum* L.) genotypes of Jammu region**” has been carried out from 2020 to 2022. Survey work for a selection of superior wild pomegranate genotypes was undertaken at various locations in the Rajouri, Poonch and Udhampur districts of Jammu province of Union territory of Jammu and Kashmir. The study comprised of extensive survey, selection and characterization of wild pomegranate genotypes in three district of Jammu province. A population of 70 genotypes was selected and subjected to morphological, biochemical and molecular characterization. The observations on various parameters were recorded and statistically analyzed. The present investigations were carried out in three separate experiments during the years. The results obtained during the course of investigations are presented in this chapter.

4.1 Morphological and biochemical characterization of wild pomegranate genotypes.

4.1.1 Tree characteristics

4.1.2 Leaf characteristics

4.1.3 Flower characteristics

4.1.4 Fruit characteristics

4.1 Morphological and biochemical characterization of wild pomegranate genotypes

The morphological characters were observed as per UPOV descriptor 2011.

4.1.1 Tree characteristics

4.1.1.1 Tree height(m)

The data presented in (Table 4.1) showed tree height ranged from 2.05 to 5.55m. Maximum tree height (5.55m) was recorded in JWPU (Sel-61) and minimum tree height (2.05m) was recorded in JWPR (Sel-27).

4.1.1.2 Tree growth habit

Two types of tree growth habit was observed spreading and upright. The data presented (Table 4.1) depicted that out of seventy genotypes spreading growth habit was observed in fifty three (75.71%) genotypes and upright growth habit was observed in seventeen (24.28%) genotypes.

4.1.1.3 Trunk type

Trunk type was categorized as multi-trunk and single-trunk as presented in (Table 4.1) and frequency percentage in (Table 4.3). Out of seventy genotypes multi-trunk was observed in sixty five genotypes (92.85%) and single trunk was observed in five (5.71%) genotypes.

Table: 4.1: Tree characteristics/Variation in Tree height, tree growth habit, trunk type, tree vigor and thorn present of wild pomegranate genotypes of Jammu region

S. No.	Genotypes	Tree Height (m)	Tree Growth Habit	Trunk type
1	JWPR (Sel-1)	5.02	Spreading	Single trunk
2	JWPR (Sel-2)	3.12	Spreading	Multitrunk
3	JWPR (Sel-3)	2.21	Spreading	Multitrunk
4	JWPR (Sel-4)	4.65	Spreading	Multitrunk
5	JWPR (Sel-5)	4.51	Spreading	Single trunk
6	JWPR (Sel-6)	5.15	Spreading	Multitrunk
7	JWPR (Sel-7)	4.23	Upright	Multitrunk
8	JWPR (Sel-8)	3.21	Upright	Single trunk
9	JWPR (Sel-9)	5.05	Upright	Multitrunk
10	JWPR (Sel-10)	4.65	Spreading	Multitrunk
11	JWPR (Sel-11)	4.15	Spreading	Multitrunk
12	JWPR (Sel-12)	4.24	Spreading	Multitrunk
13	JWPR (Sel-13)	5.16	Spreading	Multitrunk

14	JWPR (Sel-14)	5.34	Upright	Multitrunk
15	JWPR (Sel-15)	4.55	Spreading	Single trunk
16	JWPR (Sel-16)	3.36	Upright	Multitrunk
17	JWPR (Sel-17)	2.52	Upright	Single trunk
18	JWPR (Sel-18)	3.25	Spreading	Multitrunk
19	JWPR (Sel-19)	5.42	Upright	Multitrunk
20	JWPR (Sel-20)	5.22	Spreading	Multitrunk
21	JWPR (Sel-21)	4.05	Spreading	Multitrunk
22	JWPR (Sel-22)	4.06	Spreading	Multitrunk
23	JWPR (Sel-23)	4.04	Spreading	Multitrunk
24	JWPR (Sel-24)	5.04	Spreading	Multitrunk
25	JWPR (Sel-25)	3.12	Spreading	Multitrunk
26	JWPR (Sel-26)	3.22	Spreading	Multitrunk
27	JWPR (Sel-27)	2.05	Spreading	Multitrunk
28	JWPR (Sel-28)	4.23	Spreading	Multitrunk
29	JWPR (Sel-29)	5.02	Spreading	Multitrunk
30	JWPR (Sel-30)	3.08	Spreading	Multitrunk
31	JWPP (Sel-31)	3.12	Spreading	Multitrunk
32	JWPP (Sel-32)	5.04	Upright	Multitrunk
33	JWPP (Sel-33)	5.12	Upright	Multitrunk
34	JWPP (Sel-34)	5.08	Upright	Multitrunk
35	JWPP (Sel-35)	5.24	Spreading	Multitrunk
36	JWPP (Sel-35)	4.12	Spreading	Multitrunk
37	JWPP (Sel-36)	4.14	Spreading	Multitrunk
38	JWPP (Sel-38)	3.16	Spreading	Multitrunk
39	JWPP (Sel-39)	3.12	Upright	Multitrunk
40	JWPP (Sel-40)	3.15	Spreading	Multitrunk
41	JWPP (Sel-41)	4.23	Upright	Multitrunk
42	JWPP (Sel-42)	5.45	Upright	Multi-trunk

43	JWPP (Sel-43)	3.14	Spreading	Multitrunk
44	JWPP (Sel-44)	4.16	Upright	Multitrunk
45	JWPP (Sel-45)	4.14	Spreading	Multitrunk
46	JWPP (Sel-46)	5.42	Spreading	Multitrunk
47	JWPP (Sel-47)	5.39	Spreading	Multitrunk
48	JWPP (Sel-48)	5.24	Spreading	Multitrunk
49	JWPP (Sel-49)	3.12	Spreading	Multitrunk
50	JWPP (Sel-50)	5.12	Spreading	Multitrunk
51	JWPU (Sel-51)	4.12	Spreading	Multitrunk
52	JWPU (Sel-52)	5.22	Spreading	Multitrunk
53	JWPU (Sel-53)	3.24	Spreading	Multitrunk
54	JWPU (Sel-54)	5.11	Upright	Single trunk
55	JWPU (Sel-55)	3.46	Spreading	Multitrunk
56	JWPU (Sel-56)	3.95	Spreading	Multitrunk
57	JWPU (Sel-57)	4.46	Spreading	Multitrunk
58	JWPU (Sel-58)	4.58	Spreading	Multitrunk
59	JWPU (Sel-59)	5.14	Spreading	Multitrunk
60	JWPU (Sel-60)	4.50	Spreading	Multitrunk
61	JWPU (Sel-61)	5.55	Spreading	Multitrunk
62	JWPU (Sel-62)	4.23	Spreading	Multitrunk
63	JWPU (Sel-63)	4.24	Spreading	Multitrunk
64	JWPU (Sel-64)	4.18	Spreading	Multitrunk
65	JWPU (Sel-65)	5.45	Spreading	Multitrunk
66	JWPU (Sel-66)	5.42	Spreading	Multitrunk
67	JWPU (Sel-67)	4.34	Spreading	Multitrunk
68	JWPU (Sel-68)	3.67	Spreading	Multitrunk
69	JWPU (Sel-69)	3.12	Spreading	Multitrunk
70	JWPU (Sel-70)	4.56	Spreading	Multitrunk



JWPR (Sel-1)



JWPR (Sel-2)



JWPR (Sel-3)



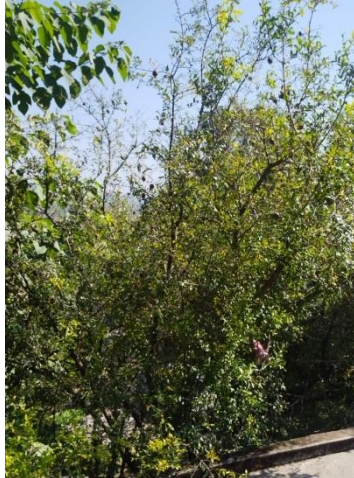
JWPR (Sel-4)



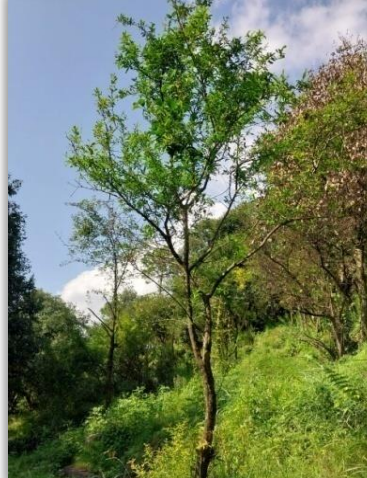
JWPR (Sel-5)



JWPR (Sel-6)



JWPR (Sel-7)



JWPR (Sel-8)



JWPR (Sel-9)



JWPR (Sel-10)

Plate 1: Variations in tree characters of wild pomegranate genotype collected during the study

Cont...



JWPR (Sel-11)



JWPR (Sel-12)



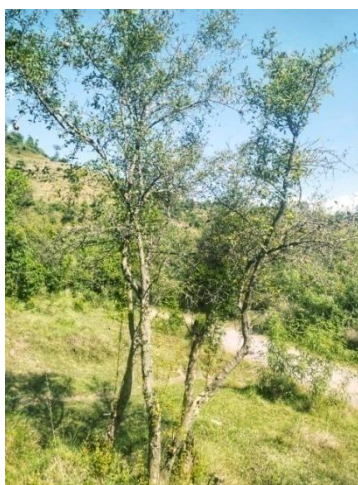
JWPR (Sel-13)



JWPR (Sel-14)



JWPR (Sel-15)



JWPR (Sel-16)



JWPR (Sel-17)



JWPR (Sel-18)



JWPR (Sel-19)



JWPR (Sel-20)

Cont...



JWPR (Sel-21)



JWPR (Sel-22)



JWPR (Sel-23)



JWPR (Sel-24)



JWPR (Sel-25)



JWPR (Sel-26)



JWPR (Sel-27)



JWPR (Sel-28)



JWPR (Sel-29)



JWPR (Sel-30)

Cont...



JWPP (Sel-31)



JWPP (Sel-32)



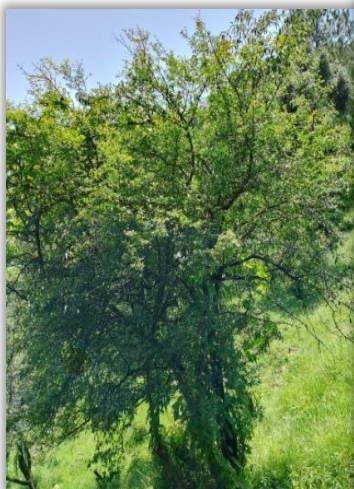
JWPP (Sel-33)



JWPP (Sel-34)



JWPP (Sel-35)



JWPP (Sel-36)



JWPP (Sel-37)



JWPP (Sel-38)



JWPP (Sel-39)



JWPP (Sel-40)

Cont...



JWPP (Sel-41)



JWP P(Sel-42)



JWPP (Sel-43)



JWPP (Sel-44)



JWPP (Sel-45)



JWPP (Sel-46)



JWPP (Sel-47)



JWPP (Sel-48)



JWPP (Sel-49)



JWPP (Sel-50)

Cont...



JWPU (Sel-51)



JWPU (Sel-52)



JWPU (Sel-53)



JWPU (Sel-54)



JWPU (Sel-55)



JWPU (Sel-56)



JWPU (Sel-57)



JWPU (Sel-58)



JWPU (Sel-59)



JWPU (Sel-60)

Cont...



JWPU (Sel-61)



JWPU (Sel-62)



JWPU (Sel-63)



JWPU (Sel-64)



JWPU (Sel-55)



JWPU (Sel-66)



JWPU (Sel-67)



JWPU (Sel-68)



JWPU (Sel-69)



JWPU (Sel-70)

4.1.2 Leaf characteristics

4.1.2.1 Leaf blade shape

Leaf blade shape of wild pomegranate was categorized as broad elliptic, elliptic lanceolate and lanceolate as depicted in Table 4.2 and is clear from the data presented in Table 4.3 that out of seventy wild pomegranate genotypes broad elliptic shape was observed in twenty six (37.14%) genotypes, elliptic lanceolate (34.28%) was observed in twenty four genotypes and twenty (28.57%) genotypes had lanceolate leaf shape.

4.1.2.2 Leaf blade length (cm)

The data presented in Table 4.2 showed that leaf blade length of seventy wild pomegranate genotypes. Significant variation was recorded in leaf blade length of wild pomegranate genotypes. Maximum leaf blade length of 6.44 cm was recorded in JWPP (Sel-40) and minimum leaf blade length of 3.45 cm was recorded in JWPR (Sel-2).

4.1.2.3 Leaf blade width (cm)

The perusal of data presented in Table 4.2 showed that there was a significant variations in leaf blade width of selected wild pomegranate genotypes. The leaf blade width ranged between 1.02 cm and 3.32 cm. Maximum leaf blade width of 3.32 cm was recorded in JWPP (Sel-48) and minimum leaf blade width of 1.02cm was recorded in JWPR (Sel-9).

4.1.2.4 Petiole length (mm)

The data presented in Table 4.2 showed a significant variation in petiole length of selected wild pomegranate genotypes. Maximum petiole length of 7.19 mm was recorded in JWPU (Sel-62) and minimum petiole length of 2.06 mm was recorded in JWPR (Sel-2).

Table: 4.2: Variation in leaf blade shape, leaf blade length, leaf blade width and petiole length of wild pomegranate genotypes of Jammu region

S. No	Genotypes	Leaf blade shape	Leaf blade length (cm)	Leaf blade width (cm)	Petiole length (mm)
1	JWPR (Sel-1)	Broad elliptic	4.08	2.57	6.37
2	JWPR (Sel-2)	Broad elliptic	3.45	1.94	2.06
3	JWPR (Sel-3)	Lanceolate	4.08	2.30	5.31
4	JWPR (Sel-4)	Lanceolate	4.52	2.29	2.44
5	JWPR (Sel-5)	Elliptic lanceolate	4.12	1.15	6.39
6	JWPR (Sel-6)	Broad elliptic	4.44	2.62	3.37
7	JWPR (Sel-7)	Broad elliptic	4.03	1.31	2.41
8	JWPR (Sel-8)	Lanceolate	5.17	1.15	3.43
9	JWPR (Sel-9)	Elliptic lanceolate	4.60	1.16	4.56
10	JWPR (Sel-10)	Elliptic lanceolate	5.12	2.78	3.56
11	JWPR (Sel-11)	Broad elliptic	4.24	1.24	3.26
12	JWPR (Sel-12)	Lanceolate	4.14	1.14	5.23
13	JWPR (Sel-13)	Broad elliptic	4.21	1.17	5.08
14	JWPR (Sel-14)	Lanceolate	3.95	1.24	3.26
15	JWPR (Sel-15)	Lanceolate	6.12	1.12	2.29
16	JWPR (Sel-16)	Elliptic lanceolate	5.27	1.23	4.24
17	JWPR (Sel-17)	Elliptic lanceolate	5.13	2.13	5.35
18	JWPR (Sel-18)	Elliptic lanceolate	3.53	1.15	5.19
19	JWPR (Sel-19)	Broad elliptic	3.57	2.09	6.35
20	JWPR (Sel-20)	Broad elliptic	5.15	2.17	4.14
21	JWPR (Sel-21)	Elliptic lanceolate	5.56	1.16	2.83
22	JWPR (Sel-22)	Broad elliptic	4.45	2.11	5.29
23	JWPR (Sel-23)	Lanceolate	4.14	1.33	6.26
24	JWPR (Sel-24)	Lanceolate	3.95	1.38	5.36
25	JWPR (Sel-25)	Elliptic lanceolate	4.11	1.69	4.42
26	JWPR (Sel-26)	Broad elliptic	4.53	1.67	5.22
27	JWPR (Sel-27)	Lanceolate	6.09	2.65	7.19
28	JWPR (Sel-28)	Lanceolate	4.63	2.47	6.14

29	JWPR (Sel-29)	Broad elliptic	4.71	1.68	5.20
30	JWPR (Sel-30)	Lanceolate	3.76	1.48	6.11
31	JWPP (Sel-31)	Broad elliptic	3.82	2.57	3.40
32	JWPP (Sel-32)	Elliptic lanceolate	5.34	2.11	3.19
33	JWPP (Sel-33)	Lanceolate	6.16	1.80	5.29
34	JWPP (Sel-34)	Broad elliptic	5.31	2.10	5.22
35	JWPP (Sel-35)	Lanceolate	5.22	1.06	6.33
36	JWPP (Sel-35)	Lanceolate	4.15	1.11	4.78
37	JWPP (Sel-36)	Elliptic lanceolate	4.64	2.12	3.30
38	JWPP (Sel-38)	Elliptic lanceolate	4.26	2.07	4.33
39	JWPP (Sel-39)	Elliptic lanceolate	5.96	1.14	4.24
40	JWPP (Sel-40)	Lanceolate	6.44	1.10	2.22
41	JWPP (Sel-41)	Elliptic lanceolate	6.13	1.07	5.41
42	JWPP (Sel-42)	Lanceolate	3.55	1.16	3.50
43	JWPP (Sel-43)	Lanceolate	3.74	1.29	5.30
44	JWPP (Sel-44)	Broad elliptic	3.84	2.23	6.16
45	JWPP (Sel-45)	Lanceolate	4.12	1.10	6.20
46	JWPP (Sel-46)	Broad elliptic	4.25	1.21	6.21
47	JWPP (Sel-47)	Lanceolate	4.15	2.27	5.42
48	JWPP (Sel-48)	Broad elliptic	4.08	3.33	5.28
49	JWPP (Sel-49)	Broad elliptic	5.13	3.16	6.16
50	JWPP (Sel-50)	Lanceolate	5.33	2.23	6.20
51	JWPU (Sel-51)	Lanceolate	6.10	2.23	5.34
52	JWPU (Sel-52)	Lanceolate	5.19	1.39	6.31
53	JWPU (Sel-53)	Elliptic lanceolate	5.18	2.23	5.26
54	JWPU (Sel-54)	Broad elliptic	4.47	2.35	5.18
55	JWPU (Sel-55)	Lanceolate	4.20	2.28	6.72
56	JWPU (Sel-56)	Lanceolate	4.32	1.70	6.24
57	JWPU (Sel-57)	Lanceolate	5.25	1.40	5.41
58	JWPU (Sel-58)	Broad elliptic	4.11	2.42	2.96
59	JWPU (Sel-59)	Broad elliptic	5.33	1.21	6.71
60	JWPU (Sel-60)	Lanceolate	4.15	1.29	5.20

61	JWPU (Sel-61)	Lanceolate	4.27	1.98	4.31
62	JWPU (Sel-62)	Elliptic lanceolate	4.98	2.24	7.19
63	JWPU (Sel-63)	Broad elliptic	4.19	1.15	5.66
64	JWPU (Sel-64)	Broad elliptic	4.46	1.20	3.22
65	JWPU (Sel-65)	Broad elliptic	6.12	2.19	4.54
66	JWPU (Sel-66)	Lanceolate	4.15	1.09	5.32
67	JWPU (Sel-67)	Lanceolate	5.03	1.49	4.19
68	JWPU (Sel-68)	Elliptic lanceolate	4.07	1.56	4.75
69	JWPU (Sel-69)	Broad elliptic	5.21	2.15	4.25
70	JWPU (Sel-70)	Lanceolate	4.54	1.57	4.27
	C.D. at 5% SE(mean)		0.10 0.03	0.14 0.07	0.11 0.04

Table: 4.3: Frequency percentages of tree growth habit, trunk type and leaf shape of wild pomegranate genotypes of Jammu region.

Tree Characteristics	Category	Number of genotypes	Percentage
Tree growth Habit	Spreading	53	75.71
	Upright	17	24.28
	Weeping	0	0
Trunk Type	Multitrunk	65	92.85
	Singletrunk	5	7.14
Leaf shape	Elliptic lanceolate	24	34.28
	Broad elliptic	26	37.14
	Lanceolate	20	28.57

4.1.3 Flowering characteristics

4.1.3.1 Start of flowering

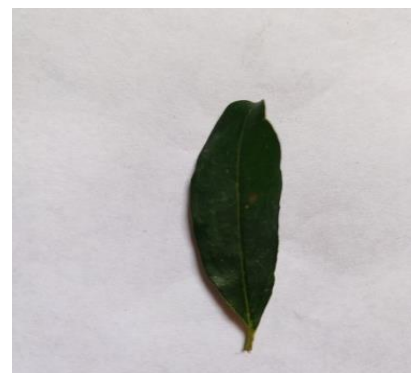
The data presented in Table 4.4 showed that different wild pomegranate genotypes start flowering from 3rd week of April to 4th week of April. Out of seventy wild pomegranate genotypes twenty seven genotypes start flowering in 3rd week of April and forty three genotypes start flowering from 4th week of April.



JWPR (Sel-1)



JWPR (Sel-2)



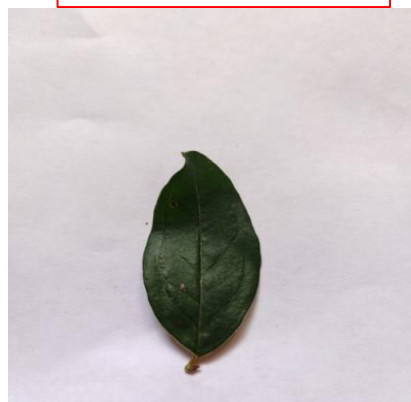
JWPR (Sel-3)



JWPR (Sel-4)



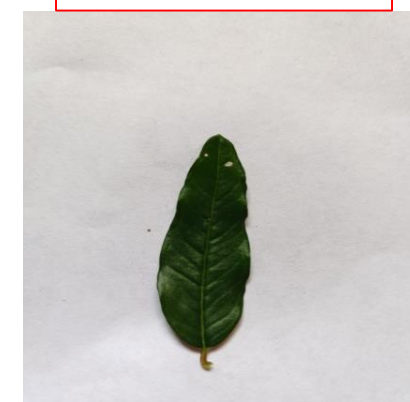
JWPR (Sel-5)



JWPR (Sel-6)



JWPR (Sel-7)



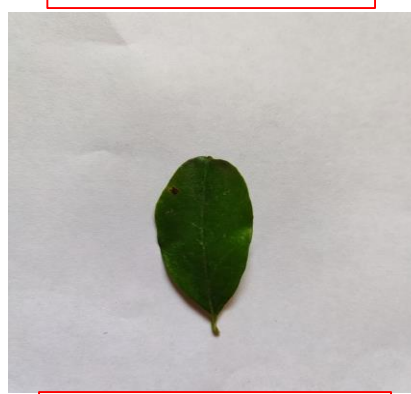
JWPR (Sel-8)



JWPR (Sel-9)



JWPR (Sel-10)



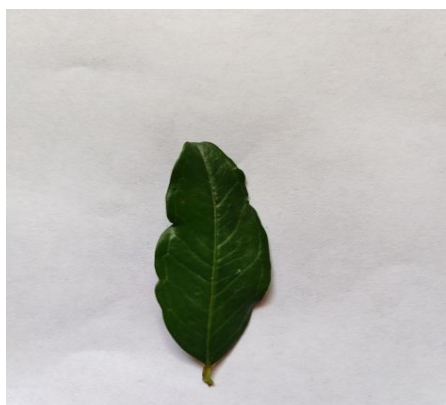
JWPR (Sel-11)



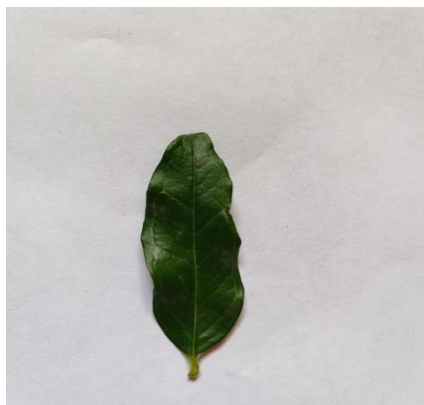
JWPR (Sel-12)

Plate 2: Variations in leaf characters of various wild pomegranate genotypes

Cont...



JWPR (Sel-13)



JWPR (Sel-14)



JWPR (Sel-15)



JWPR (Sel-16)



JWPR (Sel-17)



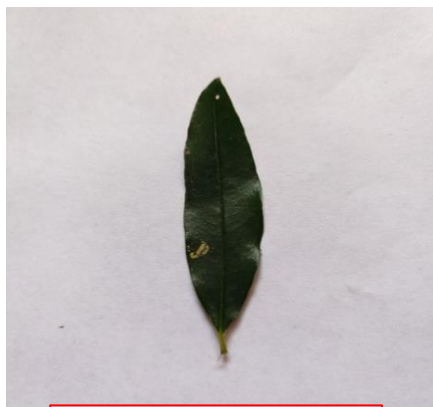
JWPR (Sel-18)



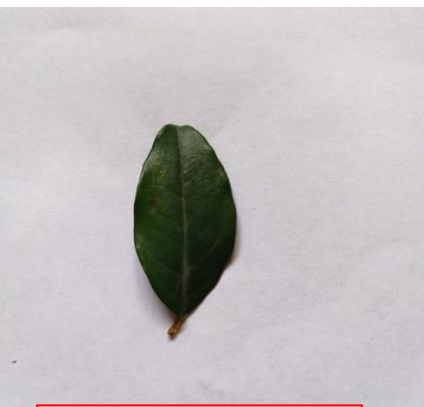
JWPR (Sel-19)



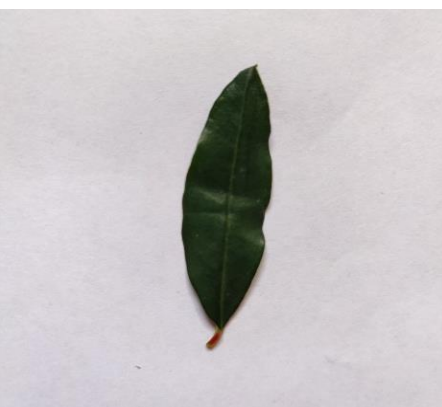
JWPR (Sel-20)



JWPR (Sel-21)



JWPR (Sel-22)



JWPR (Sel-23)



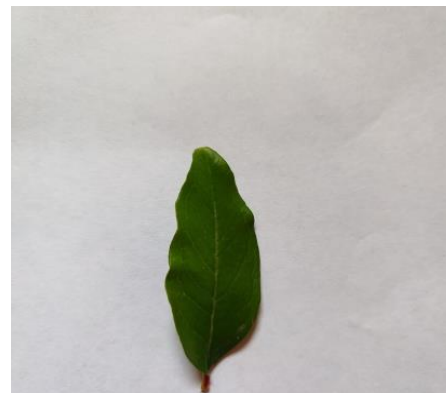
JWPR (Sel-24)



JWPR (Sel-25)



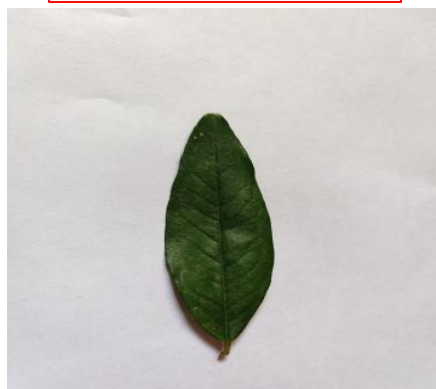
JWPR (Sel-26)



JWPR (Sel-27)



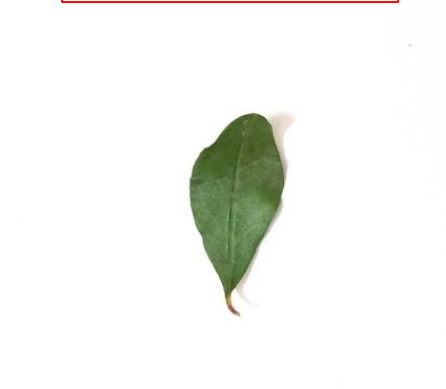
JWPR (Sel-28)



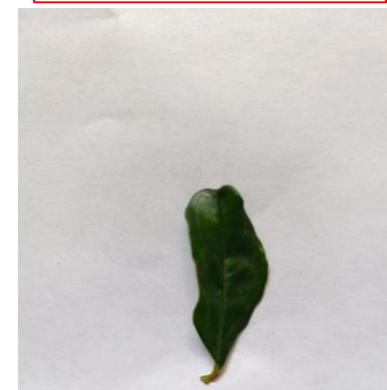
JWPR (Sel-29)



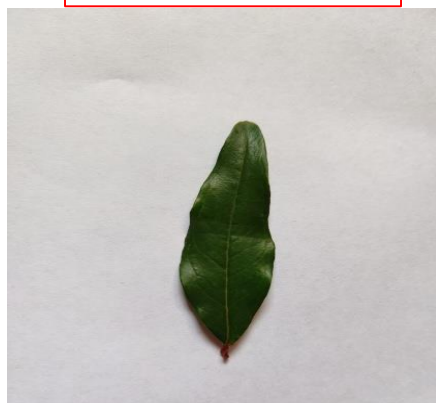
JWPR (Sel-30)



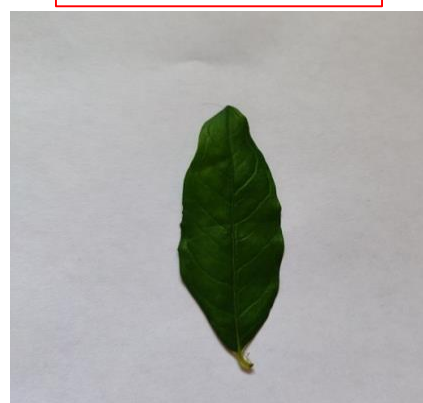
JWPR (Sel-31)



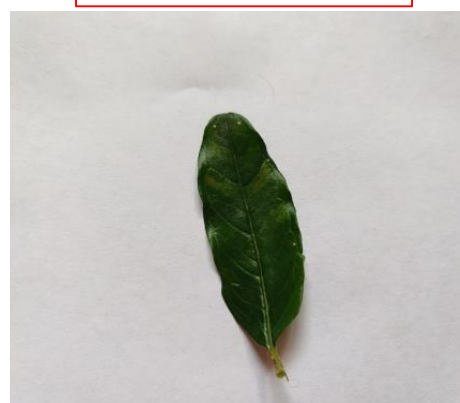
JWPP (Sel-32)



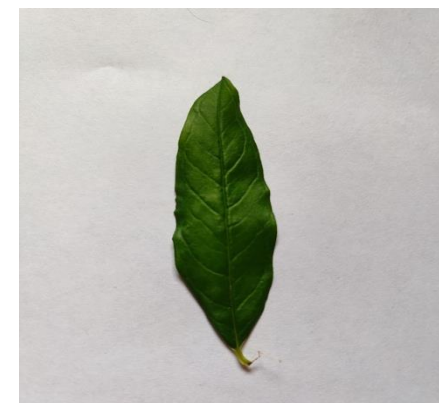
JWPP (Sel-33)



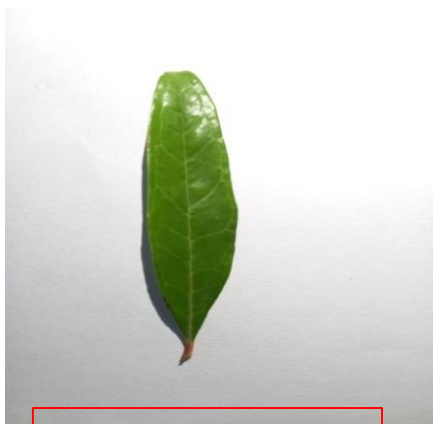
JWPP (Sel-34)



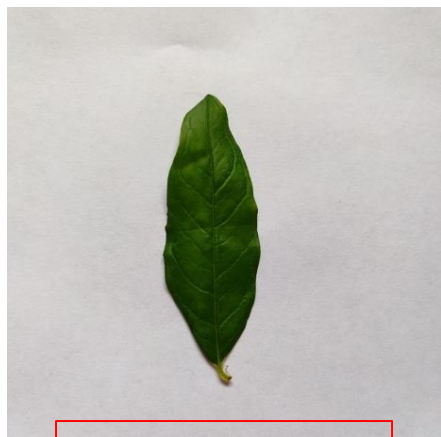
JWPP (Sel-35)



JWPP (Sel-36)



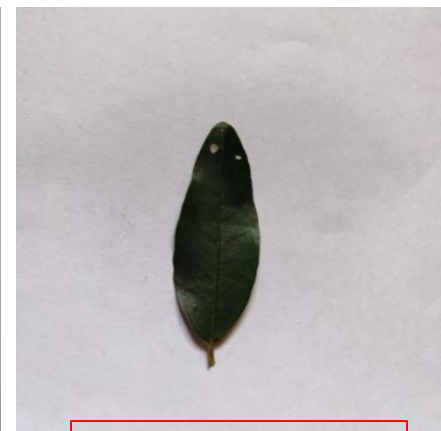
JWPP (Sel-37)



JWPP (Sel-38)



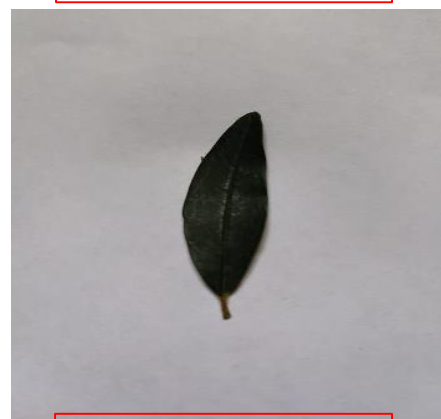
JWPP (Sel-39)



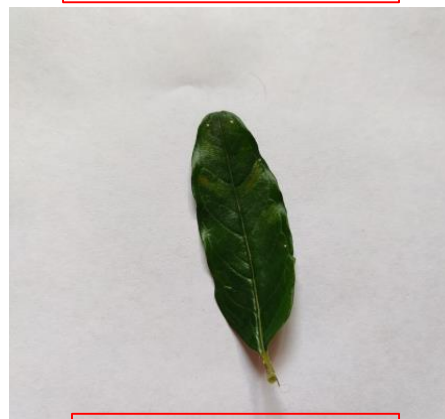
JWPP (Sel-40)



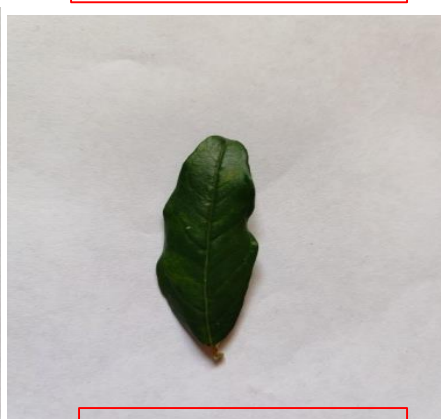
JWPP (Sel-41)



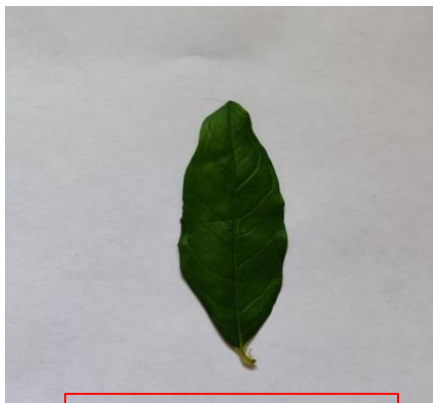
JWPP (Sel-42)



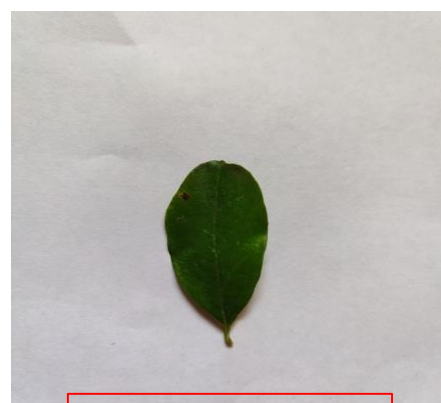
JWPP (Sel-43)



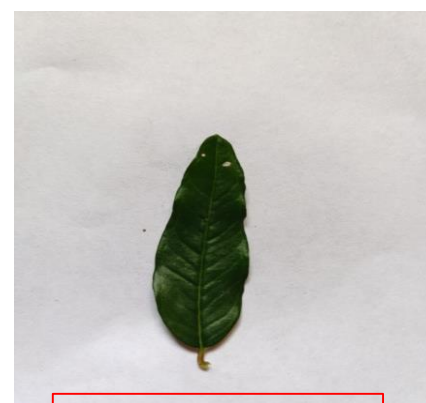
JWPP (Sel-44)



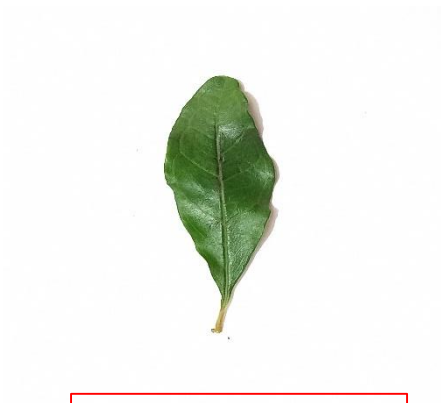
JWPP (Sel-45)



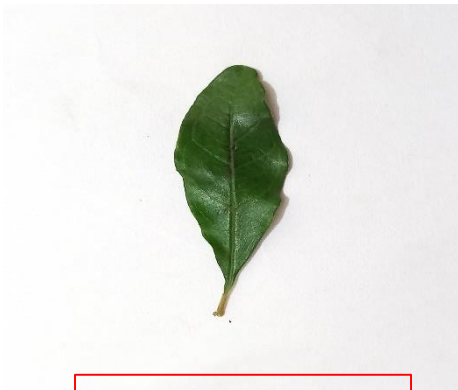
JWPP (Sel-46)



JWPP (Sel-47)



JWPP (Sel-48)



JWPP (Sel-49)



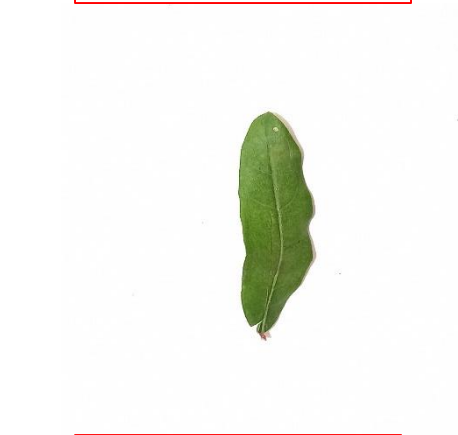
JWPP (Sel-50)



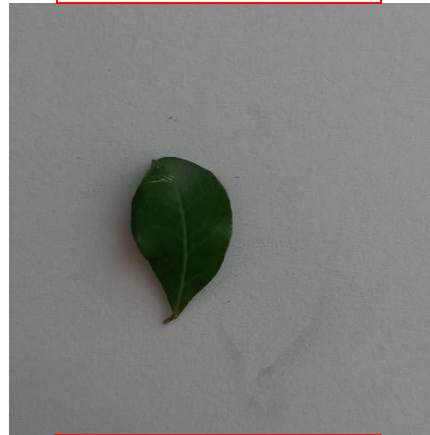
JWPU (Sel-51)



JWPU (Sel-52)



JWPU (Sel-53)



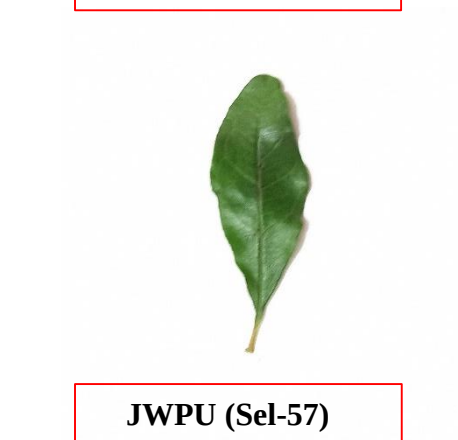
JWPU (Sel-54)



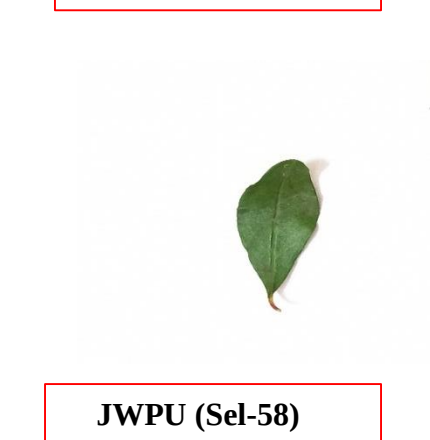
JWPU (Sel-55)



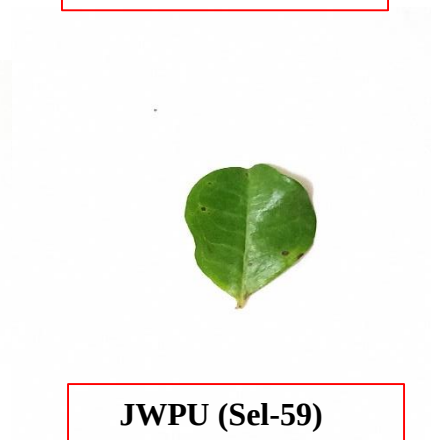
JWPU (Sel-56)



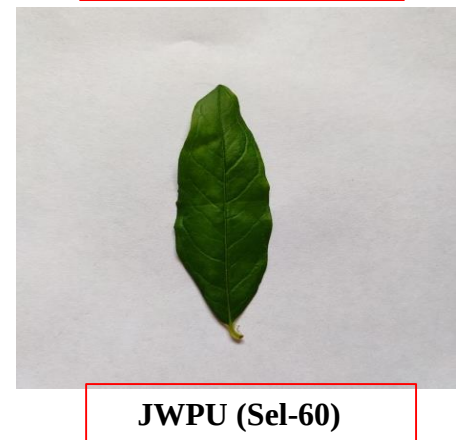
JWPU (Sel-57)



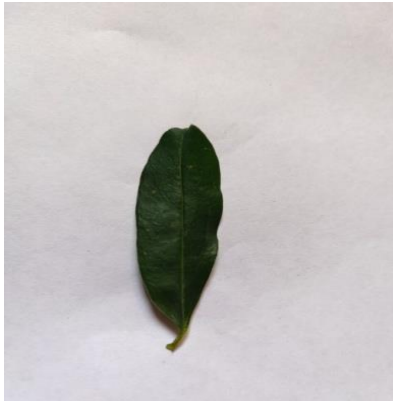
JWPU (Sel-58)



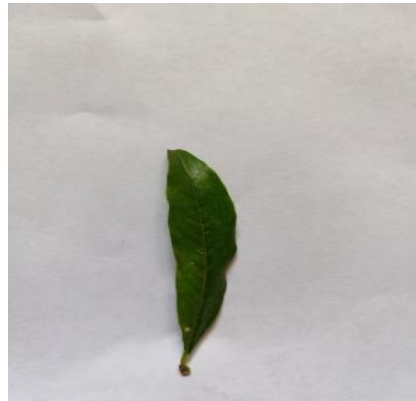
JWPU (Sel-59)



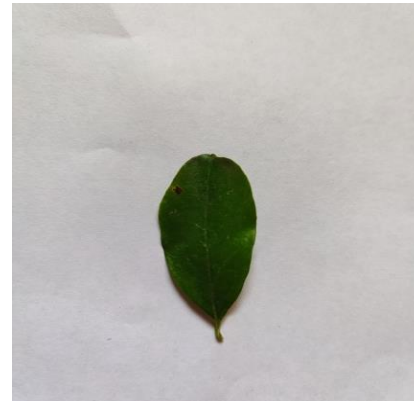
JWPU (Sel-60)



JWPU (Sel-61)



JWPU (Sel-62)



JWPU (Sel-63)



JWPU (Sel-64)



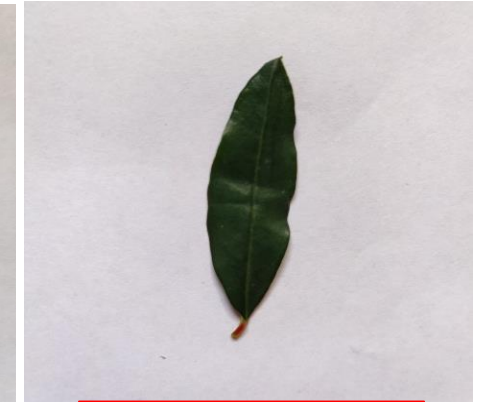
JWPU (Sel-65)



JWPU (Sel-66)



JWPU (Sel-67)



JWPU (Sel-68)



JWPU (Sel-69)



JWPU (Sel-70)

4.1.3.2 End of flowering

The data depicted in Table 4.4 showed that the end of flowering in different wild pomegranate genotypes. All seventy genotypes had shown end of flowering in 2nd week of June.

4.1.3.3 Duration of flowering

The data pertaining to duration of flowering in different genotypes are presented in Table 4.4. The data showed that duration of flowering in different wild pomegranate genotypes ranged from 47 to 54 days.

4.1.3.4 Calyx color

Perusal of the data in Table 4.4 revealed that the calyx colour in the wild pomegranate genotypes was either orange or red. Out of seventy wild pomegranate genotypes forty five (64.28%) genotypes had orange coloured calyx and twenty five (35.71%) genotypes had red coloured calyx.

4.1.3.5 Petal length (mm)

The data presented in Table 4.5 showed that petal length of seventy wild pomegranate genotypes. Significant variation was observed in petal length of different wild pomegranate genotypes studied. Maximum petal length of 25.64 mm was recorded in JWPP (Sel-36) followed by 25.60 mm in JWPR (Sel-5). Minimum petal length of 14.24 mm was recorded in JWPR (Sel-26).

4.1.3.6 Petal width (mm)

There was a significant variation in petal width of different wild pomegranate genotypes studied (Table 4.5). Maximum petal length of 22.78 mm was recorded in JWPP (Sel-36) followed by 22.53 mm in JWPR (Sel-5) and minimum of 12.34 mm was recorded in JWPR (Sel-26).

4.1.3.7 Calyx length (mm)

The data presented in Table 4.5 showed that variation in calyx length of different wild pomegranate genotypes. Maximum calyx length of 36.44 mm was recorded in JWPP (Sel-36) followed by JWPR (Sel-5) and minimum calyx length of 22.17 mm was recorded in JWPR (Sel-4).

4.1.3.8 Calyx width (mm)

The data presented in Table 4.5 showed that calyx width of wild different pomegranate genotypes. Maximum calyx width of 15.92mm was recorded in JWPR (Sel-5) followed by 15.87 mm in JWPP (Sel-36) and minimum calyx width of 10.12 mm was recorded in JWR (Sel-2).

Table 4.4: Variation in start of flowering, end of flowering, duration of flowering and calyx color of wild pomegranate genotypes of Jammu region

S. No	Genotypes	Start of flowering	End of flowering	Duration of flowering	Calyx color
1	JWPR (Sel-1)	4 th week of April	2 nd week of June	47	Orange
2	JWPR (Sel-2)	4 th week of April	2 nd week of June	47	Orange
3	JWPR (Sel-3)	4 th week of April	2 nd week of June	54	Orange
4	JWPR (Sel-4)	3 rd week of April	2 nd week of June	54	Orange
5	JWPR (Sel-5)	3 rd week of April	2 nd week of June	54	Orange
6	JWPR (Sel-6)	3 rd week of April	2 nd week of June	54	Orange
7	JWPR (Sel-7)	3 rd week of April	2 nd week of June	54	Orange
8	JWPR (Sel-8)	3 rd week of April	2 nd week of June	54	Orange
9	JWPR (Sel-9)	4 th week of April	3 rd week of June	54	Orange
10	JWPR (Sel-10)	4 th week of April	3 rd week of June	54	Orange
11	JWPR (Sel-11)	4 th week of April	2 nd week of June	47	Orange
12	JWPR (Sel-12)	4 th week of April	2 nd week of June	47	Orange
13	JWPR (Sel-13)	4 th week of April	2 nd week of June	47	Orange
14	JWPR (Sel-14)	4 th week of April	2 nd week of June	47	Orange
15	JWPR (Sel-15)	4 th week of April	2 nd week of June	47	Orange
16	JWPR (Sel-16)	4 th week of April	2 nd week of June	47	Orange
17	JWPR (Sel-17)	4 th week of April	2 nd week of June	47	Orange
18	JWPR (Sel-18)	3 rd week of April	2 nd week of June	54	Orange
19	JWPR (Sel-19)	3 rd week of April	2 nd week of June	54	Orange

20	JWPR (Sel-20)	3 rd week of April	2 nd week of June	54	Orange
21	JWPR (Sel-21)	3 rd week of April	2 nd week of June	54	Orange
22	JWPR (Sel-22)	4 th week of April	2 nd week of June	47	Orange
23	JWPR (Sel-23)	4 th week of April	2 nd week of June	47	Orange
24	JWPR (Sel-24)	4 th week of April	2 nd week of June	47	Orange
25	JWPR (Sel-25)	4 th week of April	2 nd week of June	47	Orange
26	JWPR (Sel-26)	3 rd week of April	2 nd week of June	54	Orange
27	JWPR (Sel-27)	3 rd week of April	2 nd week of June	54	Orange
28	JWPR (Sel-28)	3 rd week of April	2 nd week of June	54	Orange
29	JWPR (Sel-29)	4 th week of April	2 nd week of June	47	Orange
30	JWPR (Sel-30)	4 th week of April	2 nd week of June	47	Orange
31	JWPP (Sel-31)	4 th week of April	3 rd week of June	54	Orange
32	JWPP (Sel-32)	4 th week of April	2 nd week of June	47	Orange
33	JWPP (Sel-33)	4 th week of April	3 rd week of June	54	Orange
34	JWPP (Sel-34)	3 rd week of April	2 nd week of June	54	Orange
35	JWPP (Sel-35)	3 rd week of April	2 nd week of June	54	Orange
36	JWPP (Sel-36)	4 th week of April	2 nd week of June	47	Orange
37	JWPP (Sel-37)	4 th week of April	2 nd week of June	47	Orange
38	JWPP (Sel-38)	4 th week of April	2 nd week of June	47	Orange
39	JWPP (Sel-39)	4 th week of April	2 nd week of June	47	Orange
40	JWPP (Sel-40)	3 rd week of April	2 nd week of June	54	Orange
41	JWPP (Sel-41)	3 rd week of April	2 nd week of June	54	Orange
42	JWPP (Sel-42)	4 th week of April	2 nd week of June	47	Orange
43	JWPP (Sel-43)	4 th week of April	3 rd week of June	54	Orange
44	JWPP (Sel-44)	4 th week of April	3 rd week of June	54	Orange
45	JWPP (Sel-45)	3 rd week of April	2 nd week of June	54	Orange
46	JWPP (Sel-46)	4 th week of April	2 nd week of June	47	Red

47	JWPP (Sel-47)	4 th week of April	2 nd week of June	47	Red
48	JWPP (Sel-48)	4 th week of April	2 nd week of June	47	Red
49	JWPP (Sel-49)	4 th week of April	2 nd week of June	47	Red
50	JWPP (Sel-50)	3 rd week of April	2 nd week of June	54	Red
51	JWPU (Sel-51)	3 rd week of April	2 nd week of June	54	Red
52	JWPU (Sel-52)	4 th week of April	2 nd week of June	47	Red
53	JWPU (Sel-53)	4 th week of April	2 nd week of June	47	Red
54	JWPU (Sel-54)	4 th week of April	3 rd week of June	54	Red
55	JWPU (Sel-55)	4 th week of April	3 rd week of June	54	Red
56	JWPU (Sel-56)	4 th week of April	2 nd week of June	47	Red
57	JWPU (Sel-57)	4 th week of April	2 nd week of June	47	Red
58	JWPU (Sel-58)	4 th week of April	2 nd week of June	47	Red
59	JWPU (Sel-59)	3 rd week of April	2 nd week of June	54	Red
60	JWPU (Sel-60)	3 rd week of April	2 nd week of June	54	Red
61	JWPU (Sel-61)	4 th week of April	2 nd week of June	47	Red
62	JWPU (Sel-62)	3 rd week of April	2 nd week of June	54	Red
63	JWPU (Sel-63)	3 rd week of April	2 nd week of June	54	Red
64	JWPU (Sel-64)	3 rd week of April	2 nd week of June	54	Red
65	JWPU (Sel-65)	3 rd week of April	2 nd week of June	54	Red
66	JWPU (Sel-66)	3 rd week of April	2 nd week of June	54	Red
67	JWPU (Sel-67)	3 rd week of April	2 nd week of June	54	Red
68	JWPU (Sel-68)	4 th week of April	2 nd week of June	47	Red
69	JWPU (Sel-69)	4 th week of April	2 nd week of June	47	Red
70	JWPU (Sel-70)	3 rd week of April	2 nd week of June	54	Red

Table: 4.5: Variation in petal length, petal width, calyx length and calyx width of wild pomegranate genotypes of Jammu region

S. No	Genotypes	Petal length (mm)	Petal width (mm)	Calyx length (mm)	Calyx width (mm)
1	JWPR (Sel-1)	17.28	15.48	29.15	10.22
2	JWPR (Sel-2)	16.12	16.34	27.82	10.12
3	JWPR (Sel-3)	17.58	18.12	31.75	13.21
4	JWPR (Sel-4)	21.23	19.12	22.17	13.86
5	JWPR (Sel-5)	25.60	22.53	35.54	15.92
6	JWPR (Sel-6)	18.32	15.24	28.14	10.55
7	JWPR (Sel-7)	15.68	14.21	27.15	13.28
8	JWPR (Sel-8)	20.12	18.71	27.77	13.55
9	JWPR (Sel-9)	20.45	18.81	31.18	14.14
10	JWPR (Sel-10)	17.73	14.56	32.35	14.78
11	JWPR (Sel-11)	21.45	19.34	33.35	13.12
12	JWPR (Sel-12)	21.86	19.56	34.18	13.98
13	JWPR (Sel-13)	18.56	16.43	27.33	11.44
14	JWPR (Sel-14)	22.45	21.12	29.21	11.13
15	JWPR (Sel-15)	14.56	13.76	20.15	12.87
16	JWPR (Sel-16)	14.85	13.95	22.45	12.95
17	JWPR (Sel-17)	17.93	16.42	32.67	10.64
18	JWPR (Sel-18)	21.64	21.24	32.48	14.87
19	JWPR (Sel-19)	22.84	21.56	31.57	14.94
20	JWPR (Sel-20)	18.56	16.82	34.66	15.14
21	JWPR (Sel-21)	16.12	14.67	30.46	15.67
22	JWPR (Sel-22)	18.17	16.21	28.14	12.67
23	JWPR (Sel-23)	21.53	20.87	29.27	11.88
24	JWPR (Sel-24)	21.59	21.04	30.17	14.34
25	JWPR (Sel-25)	14.98	12.51	32.46	12.58
26	JWPR (Sel-26)	14.24	12.34	23.57	10.68
27	JWPR (Sel-27)	20.16	19.45	34.87	12.16
28	JWPR (Sel-28)	23.24	22.53	29.53	13.09
29	JWPR (Sel-29)	21.54	20.45	29.65	12.08
30	JWPR (Sel-30)	17.43	15.17	20.37	14.35

31	JWPP (Sel-31)	18.57	16.31	33.16	15.13
32	JWPP (Sel-32)	19.24	18.25	31.17	12.28
33	JWPP (Sel-33)	20.18	19.43	33.56	15.42
34	JWPP (Sel-34)	20.63	19.56	29.29	10.77
35	JWPP (Sel-35)	18.93	16.58	32.76	11.67
36	JWPP (Sel-36)	25.64	22.78	36.44	15.87
37	JWPP (Sel-37)	25.36	19.87	26.12	13.88
38	JWPP (Sel-38)	18.65	17.28	30.54	11.15
39	JWPP (Sel-39)	22.14	20.34	30.87	14.74
40	JWPP (Sel-40)	24.56	22.71	30.13	13.95
41	JWPP (Sel-41)	18.98	16.24	30.76	14.79
42	JWPP (Sel-42)	19.23	17.24	34.18	12.77
43	JWPP (Sel-43)	19.56	16.87	34.86	13.07
44	JWPP (Sel-44)	19.12	16.87	30.97	12.33
45	JWPP (Sel-45)	18.57	15.43	35.37	10.14
46	JWPP (Sel-46)	18.19	15.42	31.67	10.97
47	JWPP (Sel-47)	19.89	16.21	31.15	15.22
48	JWPP (Sel-48)	20.57	17.24	29.44	15.07
49	JWPP (Sel-49)	20.34	18.64	30.27	12.66
50	JWPP (Sel-50)	23.43	20.95	29.73	13.22
51	JWPU (Sel-51)	18.23	14.56	30.26	13.03
52	JWPU (Sel-52)	18.56	14.78	33.11	11.06
53	JWPU (Sel-53)	20.25	19.17	30.14	11.86
54	JWPU (Sel-54)	20.12	19.21	21.26	11.56
55	JWPU (Sel-55)	19.25	16.78	29.35	11.62
56	JWPU (Sel-56)	20.61	18.38	29.58	13.77
57	JWPU (Sel-57)	21.14	19.06	31.65	12.55
58	JWPU (Sel-58)	21.53	19.16	30.74	13.44
59	JWPU (Sel-59)	20.63	17.86	23.56	15.07
60	JWPU (Sel-60)	19.83	17.43	21.76	13.03
61	JWPU (Sel-61)	22.56	20.34	23.36	12.17
62	JWPU (Sel-62)	22.12	20.16	29.73	12.98

63	JWPU (Sel-63)	19.93	15.23	33.43	15.82
64	JWPU (Sel-64)	19.12	15.65	34.97	15.18
65	JWPU (Sel-65)	22.83	19.20	26.34	13.95
66	JWPU (Sel-66)	19.69	17.56	33.13	10.76
67	JWPU (Sel-67)	20.34	18.13	31.25	10.92
68	JWPU (Sel-68)	22.53	17.45	22.46	15.81
69	JWPU (Sel-69)	23.45	20.24	30.45	14.84
70	JWPU (Sel-70)	20.19	18.23	29.87	14.37
	C.D at 5% SE (mean)	0.53 0.15	0.61 0.12	0.56 0.20	0.14 0.06

4.1.4 Fruit characteristics

4.1.4.1 Fruit shape

The data given in Table 4.6 showed that on the basis of fruit shape three types of fruits viz., round, elliptical and oval were observed in different wild pomegranate genotypes studied. Out of seventy wild pomegranate genotypes thirty eight (54.28%) genotypes were round shaped, eleven genotypes (15.71%) were elliptical shaped and twenty one (30%) were oval shaped.

4.1.4.2 Fruit color

Data presented in Table 4.6 revealed that on the basis of fruit color different wild pomegranate genotypes can be categorized into green, reddish green, greenish yellow and reddish yellow. Out of seventy wild pomegranate genotypes three genotypes (4.28%) had green coloured fruits color, thirteen genotypes (18.57%) had reddish green coloured fruits, twenty five (35.71%) genotypes had greenish yellow coloured fruits and thirty two genotypes (45.71%) had reddish yellow coloured fruits.

4.1.4.3.1 Crown length (mm)

The data presented in Table 4.6 showed that there was a significant variation in crown length of seventy wild pomegranate genotypes studied. Maximum crown length of 20.43 mm was recorded in JWPU (Sel-52) and minimum crown length of 9.24 mm was recorded in JWPU (Sel-59).

4.1.4.4 Rind thickness (mm)

A significant variation in rind thickness of different wild pomegranate genotypes was observed (Table 4.6). Rind thickness of wild pomegranate genotypes ranged from 1.69 to 4.97 mm. Maximum rind thickness of 4.97 mm was recorded in genotype JWPR (Sel-6) and was significantly higher as compared to other genotypes. The minimum rind thickness of 1.69 mm was recorded in JWPR (Sel-18).

4.1.4.5 Fruit weight (g)

Perusal of data from Table 4.7 revealed that there was a significant variation in fruit weight of seventy wild pomegranate genotype studied and it ranged from 28.36 to 111.51g. Maximum fruit weight of 111.51g was recorded in JWPP (Sel-36) closely followed by 110.08 g in JWPR (Sel-5) and were at par with each other. Minimum fruit weight of 28.35g was recorded in JWPR (Sel-9).

4.1.4.6 Fruit length (mm)

The data presented in Table 4.7 revealed that fruit length of different wild pomegranate genotypes varied significantly. It ranged from 30.17 to 57.54 mm. The maximum fruit length of 57.54 mm was recorded in JWPP (Sel-36) followed by 56.33 mm in JWPR (Sel-5) and were at par with each other. Minimum fruit length of 30.17 mm was recorded in JWPR (Sel-9).

4.1.4.7 Fruit width (mm)

The data presented in Table 4.7 showed that fruit width of different wild pomegranate genotypes studied, varied significantly ranged from 29.72 to 55.65 mm. The maximum fruit width of 55.65 mm was recorded in JWPP (Sel-36) closely followed by 55.30 mm in JWPR (Sel-5) and were at par with each other. The minimum fruit width of 29.72 mm was recorded in JWPR (Sel-9).

4.1.4.8 Juice percent

It is evident from the data presented in Table 4.7 that the juice content of fruits in different genotypes varied significantly. Maximum juice content of 46.77 per cent was recorded in JWPP (Sel-36) closely followed by 46.34 per cent in JWPR (Sel-5). The minimum juice per cent of 12.15 per cent was recorded in JWPR (Sel-18).

Table 4.6: Variation in fruit shape, fruit color, crown length and rind thickness of wild pomegranate genotypes of Jammu region

S. No	Genotypes	Fruit shape	Fruit color	Crown length (mm)	Rind thickness (mm)
1	JWPR (Sel-1)	Round	Reddish yellow	14.31	2.36
2	JWPR (Sel-2)	Oval	Reddish yellow	19.78	3.67
3	JWPR (Sel-3)	Round	Reddish green	10.66	3.65
4	JWPR (Sel-4)	Oval	Reddish green	10.23	2.88
5	JWPR (Sel-5)	Round	Reddish yellow	13.23	3.91
6	JWPR (Sel-6)	Round	Greenish yellow	10.09	4.97
7	JWPR (Sel-7)	Elliptical	Greenish yellow	10.16	3.25
8	JWPR (Sel-8)	Oval	Reddish yellow	20.02	3.87
9	JWPR (Sel-9)	Oval	Reddish yellow	20.12	3.78
10	JWPR (Sel-10)	Oval	Reddish yellow	17.46	3.36
11	JWPR (Sel-11)	Round	Reddish green	19.48	3.56
12	JWPR (Sel-12)	Round	Reddish green	13.26	3.46
13	JWPR (Sel-13)	Oval	Reddish green	17.47	3.78
14	JWPR (Sel-14)	Oval	Reddish yellow	15.27	3.61
15	JWPR (Sel-15)	Round	Greenish yellow	11.28	3.87
16	JWPR (Sel-16)	Oval	Green	16.26	2.94
17	JWPR (Sel-17)	Round	Greenish yellow	13.53	3.66
18	JWPR (Sel-18)	Elliptical	Reddish green	12.85	1.69
19	JWPR (Sel-19)	Round	Greenish yellow	10.98	3.66
20	JWPR (Sel-20)	Elliptical	Reddish yellow	10.06	3.96
21	JWPR (Sel-21)	Round	Greenish yellow	10.33	3.05
22	JWPR (Sel-22)	Round	Reddish yellow	14.05	2.83
23	JWPR (Sel-23)	Elliptical	Reddish green	10.18	3.03
24	JWPR (Sel-24)	Oval	Greenish yellow	12.86	2.69
25	JWPR (Sel-25)	Elliptical	Greenish yellow	16.56	2.73
26	JWPR (Sel-26)	Oval	Reddish green	10.24	3.15
27	JWPR (Sel-27)	Round	Reddish yellow	20.12	3.13
28	JWPR (Sel-28)	Round	Reddish yellow	18.22	2.74
29	JWPR (Sel-29)	Round	Reddish yellow	10.93	2.94
30	JWPR (Sel-30)	Round	Greenish yellow	10.07	2.93
31	JWPP (Sel-31)	Oval	Greenish yellow	12.18	2.87
32	JWPP (Sel-32)	Round	Reddish yellow	15.79	2.24
33	JWPP (Sel-33)	Round	Reddish yellow	18.37	2.35
34	JWPP (Sel-34)	Oval	Reddish yellow	14.76	2.17

35	JWPP (Sel-35)	Elliptical	Reddish yellow	15.05	2.28
36	JWPP (Sel-36)	Round	Reddish green	17.55	2.85
37	JWPP (Sel-37)	Oval	Greenish yellow	18.44	2.14
38	JWPP (Sel-38)	Round	Reddish yellow	15.45	2.73
39	JWPP (Sel-39)	Oval	Reddish green	14.36	2.78
40	JWPP (Sel-40)	Round	Greenish yellow	13.26	2.78
41	JWPP (Sel-41)	Round	Reddish yellow	14.27	2.17
42	JWPP (Sel-42)	Round	Greenish yellow	15.26	2.24
43	JWPP (Sel-43)	Oval	Green	20.01	2.27
44	JWPP (Sel-44)	Round	Reddish yellow	18.17	2.98
45	JWPP (Sel-45)	Round	Greenish yellow	15.27	2.98
46	JWPP (Sel-46)	Oval	Greenish yellow	12.76	2.19
47	JWPP (Sel-47)	Round	Greenish yellow	10.44	2.69
48	JWPP (Sel-48)	Elliptical	Greenish yellow	16.15	2.10
49	JWPP (Sel-49)	Round	Reddish yellow	13.14	2.67
50	JWPP (Sel-50)	Oval	Greenish yellow	18.83	2.61
51	JWPU (Sel-51)	Round	Greenish yellow	17.35	2.10
52	JWPU (Sel-52)	Round	Red	18.04	2.66
53	JWPU (Sel-53)	Round	Reddish yellow	20.43	3.87
54	JWPU (Sel-54)	Round	Reddish green	14.73	2.91
55	JWPU (Sel-55)	Oval	Reddish yellow	12.16	2.61
56	JWPU (Sel-56)	Round	Reddish yellow	14.97	3.39
57	JWPU (Sel-57)	Elliptical	Reddish green	12.94	3.83
58	JWPU (Sel-58)	Elliptical	Greenish yellow	13.17	2.21
59	JWPU (Sel-59)	Oval	Greenish yellow	9.24	3.20
60	JWPU (Sel-60)	Round	Reddish yellow	11.67	2.30
61	JWPU (Sel-61)	Round	Greenish yellow	10.77	3.71
62	JWPU (Sel-62)	Round	Greenish yellow	12.37	2.93
63	JWPU (Sel-63)	Elliptical	Greenish yellow	14.23	3.09
64	JWPU (Sel-64)	Round	Greenish yellow	14.96	2.59
65	JWPU (Sel-65)	Round	Reddish green	13.57	2.78
66	JWPU (Sel-66)	Round	Reddish yellow	12.68	2.44
67	JWPU (Sel-67)	Round	Reddish yellow	13.44	2.49
68	JWPU (Sel-68)	Round	Reddish yellow	11.24	3.38
69	JWPU (Sel-69)	Round	Greenish yellow	12.14	2.29
70	JWPU (Sel-70)	Elliptical	Reddish yellow	11.17	3.17
C.D. at 5% SE(mean)				0.29 0.10	0.74 0.26

Table 4.7: Variation in fruit weight, fruit length, fruit width and juice percent of wild pomegranate genotypes of Jammu region

S. No.	Genotypes	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Juice (%)
1	JWPR (Sel-1)	47.67	42.44	40.12	22.45
2	JWPR (Sel-2)	84.60	50.63	50.07	32.68
3	JWPR (Sel-3)	41.44	39.59	40.05	19.31
4	JWPR (Sel-4)	52.66	45.11	40.04	25.40
5	JWPR (Sel-5)	110.08	56.53	55.30	46.34
6	JWPR (Sel-6)	92.74	51.62	50.03	33.09
7	JWPR (Sel-7)	81.43	50.40	50.05	28.88
8	JWPR (Sel-8)	38.46	35.18	39.68	18.33
9	JWPR (Sel-9)	28.35	30.17	29.72	12.40
10	JWPR (Sel-10)	51.64	44.93	39.78	24.16
11	JWPR (Sel-11)	33.96	30.97	30.08	16.28
12	JWPR (Sel-12)	63.60	49.38	40.09	27.45
13	JWPR (Sel-13)	29.73	37.57	37.55	13.97
14	JWPR (Sel-14)	54.93	45.57	49.88	27.03
15	JWPR (Sel-15)	59.55	46.52	41.24	29.81
16	JWPR (Sel-16)	44.53	41.14	32.21	20.90
17	JWPR (Sel-17)	32.64	30.51	40.24	17.17
18	JWPR (Sel-18)	28.65	39.03	39.19	12.15
19	JWPR (Sel-19)	54.94	45.90	40.11	26.32
20	JWPR (Sel-20)	37.84	32.01	30.10	19.24
21	JWPR (Sel-21)	61.61	48.37	40.07	13.21
22	JWPR (Sel-22)	60.44	47.34	38.36	32.41
23	JWPR (Sel-23)	71.63	49.92	50.78	18.53
24	JWPR (Sel-24)	47.73	42.65	40.06	28.10
25	JWPR (Sel-25)	43.75	40.41	40.79	25.50
26	JWPR (Sel-26)	65.22	45.34	40.47	34.29
27	JWPR (Sel-27)	68.15	46.85	40.26	17.13
28	JWPR (Sel-28)	43.74	40.13	39.86	18.31
29	JWPR (Sel-29)	38.48	30.78	30.51	14.60

30	JWPR (Sel-30)	35.63	30.62	29.72	13.12
31	JWPP (Sel-31)	91.26	50.18	50.19	28.91
32	JWPP (Sel-32)	35.56	31.88	40.01	13.12
33	JWPP (Sel-33)	80.63	46.30	50.22	36.28
34	JWPP (Sel-34)	68.91	42.23	48.19	28.60
35	JWPP (Sel-35)	93.19	50.39	50.54	30.91
36	JWPP (Sel-36)	111.51	57.54	55.65	46.77
37	JWPP (Sel-37)	90.34	50.09	50.11	35.00
38	JWPP (Sel-38)	85.51	50.52	50.53	26.76
39	JWPP (Sel-39)	71.15	50.04	50.06	26.61
40	JWPP (Sel-40)	75.09	49.50	50.05	18.80
41	JWPP (Sel-41)	68.83	50.10	50.16	30.00
42	JWPP (Sel-42)	65.88	49.85	49.86	25.00
43	JWPP (Sel-43)	93.62	55.31	53.65	23.03
44	JWPP (Sel-44)	88.13	50.18	50.19	38.6
45	JWPP (Sel-45)	40.13	38.11	38.13	15.73
46	JWPP (Sel-46)	55.22	49.69	49.86	18.08
47	JWPP (Sel-47)	60.26	50.05	50.07	21.83
48	JWPP (Sel-48)	75.26	50.48	50.90	32.56
49	JWPP (Sel-49)	80.53	54.07	54.10	36.47
50	JWPP (Sel-50)	38.42	30.17	30.20	14.02
51	JWPU (Sel-51)	78.46	45.41	48.12	29.18
52	JWPU (Sel-52)	66.45	50.10	50.12	26.23
53	JWPU (Sel-53)	79.21	50.32	51.62	32.25
54	JWPU (Sel-54)	33.05	50.58	55.86	33.98
55	JWPU (Sel-55)	61.15	51.69	56.65	22.66
56	JWPU (Sel-56)	56.17	42.09	41.36	36.45
57	JWPU (Sel-57)	56.19	50.23	50.26	30.05
58	JWPU (Sel-58)	80.93	49.99	50.06	28.79
59	JWPU (Sel-59)	66.72	48.32	51.69	24.70
60	JWPU (Sel-60)	60.32	53.82	55.93	28.46
61	JWPU (Sel-61)	99.63	51.63	50.47	29.37



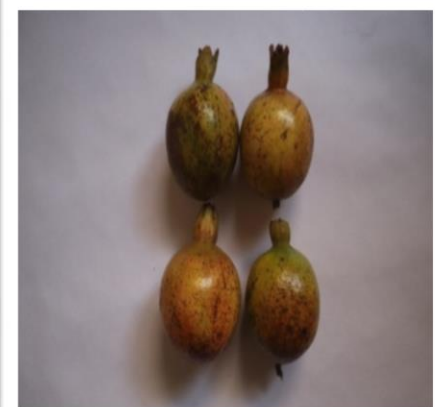
JWPR (Sel-1)



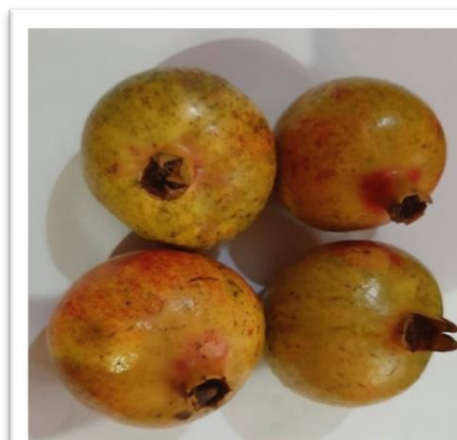
JWPR (Sel-2)



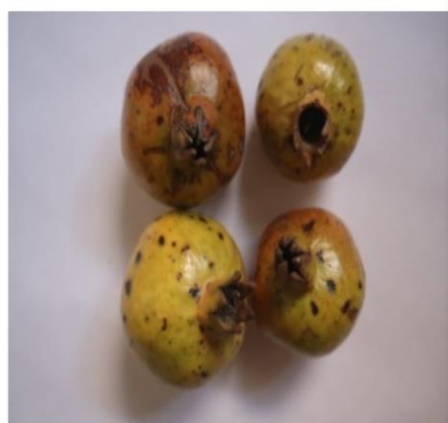
JWPR (Sel-3)



JWPR (Sel-4)



JWPR (Sel-5)



JWPR (Sel-6)

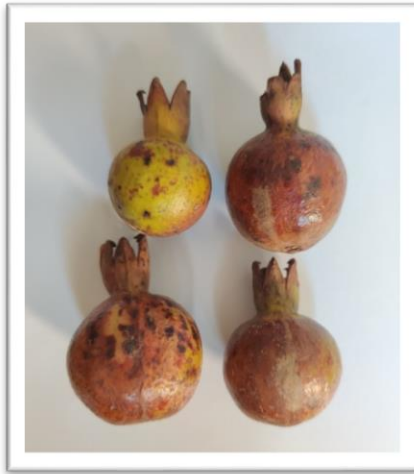


JWPR (Sel-7)



JWPR (Sel-8)

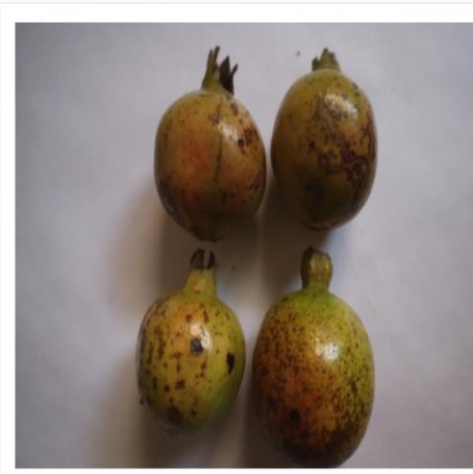
Plate 3: Variation in fruit characters of wild pomegranate genotypes



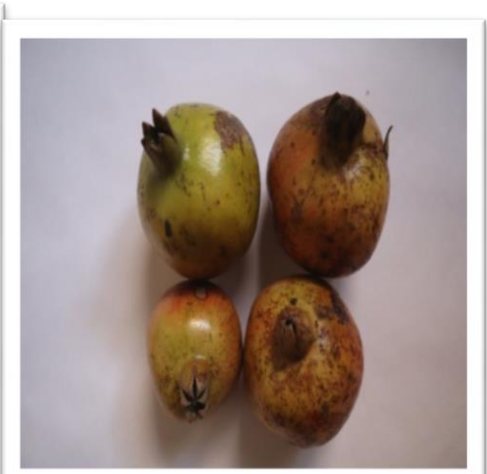
JWPR (Sel-9)



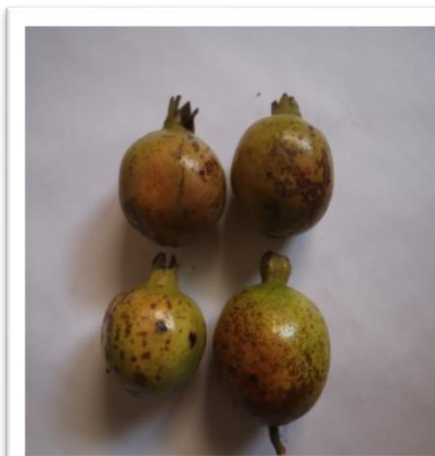
JWPR (Sel-10)



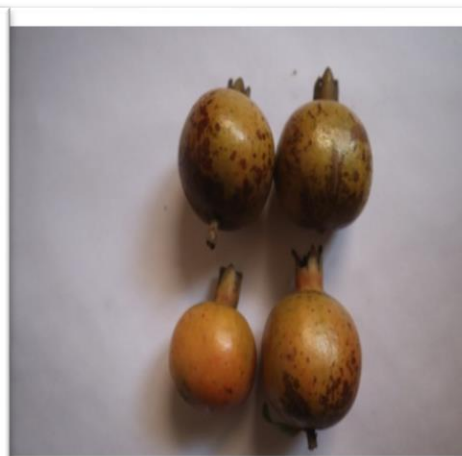
JWPR (Sel-11)



JWPR (Sel-12)



JWPR (Sel-13)



JWPR (Sel-14)



JWPR (Sel-15)



JWPR (Sel-16)



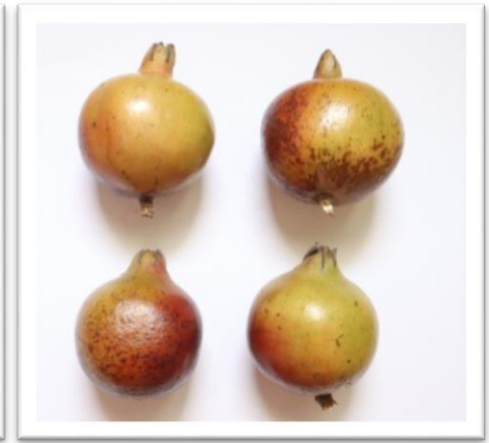
JWPR (Sel-17)



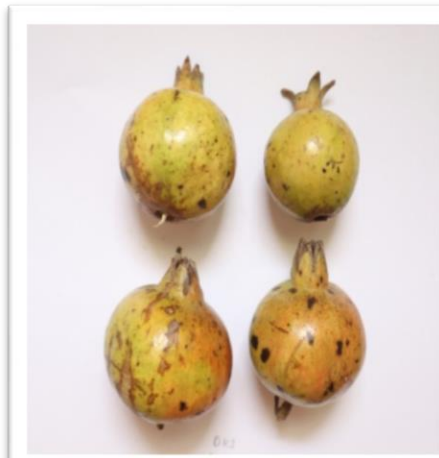
JWPR (Sel-18)



JWPR (Sel-19)



JWPR (Sel-20)



JWPR (Sel-21)



JWPR (Sel-22)



JWPR (Sel-23)



JWPR (Sel-24)



JWPR (Sel-25)



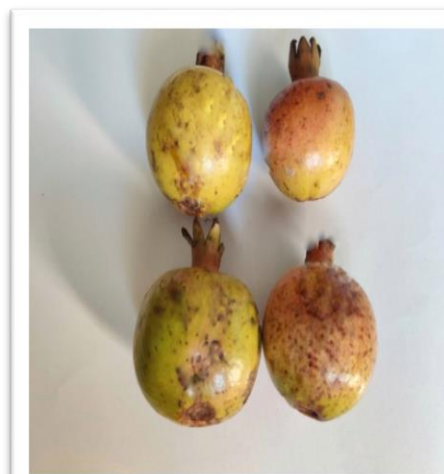
JWPR (Sel-26)



JWPR (Sel-27)



JWPR (Sel-28)



JWPR (Sel-29)



JWPR (Sel-30)



JWPP (Sel-31)



JWPP (Sel-32)



JWPP (Sel-33)



JWPP (Sel-34)



JWPP (Sel-35)



JWPP (Sel-36)



JWPP (Sel-37)



JWPP (Sel-38)



JWPP (Sel-39)



JWPP (Sel-40)



JWPP (Sel-41)



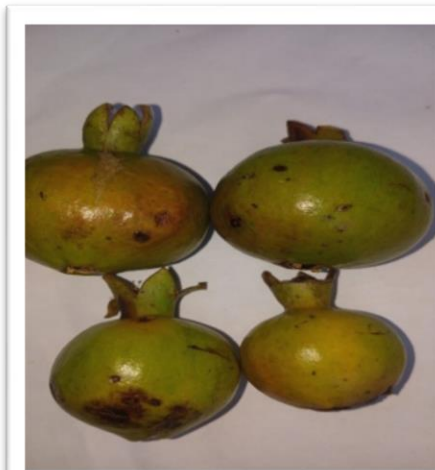
JWPP (Sel-42)



JWPP (Sel-43)



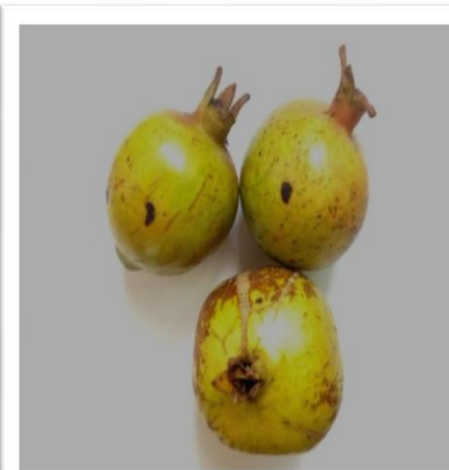
JWPP (Sel-44)



JWPP (Sel-45)



JWPP (Sel-46)



JWPP (Sel-47)



JWPP (Sel-48)



JWPP (Sel-49)



JWPP (Sel-50)



JWPU (Sel-51)



JWPU (Sel-52)



JWPU (Sel-53)



JWPU (Sel-54)



JWPU (Sel-55)



JWPU (Sel-56)



JWPU (Sel-57)



JWPU (Sel-58)



JWPU (Sel-59)



JWPU (Sel-60)



JWPU (Sel-61)



JWPU (Sel-62)



JWPU (Sel-63)



JWPU (Sel-64)



JWP (Sel-65)



JWP (Sel-66)



JWP (Sel-67)



JWP (Sel-68)



JWP (Sel-69)



JWP (Sel-70)

62	JWPU (Sel-62)	71.54	38.85	42.56	18.68
63	JWPU (Sel-63)	46.20	50.28	50.16	22.51
64	JWPU (Sel-64)	70.35	53.65	49.56	28.15
65	JWPU (Sel-65)	53.22	40.12	43.27	12.27
66	JWPU (Sel-66)	48.26	40.74	42.77	17.67
67	JWPU (Sel-67)	54.15	45.23	44.20	23.89
68	JWPU (Sel-68)	47.26	44.39	44.70	18.70
69	JWPU (Sel-69)	51.26	51.46	54.32	22.81
70	JWPU (Sel-70)	60.15	50.24	46.18	22.26
C.D. at 5%		8.14	1.49	0.76	1.42
SE(mean)		2.90	0.53	0.27	0.50

4.1.5 Aril characteristics

4.1.5.1 Aril color

A wide variation in aril colour of different genotypes of wild pomegranate was observed as evident from Table 4.8. The different colour of aril observed were red, light red, dark red, white red, medium red, pink, light pink, medium pink, light yellow and white.

4.1.5.2 Seed texture

Seed texture of different wild pomegranate genotypes studied was categorized into hard, semi hard and soft and is presented in Table 4.8 and frequency percentage in Table 4.9. It is clear from the data presented in table 4.9 that seventy wild pomegranate genotypes studied the seed texture was hard in fifty four (77.14%) and semi hard in sixteen genotypes (22.85%).

4.1.5.3 Number of arils

The data presented in Table 4.8 showed that number of arils in fruits of different wild pomegranate genotypes varied significantly. Number of arils in wild pomegranate genotypes ranged from 104.33 to 452.00. Maximum number of arils of 452.00 was recorded in genotype JWPP (Sel-36) which was significantly higher as compared to other genotypes and minimum number of arils of 104.33 was recorded in JWPR (Sel-18).

4.1.5.3 Weight of 100 arils (g)

The data presented in Table 4.8 reveal that weight of 100 arils in different wild pomegranate genotypes varied significantly ranged from 6.12g to 16.18g. Maximum weight of 100 aril of 16.18 g was recorded in JWPP (Sel-36) followed by (15.13g) JWPR (Sel-5) and minimum aril weight (6.12g) was recorded in JWPR (Sel-18).

4.1.5.4 Aril length (mm)

Aril length in different wild pomegranate genotypes varied significantly (Table 4.8). Maximum aril length of 7.35 mm was recorded in JWPP (Sel-36) followed by (6.44mm) in genotype JWPR (Sel-5) while as minimum aril length of 2.25 mm was recorded in JWPR (Sel-18).

4.1.5.5 Aril width (mm)

It is evident from the Table 4.8 that aril width in wild pomegranate genotypes ranged from 2.98 to 9.14 mm and maximum aril width of 9.14 mm was recorded in JWPP (Sel-36) followed by 8.75mm in JWPR (Sel-5) while as minimum aril width of 2.98 mm was recorded in JWPR (Sel-18).

Table 4.8: Variations in aril color, seed texture, number of arils, weight of 100 arils, aril length and aril width of wild pomegranate genotypes of Jammu region

S. No.	Genotypes	Aril color	Seed texture	Number of arils	Weight of 100 aril (g)	Aril length (mm)	Aril width (mm)
1	JWPR (Sel-1)	Light red	Hard	234.66	14.14	4.11	4.35
2	JWPR (Sel-2)	Light red	Hard	367.00	13.16	6.23	7.54
3	JWPR (Sel-3)	Light red	Hard	226.33	12.24	4.28	5.15
4	JWPR (Sel-4)	Light pink	Hard	262.00	13.42	3.30	4.14
5	JWPR (Sel-5)	Red	Semi hard	394.00	15.13	6.44	8.75
6	JWPR (Sel-6)	Light yellow	Hard	362.33	14.84	5.57	6.14
7	JWPR (Sel-7)	Light yellow	Hard	327.66	12.17	5.18	5.95
8	JWPR (Sel-8)	Light pink	Hard	152.00	10.33	2.77	3.85
9	JWPR (Sel-9)	Dark red	Hard	104.66	6.15	2.25	3.25

10	JWPR (Sel-10)	Red	Hard	264.33	15.25	4.35	5.27
11	JWPR (Sel-11)	Medium pink	Hard	208.23	9.17	2.44	2.64
12	JWPR (Sel-12)	Light pink	Semi hard	285.13	12.17	3.64	4.25
13	JWPR (Sel-13)	White red	Semi hard	112.66	11.57	2.61	2.94
14	JWPR (Sel-14)	White red	Hard	266.33	15.24	4.23	4.97
15	JWPR (Sel-15)	White red	Hard	270.66	17.15	4.17	4.85
16	JWPR (Sel-16)	White red	Hard	253.62	12.56	3.86	4.10
17	JWPR (Sel-17)	White red	Hard	112.66	14.26	2.94	3.15
18	JWPR (Sel-18)	White red	Hard	104.33	6.12	2.25	2.98
19	JWPR (Sel-19)	White red	Hard	266.00	13.53	4.35	5.46
20	JWPR (Sel-20)	White red	Hard	113.21	15.17	3.43	5.16
21	JWPR (Sel-21)	White red	Hard	282.61	11.17	4.55	5.57
22	JWPR (Sel-22)	White red	Hard	279.21	16.17	4.47	5.26
23	JWPR (Sel-23)	White red	Hard	290.00	15.35	5.24	6.55
24	JWPR (Sel-24)	White red	Hard	255.66	16.18	3.57	4.29
25	JWPR (Sel-25)	White red	Hard	244.66	11.14	3.57	4.06
26	JWPR (Sel-26)	White	Hard	253.33	10.15	2.57	3.18
27	JWPR (Sel-27)	Red	Hard	275.00	11.23	3.86	4.56
28	JWPR (Sel-28)	White red	Hard	247.66	13.17	3.94	4.76
29	JWPR (Sel-29)	White red	Hard	115.00	12.15	2.86	4.25
30	JWPR (Sel-30)	White red	Hard	113.33	14.27	2.59	3.96
31	JWPP (Sel-31)	White red	Hard	387.33	15.16	7.15	8.13
32	JWPP (Sel-32)	White red	Hard	114.00	14.08	5.25	7.44
33	JWPP (Sel-33)	Medium pink	Hard	377.00	9.26	4.82	5.78
34	JWPP (Sel-34)	Dark red	Hard	286.66	10.27	5.14	6.55
35	JWPP (Sel-35)	Light yellow	Hard	410.33	10.14	5.71	6.26
36	JWPP (Sel-36)	Dark red	Semi hard	452.00	16.18	7.35	9.14
37	JWPP (Sel-37)	Light yellow	Semi hard	391.66	13.17	6.14	7.46

38	JWPP (Sel-38)	Light red	Semi hard	317.33	14.67	5.96	6.27
39	JWPP (Sel-39)	Light red	Semi hard	293.66	15.17	5.82	7.45
40	JWPP (Sel-40)	White red	Semi hard	296.33	17.84	4.23	5.09
41	JWPP (Sel-41)	White red	Semi hard	274.00	11.24	4.11	5.91
42	JWPP (Sel-42)	White red	Semi hard	270.33	13.46	4.17	5.99
43	JWPP (Sel-43)	White red	Semi hard	447.12	14.47	7.35	7.09
44	JWPP (Sel-44)	Light pink	Hard	420.33	9.23	6.21	7.44
45	JWPP (Sel-45)	Medium pink	Hard	131.66	14.55	3.15	4.05
46	JWPP (Sel-46)	Medium pink	Hard	197.13	15.67	3.54	4.28
47	JWPP (Sel-47)	Medium pink	Hard	212.66	13.87	4.13	5.03
48	JWPP (Sel-48)	Medium pink	Hard	276.00	6.14	4.18	5.43
49	JWPP (Sel-49)	Pink	Hard	317.66	12.16	4.36	6.43
50	JWPP (Sel-50)	Pink	Hard	110.33	6.15	2.87	3.67
51	JWPU (Sel-51)	Pink	Hard	292.16	15.10	5.87	7.33
52	JWPU (Sel-52)	White red	Hard	270.33	11.15	5.15	6.27
53	JWPU (Sel-53)	Light yellow	Hard	313.33	12.56	5.85	6.75
54	JWPU (Sel-54)	Light yellow	Hard	373.66	10.13	6.14	8.07
55	JWPU (Sel-55)	White red	Hard	271.66	9.87	5.56	7.17
56	JWPU (Sel-56)	White red	Hard	197.33	13.45	5.66	6.73
57	JWPU (Sel-57)	Light yellow	Semi hard	317.00	15.68	5.58	7.84
58	JWPU (Sel-58)	Light red	Semi hard	275.33	12.87	4.94	5.98
59	JWPU (Sel-59)	Red	Hard	267.00	14.18	3.91	4.04
60	JWPU (Sel-60)	Red	Hard	432.33	11.33	5.75	6.98
61	JWPU (Sel-61)	White red	Hard	282.36	14.18	5.34	7.08
62	JWPU (Sel-62)	Light yellow	Hard	135.66	9.57	3.92	4.94
63	JWPU (Sel-63)	Light yellow	Hard	255.61	8.97	5.67	7.38
64	JWPU (Sel-64)	Light yellow	Hard	183.00	14.18	5.48	7.08
65	JWPU (Sel-65)	Red	Semi hard	135.66	12.36	4.63	6.06



JWPR (Sel-1)



JWPR (Sel-2)



JWPR (Sel-3)



JWPR (Sel-4)



JWPR (Sel-5)



JWPR (Sel-6)



JWPR (Sel-7)



JWPR (Sel-8)



JWPR (Sel-9)



JWPR (Sel-10)



JWPR (Sel-11)



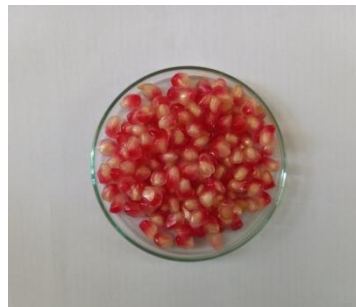
JWPR (Sel-12)

Plate 4: Variations in Aril color of various wild pomegranate genotypes

Cont...



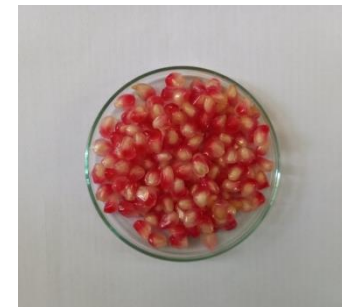
JWPR (Sel-13)



JWPR (Sel-14)



JWPR (Sel-15)



JWPR (Sel-16)



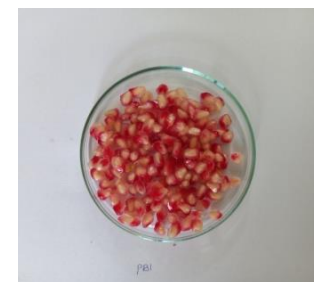
JWPR (Sel-17)



JWPR (Sel-18)



JWPR (Sel-19)



JWPR (Sel-20)



JWPR (Sel-21)



JWPR (Sel-22)



JWPR (Sel-23)



JWPR (Sel-24)



JWPR (Sel-25)



JWPR (Sel-26)



JWPR (Sel-27)



JWPR (Sel-28)



JWPR (Sel-29)



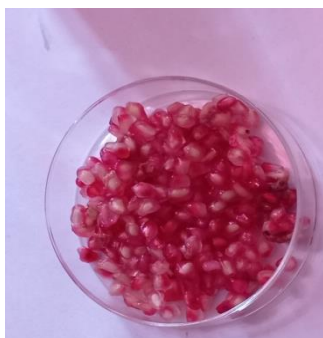
JWPR (Sel-30)



JWPP (Sel-31)



JWPP (Sel-32)



JWPP (Sel-33)



JWPP (Sel-34)



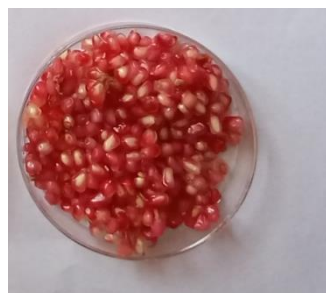
JWPP (Sel-35)



JWPP (Sel-36)



JWPP (Sel-37)



JWPP (Sel-38)



JWPP (Sel-39)



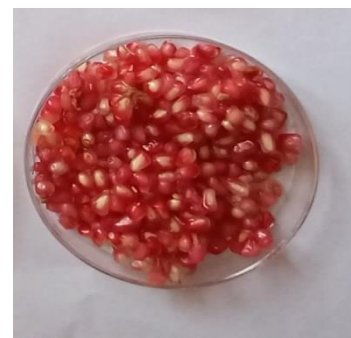
JWPP (Sel-40)



JWPP (Sel-41)



JWPP (Sel-42)



JWPP (Sel-43)



JWPP (Sel-44)



JWPP (Sel-45)



JWPP (Sel-46)



JWPP (Sel-47)



JWPP (Sel-48)



JWPP (Sel-49)



JWPP (Sel-50)



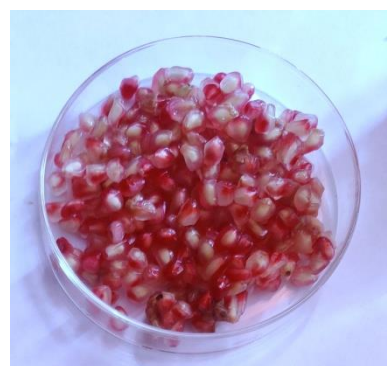
JWPU (Sel-51)



JWPU (Sel-52)



JWPU (Sel-53)



JWPU (Sel-54)



JWPU (Sel-55)



JWPU (Sel-56)



JWPU (Sel-57)



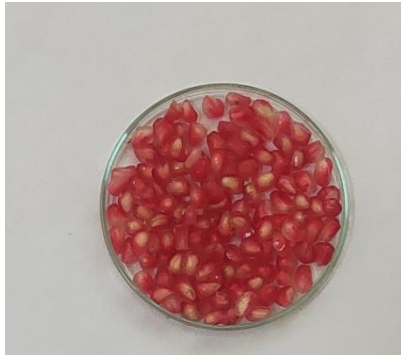
JWPU (Sel-58)



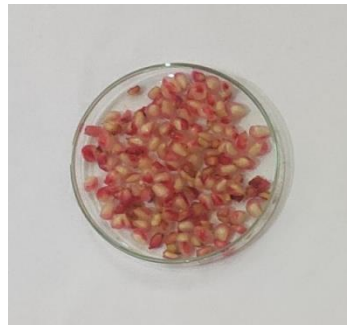
JWPU (Sel-59)



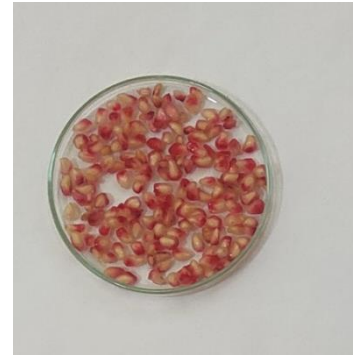
JWPU (Sel-60)



JWPU (Sel-61)



JWPU (Sel-62)



JWPU (Sel-63)



JWPU (Sel-64)



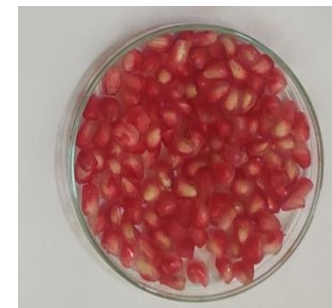
JWPU (Sel-65)



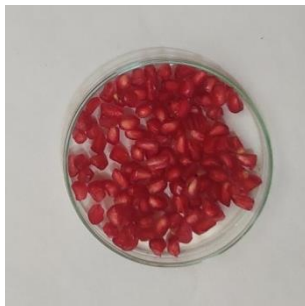
JWPU (Sel-66)



JWPU (Sel-67)



JWPU (Sel-68)



JWPU (Sel-69)



JWPU (Sel-70)

66	JWPU (Sel-66)	Red	Semi hard	186.24	11.38	4.53	5.17
67	JWPU (Sel-67)	Medium red	Semi hard	135.66	13.24	3.87	4.87
68	JWPU (Sel-68)	Medium red	Hard	174.52	13.58	4.53	5.17
69	JWPU (Sel-69)	Red	Hard	263.66	10.37	4.72	5.84
70	JWPU (Sel-70)	Light pink	Semi hard	177.33	9.27	4.67	5.02
C.D. at 5%				3.20	0.58	0.35	0.37
SE (mean)				1.14	0.18	0.16	0.12

Table 4.9: Frequency distribution of fruit shape, fruit color, aril color and seed texture of wild pomegranate genotypes of Jammu region

Characteristics	Category	Number of genotypes	Percentage (%)
Fruit shape	Round	27	38.57
	Elliptic	11	15.71
	Ovate	11	15.71
	Oval	21	30
Fruit color	Green	3	4.28
	Reddish green	13	18.57
	Greenish yellow	25	35.71
	Reddish yellow	32	45.71
Aril color	Red	8	11.42
	Light red	5	7.14
	Medium red	2	2.85
	Pink	3	4.28
	Dark pink	0	0
	Medium pink	6	8.57
	Light pink	5	7.14
	White red	31	44.28
	Light yellow	10	14.28
Seed texture	Semi hard	16	22.85
	Hard	54	77.14
	Soft	0	0

4.1.6 Yield characteristics

4.1.6.1 Number of fruits

The data presented in Table 4.10 shows number of fruits of different wild pomegranate genotypes studied. Maximum number of fruits per bush (88) was recorded in JWPR (Sel-13) and minimum number of fruits per bush (16) was recorded in JWPP (Sel-35).

4.1.6.2 Yield (kg/bush)

The data presented in Table 4.10 shows yield of different wild pomegranate genotypes. Maximum yield of 7.02 kg/bush was recorded in JWPP (Sel-36) followed by 6.27 kg/bush in JWPR (Sel-5) and minimum yield of 1.49 kg/tree was recorded in JWPP (Sel-35).

Table 4.10: Variation in number of fruits and yield (kg/tree) of wild pomegranate genotypes of Jammu region

S. No.	Genotypes	No. of fruits per bush	Yield (Kg /bush)
1	JWPR (Sel-1)	50	2.38
2	JWPR (Sel-2)	58	4.90
3	JWPR (Sel-3)	47	1.94
4	JWPR (Sel-4)	56	2.95
5	JWPR (Sel-5)	57	6.27
6	JWPR (Sel-6)	54	5.00
7	JWPR (Sel-7)	64	5.21
8	JWPR (Sel-8)	76	2.92
9	JWPR (Sel-9)	69	2.52
10	JWPR (Sel-10)	61	4.13
11	JWPR (Sel-11)	63	2.14
12	JWPR (Sel-12)	60	3.82
13	JWPR (Sel-13)	88	2.61
14	JWPR (Sel-14)	59	5.38
15	JWPR (Sel-15)	78	4.64
16	JWPR (Sel-16)	85	3.78

17	JWPR (Sel-17)	77	2.51
18	JWPR (Sel-18)	75	2.15
19	JWPR (Sel-19)	72	3.96
20	JWPR (Sel-20)	74	2.80
21	JWPR (Sel-21)	55	3.58
22	JWPR (Sel-22)	75	4.53
23	JWPR (Sel-23)	77	5.52
24	JWPR (Sel-24)	73	3.48
25	JWPR (Sel-25)	62	2.71
26	JWPR (Sel-26)	58	3.78
27	JWPR (Sel-27)	66	4.50
28	JWPR (Sel-28)	53	2.32
29	JWPR (Sel-29)	55	2.11
30	JWPR (Sel-30)	61	2.17
31	JWPP (Sel-31)	59	3.55
32	JWPP (Sel-32)	78	2.77
33	JWPP (Sel-33)	56	4.51
34	JWPP (Sel-34)	23	1.58
35	JWPP (Sel-35)	16	1.49
36	JWPP (Sel-36)	63	7.02
37	JWPP (Sel-37)	67	6.05
38	JWPP (Sel-38)	56	4.79
39	JWPP (Sel-39)	78	5.55
40	JWPP (Sel-40)	56	4.21
41	JWPP (Sel-41)	58	3.99
42	JWPP (Sel-42)	54	5.53
43	JWPP (Sel-43)	65	6.73
44	JWPP (Sel-44)	53	4.67
45	JWPP (Sel-45)	45	1.80
46	JWPP (Sel-46)	67	3.70
47	JWPP (Sel-47)	77	4.64
48	JWPP (Sel-48)	49	3.69

49	JWPP (Sel-49)	58	4.67
50	JWPP (Sel-50)	65	2.50
51	JWPU (Sel-51)	48	3.77
52	JWPU (Sel-52)	59	5.91
53	JWPU (Sel-53)	64	5.07
54	JWPU (Sel-54)	55	4.49
55	JWPU (Sel-55)	49	2.99
56	JWPU (Sel-56)	80	4.49
57	JWPU (Sel-57)	71	5.75
58	JWPU (Sel-58)	75	5.01
59	JWPU (Sel-59)	76	4.58
60	JWPU (Sel-60)	52	5.18
61	JWPU (Sel-61)	83	5.94
62	JWPU (Sel-62)	85	3.93
63	JWPU (Sel-63)	49	3.45
64	JWPU (Sel-64)	50	2.66
65	JWPU (Sel-65)	51	2.46
66	JWPU (Sel-66)	67	3.63
67	JWPU (Sel-67)	59	2.79
68	JWPU (Sel-68)	71	3.64
69	JWPU (Sel-69)	55	2.86
70	JWPU (Sel-70)	68	3.55

4.1.7 Biochemical characteristics

4.1.7.1 TSS (°Brix)

The data obtained from TSS show significant variation among all wild pomegranate genotypes as depicted in Table 4.11 whereas maximum TSS of 17.90°Brix was recorded in JWPP (Sel-36) followed by 17.80°Brix in JWPR (Sel-5) and minimum TSS of 12.24°Brix was recorded in JWPR (Sel-26).

4.1.7.2 Total sugars (%)

The data on total sugars per cent in different wild pomegranate genotypes are presented in the Table 4.11 showed that the highest total sugar of 14.47% was in JWPP (Sel-36) followed by 14.25% in JWPR (Sel-5) while minimum total sugar 8.19% was found in JWPR (Sel-26).

4.1.7.3 Reducing sugars (%)

The data presented in Table 4.11 shows the percentage of reducing sugars in various wild pomegranate genotypes. The highest reducing sugar content was recorded in JWPP (Sel-36) at 9.74%, followed closely by JWPR (Sel-5) at 9.71%. The lowest reducing sugar percentage, 5.58%, was found in JWPR (Sel-26).

4.1.7.4 Non reducing sugars (%)

The data obtained for non reducing sugars shows significant variation among all wild pomegranate genotypes studied as depicted in Table 4.11 whereas maximum (4.73%) non reducing sugar recorded in JWPP (Sel-36) followed by (4.54%) in JWPR (Sel-5) and minimum non reducing sugar (2.60%) was recorded in JWPR (Sel-26).

4.1.7.5 Titratable acidity (%)

The data presented in Table 4.12 shows the titratable acidity of different wild pomegranate genotypes. Significant variation was observed among the genotypes, with the highest titratable acidity 6.93% was recorded in JWPP (Sel-36) followed by 6.86% in JWPR (Sel-5). The minimum titratable acidity 4.16%, was found in JWPR (Sel-26).

4.1.7.6 Ascorbic acid (mg/100g)

The data obtained for ascorbic acid in Table 4.12 shows significant variation among all wild pomegranate genotypes whereas maximum (24.74%) ascorbic acid recorded in JWPP (Sel-36) followed by (24.69%) in JWPR (Sel-5) and minimum (14.08%) ascorbic acid was recorded in JWPR (Sel-26).

Table 4.11: Variation in TSS, total sugars, reducing sugar and non reducing sugar of wild pomegranate genotypes of Jammu region

S. No.	Genotypes	TSS (°Brix)	Total sugar (%)	Reducing sugar (%)	Non reducing sugar (%)
1	JWPR (Sel-1)	15.47	9.24	6.03	3.21
2	JWPR (Sel-2)	15.20	9.33	6.07	3.26
3	JWPR (Sel-3)	16.05	10.31	7.20	3.11
4	JWPR (Sel-4)	14.61	9.54	6.54	3.00
5	JWPR (Sel-5)	17.80	14.25	9.71	4.54
6	JWPR (Sel-6)	17.16	12.18	8.16	4.02
7	JWPR (Sel-7)	15.30	9.46	6.49	2.97
8	JWPR (Sel-8)	14.23	8.93	5.87	3.06
9	JWPR (Sel-9)	17.20	12.10	8.09	4.09
10	JWPR (Sel-10)	15.37	9.54	6.55	2.99
11	JWPR (Sel-11)	16.23	10.25	7.18	3.07
12	JWPR (Sel-12)	15.04	9.23	6.08	3.15
13	JWPR (Sel-13)	14.16	8.83	5.83	3.00
14	JWPR (Sel-14)	15.22	9.25	6.01	3.24
15	JWPR (Sel-15)	13.64	9.27	6.36	2.91
16	JWPR (Sel-16)	13.06	8.24	5.61	2.64
17	JWPR (Sel-17)	13.33	8.84	5.58	3.31
18	JWPR (Sel-18)	14.65	8.23	5.58	2.65
19	JWPR (Sel-19)	16.53	8.54	5.74	2.80
20	JWPR (Sel-20)	16.90	10.44	7.26	3.18
21	JWPR (Sel-21)	12.32	8.35	5.64	2.71
22	JWPR (Sel-22)	14.53	9.15	6.00	3.15
23	JWPR (Sel-23)	16.65	11.23	7.18	4.04
24	JWPR (Sel-24)	14.06	10.71	7.45	3.27
25	JWPR (Sel-25)	13.50	8.55	5.71	2.83
26	JWPR (Sel-26)	12.24	8.19	5.53	2.60
27	JWPR (Sel-27)	16.70	11.35	7.57	3.78
28	JWPR (Sel-28)	14.43	8.96	5.93	3.03

29	JWPR (Sel-29)	16.40	12.41	8.26	4.15
30	JWPR (Sel-30)	15.02	9.26	6.09	3.17
31	JWPP (Sel-31)	16.21	10.20	7.11	3.09
32	JWPP (Sel-32)	16.14	10.14	7.16	2.99
33	JWPP (Sel-33)	17.25	12.10	8.15	3.95
34	JWPP (Sel-34)	17.12	13.12	8.95	4.17
35	JWPP (Sel-35)	16.72	11.70	7.78	3.92
36	JWPP (Sel-36)	17.90	14.47	9.74	4.73
37	JWPP (Sel-37)	16.60	10.14	7.04	3.10
38	JWPP (Sel-38)	17.25	12.65	8.64	4.01
39	JWPP (Sel-39)	17.04	12.03	8.03	4.00
40	JWPP (Sel-40)	15.40	9.57	6.66	2.91
41	JWPP (Sel-41)	15.70	9.62	6.86	2.76
42	JWPP (Sel-42)	16.80	11.81	7.84	3.97
43	JWPP (Sel-43)	16.39	13.03	8.83	4.20
44	JWPP (Sel-44)	16.12	10.03	6.96	3.07
45	JWPP (Sel-45)	17.03	13.24	9.68	3.48
46	JWPP (Sel-46)	15.06	10.86	9.15	1.71
47	JWPP (Sel-47)	17.04	13.00	9.57	3.43
48	JWPP (Sel-48)	17.32	12.93	8.78	4.15
49	JWPP (Sel-49)	15.60	9.64	6.87	2.77
50	JWPP (Sel-50)	17.09	12.05	8.05	4.00
51	JWPU (Sel-51)	13.06	8.81	5.70	3.12
52	JWPU (Sel-52)	15.74	9.63	6.85	2.78
53	JWPU (Sel-53)	16.16	10.06	7.02	3.04
54	JWPU (Sel-54)	17.40	12.96	8.94	4.02
55	JWPU (Sel-55)	17.20	12.06	8.13	3.93
56	JWPU (Sel-56)	15.32	9.08	5.95	3.13
57	JWPU (Sel-57)	14.25	9.02	6.13	2.89
58	JWPU (Sel-58)	16.40	10.45	7.23	3.21
59	JWPU (Sel-59)	17.32	12.93	8.54	4.38
60	JWPU (Sel-60)	17.53	13.07	9.33	3.74
61	JWPU (Sel-61)	16.53	10.54	7.35	3.19
62	JWPU (Sel-62)	15.44	9.34	6.47	2.88

63	JWPU (Sel-63)	17.52	13.05	9.16	3.89
64	JWPU (Sel-64)	17.23	12.34	8.23	4.11
65	JWPU (Sel-65)	16.30	10.46	7.16	3.30
66	JWPU (Sel-66)	15.60	9.64	6.83	2.80
67	JWPU (Sel-67)	16.53	10.44	7.23	3.21
68	JWPU (Sel-68)	16.22	10.24	7.12	3.12
69	JWPU (Sel-69)	15.22	9.26	6.32	2.95
70	JWPU (Sel-70)	16.85	11.92	7.94	3.97
C.D. at 5%		0.27	0.15	0.14	0.10
SE (mean)		0.10	0.05	0.05	0.03

Table 4.12: Variation in titratable acidity and ascorbic acid of wild pomegranate genotypes of Jammu region

S. No.	Genotypes	Titratable Acidity (%)	Ascorbic acid (mg/100g)
1	JWPR (Sel-1)	5.37	16.45
2	JWPR (Sel-2)	5.25	15.26
3	JWPR (Sel-3)	5.63	20.45
4	JWPR (Sel-4)	5.17	15.87
5	JWPR (Sel-5)	6.86	24.71
6	JWPR (Sel-6)	5.56	15.46
7	JWPR (Sel-7)	5.43	19.25
8	JWPR (Sel-8)	5.08	15.37
9	JWPR (Sel-9)	6.28	21.15
10	JWPR (Sel-10)	5.45	15.05
11	JWPR (Sel-11)	5.55	20.05
12	JWPR (Sel-12)	5.22	15.04
13	JWPR (Sel-13)	5.06	15.25
14	JWPR (Sel-14)	5.36	15.48

15	JWPR (Sel-15)	4.90	16.14
16	JWPR (Sel-16)	4.95	17.94
17	JWPR (Sel-17)	4.90	16.34
18	JWPR (Sel-18)	5.16	15.43
19	JWPR (Sel-19)	4.59	17.25
20	JWPR (Sel-20)	5.15	15.65
21	JWPR (Sel-21)	6.14	18.26
22	JWPR (Sel-22)	5.04	18.12
23	JWPR (Sel-23)	4.41	16.76
24	JWPR (Sel-24)	4.78	17.59
25	JWPR (Sel-25)	6.17	14.85
26	JWPR (Sel-26)	4.16	14.08
27	JWPR (Sel-27)	6.13	20.85
28	JWPR (Sel-28)	5.28	15.68
29	JWPR (Sel-29)	5.55	20.18
30	JWPR (Sel-30)	5.51	15.06
31	JWPP (Sel-31)	6.33	20.53
32	JWPP (Sel-32)	6.40	15.14
33	JWPP (Sel-33)	5.53	16.44
34	JWPP (Sel-34)	6.22	22.18
35	JWPP (Sel-35)	6.05	15.83
36	JWPP (Sel-36)	6.93	24.75
37	JWPP (Sel-37)	6.27	15.25
38	JWPP (Sel-38)	5.27	21.85
39	JWPP (Sel-39)	5.27	21.22
40	JWPP (Sel-40)	6.15	15.28
41	JWPP (Sel-41)	5.53	15.06
42	JWPP (Sel-42)	6.06	20.95
43	JWPP (Sel-43)	6.25	21.25

44	JWPP (Sel-44)	6.32	22.26
45	JWPP (Sel-45)	6.09	24.18
46	JWPP (Sel-46)	5.44	22.07
47	JWPP (Sel-47)	6.42	23.06
48	JWPP (Sel-48)	6.47	21.04
49	JWPP (Sel-49)	5.48	21.26
50	JWPP (Sel-50)	5.53	23.16
51	JWPU (Sel-51)	4.17	15.31
52	JWPU (Sel-52)	4.37	16.84
53	JWPU (Sel-53)	5.34	18.45
54	JWPU (Sel-54)	6.20	20.76
55	JWPU (Sel-55)	6.85	21.33
56	JWPU (Sel-56)	5.43	19.22
57	JWPU (Sel-57)	4.90	17.07
58	JWPU (Sel-58)	6.14	20.62
59	JWPU (Sel-59)	6.31	23.42
60	JWPU (Sel-60)	6.60	23.18
61	JWPU (Sel-61)	6.02	20.64
62	JWPU (Sel-62)	5.23	15.28
63	JWPU (Sel-63)	6.64	21.23
64	JWPU (Sel-64)	6.42	22.06
65	JWPU (Sel-65)	6.29	20.85
66	JWPU (Sel-66)	5.24	18.04
67	JWPU (Sel-67)	6.05	21.64
68	JWPU (Sel-68)	6.17	16.18
69	JWPU (Sel-69)	5.21	15.84
70	JWPU (Sel-70)	6.21	19.26
	C.D at 5%	0.08	1.11
	SE(m)	0.02	0.39

4.1.8 Descriptive statistical analysis

Twenty one quantitative traits were evaluated for diversity estimation in wild and domesticated pomegranate accessions. Minimum, maximum, means, standard deviation (SD) and coefficient of variance (CV) was calculated and presented in Table 4.5. There was a large extent of diversity in wild pomegranate accessions. In wild accessions, high CV (36.01%) was recorded for number of arils followed by leaf width (34.73%), juice per cent (34.27%), fruit weight (32.35%), aril length (27.25%), aril width (26.98%), crown length (22.00%), rind thickness (21.49%) and non reducing sugars (20.54%) however the least CV was recorded for TSS (8.86%). Number of arils range from 104.33 to 452.00, juice percent range from 12.15 to 46.77 per cent, leaf width range from 1.01 to 3.23cm, fruit weight range from 28.36 to 111.51g.

Table 4.13: Descriptive statistical analysis of different characters of wild pomegranate genotypes of Jammu region

S. No.	Traits	Min.	Max.	Mean	Std Dev.	CV (%)
1	Leaf length (cm)	3.45	6.44	4.65	0.75	16.12
2	Leaf width (cm)	1.01	3.23	1.67	0.58	34.73
3	Petiole length (mm)	2.00	7.70	4.98	1.30	26.10
4	Calyx length (mm)	22.17	36.44	30.48	5.26	17.25
5	Calyx width (mm)	10.11	15.87	13.02	1.60	12.29
6	Petal length (mm)	14.24	25.63	19.86	4.97	19.39
7	Rind thickness (mm)	1.69	4.97	2.95	0.63	21.49
8	Fruit weight (g)	28.36	111.51	62.28	20.15	32.35
9	Fruit length (mm)	30.17	57.54	45.47	7.08	15.58
10	Fruit width (mm)	29.72	56.65	45.12	7.30	16.17
11	Crown length (mm)	9.24	20.43	14.29	3.14	22.00
12	Juice percent	12.15	46.77	25.89	8.61	34.27
13	No of arils	104.33	452.00	256.68	92.45	36.01
14	Aril length (mm)	2.64	9.14	5.59	1.24	27.25
15	Aril width (mm)	2.25	7.35	4.55	1.22	26.98
16	TSS (° Brix)	12.27	18.25	15.91	1.41	8.86
17	Acidity (%)	4.17	6.91	5.54	0.66	11.18
18	Total sugars (%)	7.96	13.65	12.78	2.79	21.67
19	Reducing sugar (%)	5.15	9.83	7.18	1.14	14.49
20	Non reducing sugar (%)	2.67	4.29	3.04	0.71	20.54
21	Ascorbic acid (mg/100g)	14.08	24.61	18.65	3.02	16.23

4.1.9 Parameters of variability

The estimates of coefficients of variability (phenotypic and genotypic), heritability (broad sense), genetic advance and genetic advance as percentage of mean were worked out to facilitate selection for various characters. The results are given in Table 4.14.

4.1.9.1 Coefficient of variability

Estimates of genetic variability parameters for various characters in wild pomegranate genotypes revealed that the phenotypic coefficient of variability were higher in magnitude than the genotypic coefficient of variability for all the characters under study. The phenotypic and genotypic coefficient of variability were found to be highest for petiole length (36.74 % and 36.65 %) which was followed by leaf width (34.16 % and 32.88 %), fruit weight (32.69 % and 32.60 %), rind thickness (29.40 % and 29.39 %), 100 aril weight (27.56 % and 27.56 %), aril length (26.97 % and 26.96 %), aril width (26.94 % and 26.93 %), crown length (22.00 % and 21.98 %), non reducing sugars (18.77 % and 18.74 %), reducing sugars (16.91 % and 16.01 %), leaf length (16.14 % and 16.09 %), fruit width (15.98 % and 15.97 %) and fruit length (15.46 % and 15.41 %) whereas lowest phenotypic and genotypic coefficient of variability were observed in TSS (8.90 % and 8.88 %), respectively.

4.1.9.2 Heritability

Heritability in broad sense varied from 63.52 per cent in seed width to 93.83 per cent in fruit length. High heritability was observed for all twenty characters namely calyx width (99.99 %), 100 aril weight (99.97 %), fruit weight (99.95 %), aril length (99.90 %), calyx length (99.89 %), Total sugars (99.86 %) fruit length (93.83 %), non reducing sugar (99.84 %), crown length (99.80 %), titratable acidity (99.75 %), fruit width (90.71 %), fruit length (90.67 %), TSS (99.50 %), ascorbic acid (99.19 %), rind thickness (99.00 %), leaf blade length (99.00 %), aril width (99.00 %), petiole length (99.56 %), reducing sugars (94.67 %) and leaf width (92.00 %).

4.1.9.3 Genetic Advance

Table 4.14 shows genetic advance as percentage of mean which varied from 18.25 per cent (TSS) to 75.33 per cent (petiole length). High genetic advance as percentage of mean were found for petiole length (75.33 %), fruit weight (67.35 %), leaf width (65.21 %), rind thickness (60.53 %), 100 aril weight (56.78 %), aril width (55.46 %), aril length (51.52 %), crown length (45.27 %), non reducing sugar (38.55

%), leaf length (33.03%), reducing sugars (33.00 %), calyx length (33.34 %), fruit width (32.89 %), ascorbic acid (31.89 %), fruit length (31.67 %), total sugars (31.45 %) and moderate genetic advance as percentage of mean for TSS (18.25 %).

Table. 4.14: Estimates of variability parameters for various characters in wild pomegranate genotypes.

S. No	Characters	Coefficient of variation (%)		Heritability (Broad sense %)	Genetic advance	Genetic advance at percentage of means
		GCV (%)	PCV (%)			
1	Leaf blade length (cm)	16.09	16.14	99.00	1.53	33.03
2	Leaf width (cm)	32.88	34.16	92.00	2.01	65.21
3	Petiole length (mm)	36.65	36.74	99.56	2.88	75.33
4	Calyx length (mm)	15.70	15.71	99.89	10.83	32.34
5	Calyx width (mm)	12.34	12.34	99.99	3.31	25.42
6	Rind thickness (mm)	29.39	29.40	99.00	1.77	60.53
7	Fruit weight (g)	32.60	32.69	99.95	42.05	67.35
8	Fruit length (mm)	15.41	15.46	99.67	14.39	31.67
9	Fruit width (mm)	15.97	15.98	99.71	14.82	32.89
11	Crown length (mm)	21.98	22.00	99.80	6.47	45.27
12	100 aril weight (g)	27.56	27.56	99.97	11.45	56.78
13	Aril length (mm)	26.96	26.97	99.90	3.11	51.52
14	Aril width (mm)	26.93	26.94	99.00	2.53	55.46
15	TSS ($^{\circ}$ B)	8.88	8.90	99.50	2.90	18.25
16	Titration acidity (%)	12.33	12.36	99.75	1.45	25.41
17	Total Sugars (%)	15.26	15.28	99.86	3.31	31.45
18	Reducing sugars (%)	16.01	16.91	94.67	2.36	33.00
19	Non reducing sugars (%)	18.74	18.77	99.84	1.29	38.55
20	Ascorbic acid (mg/100)	15.53	15.53	99.19	5.66	31.99

4.1.11 Correlations

4.1.11.1 Phenotypic correlation

The results pertaining to phenotypic correlation among ten characters of seventy wild pomegranate genotypes is presented in (Table 4.15). Fruit weight was significantly correlated with fruit length ($r=0.732$), fruit width (0.660), aril weight (0.2191), aril width (0.711) and juice per cent (0.441). Fruit length is positively and significantly correlated with fruit width (0.861), aril weight (0.408), aril length (0.647), aril width (0.708) and juice per cent (0.411). Fruit width was positively and highly significantly correlated with aril weight (0.396), aril length (0.704), aril width (0.738) and juice per cent (0.542). Aril weight was positively and significantly correlated with aril length (0.350), aril width (0.548) and juice per cent (0.171). Aril length was positively and significantly correlated with aril width (0.938) and Juice per cent (0.542). Aril width was positively and significantly correlated with juice per cent (0.476).

4.1.11.2 Genotypic correlation

The results pertaining to genotypic correlation among ten characters of seventy wild pomegranate genotypes is presented in (Table 4.15). Genotypic correlation coefficients for all the characters were higher than phenotypic correlation coefficients. Fruit weight was and highly significantly correlated with fruit length ($r=0.770$), fruit width (0.682), aril weight (0.293), aril width (0.733) and juice per cent (0.574). Fruit length is positively and significantly correlated with fruit width (0.870), aril weight (0.428), aril length (0.654), aril width (0.714) and juice per cent (0.726). Fruit width was positively and highly significantly correlated with aril weight (0.624), aril length (0.706), aril width (0.740) and juice per cent (0.697). Aril weight was positively and significantly correlated with aril length (0.373), aril width (0.578) and juice per cent (0.401). Aril length was positively and significantly correlated with aril width (0.938) and Juice per cent (0.5584). Aril width was positively and significantly correlated with juice per cent (0.612).

Table 4.15: Phenotypic (P) and genotypic (G) correlation coefficients among ten characters of 70 wild pomegranate genotypes of Jammu region

		Leaf blade length (cm)	Leaf blade width (cm)	Petiole length (mm)	Fruit weight (g)	Fruit Length (mm)	Fruit width (mm)	Aril weight (mm)	Aril length (mm)	Aril width (mm)	Juice percent
Leaf blade length (cm)	(P) (G)	1.000 1.000	0.018 0.019	-0.062 -0.063	-0.001 -0.002	-0.080 -0.083	0.030 0.031	-0.079 -0.149	-0.014 -0.014	-0.062 -0.063	0.011 0.003
Leaf blade width (cm)	(P) (G)		1.000 1.000	0.108 0.111	0.094 0.102	0.011 0.013	0.0681 0.0698	0.136 0.202	0.126 0.128	0.109 0.111	0.029 0.043
Petiole length (mm)	(P) (G)			1.000 1.000	-0.054 -0.055	-0.048 -0.049	0.007 0.007	0.093 0.149	-0.041 -0.042	-0.063 -0.064	0.002 0.004
Fruit weight (g)	(P) (G)				1.000 1.000	0.739* * 0.770* *	0.660** 0.682**	0.219* 0.293*	0.645** 0.665**	0.711** 0.733**	0.441** 0.574**
Fruit Length (mm)	(P) (G)					1.000 1.000	0.861** 0.870**	0.408** 0.428**	0.648** 0.654**	0.708** 0.714**	0.569** 0.726**
Fruit width (mm)	(P) (G)						1.000 1.000	0.396** 0.624**	0.704** 0.706**	0.738** 0.740**	0.542** 0.697**
Aril weight (mm)	(P) (G)							1.000 1.000	0.350* 0.373*	0.548* 0.578*	0.171** 0.401**
Aril length (mm)	(P) (G)								1.000 1.000	0.938** 0.939**	0.452** 0.584**
Aril width (mm)	(P) (G)									1.000 1.000	0.476** 0.612**
Juice percent	(P) (G)										1.000 1.000

*P=0.05 Significance at 5% and**P=0.01 Significance at 1%

4.1.12 Principal component analysis

4.1.12.1 Principal component analysis of different characters of wild pomegranate genotypes

Principal component analysis, of fourteen characters of wild pomegranate genotypes revealed maximum variations and five component shows Eigen value > 1.0 where PC1 possessed the highest Eigen value (4.90). The data presented in (Table 4.16) and fig (2) showed fourteen characters accounted for 73.88 per cent of the cumulative variance as the five main principal component. The first component accounted for 32.73% variance with maximum variation contribution by fruit weight (0.947), aril width (0.907), fruit length (0.891), fruit width (0.890), aril length (0.865) and juice per cent (0.822). The second component explained 15.44% diversity and recorded the highest contribution by petiole length (0.930), leaf width (0.466), petal length (0.379), rind thickness (0.317) and leaf length (0.276). Third component possessed 9.36% variance with the highest contribution of calyx width (0.720), petal length (0.516), leaf length (0.500) and leaf width (0.318). The fourth component recorded 8.73% variance with major contribution of calyx length (0.657), calyx width (0.385) and petiole length (0.122). The fifth component, explained 7.60% variability with major share of calyx length (0.589), leaf length (0.407) and crown length (0.399).

4.1.12.2 Principal component analysis of biochemical characters of wild pomegranate genotypes

Principal component analysis, of biochemical characters of wild pomegranate genotypes revealed significant variations and only one component showed Eigen value > 1.0 where PC1 possessed the highest Eigen value = 4.00. The data presented in table (4.17) and fig (3) showed six characters accounted for 66.71 per cent of the cumulative variance as one main principal component. The component accounted for 66.71% variance with maximum variation contributions by reducing sugars (0.941), non reducing sugars (0.937), TSS (0.858), ascorbic acid (0.858), titratable acidity (0.844) and total sugars (0.334).

Table 4.16: Principal component analysis for different character of wild pomegranate genotypes of Jammu region

Characters	PC1	PC2	PC3	PC4	PC5
Leaf length (cm)	-0.025	0.276*	0.500	-0.353	0.407
Leaf width (cm)	0.042	0.466*	0.318	-0.011	-0.081
Petiole length (mm)	0.053	0.930*	-0.273	0.122*	-0.021
Calyx length (mm)	0.021	0.055	-0.046	0.657*	0.589*
Calyx width (mm)	-0.072	-0.090	0.720*	0.385*	0.070
Petal length (mm)	0.059	0.379*	0.516*	-0.121	-0.522
Rind thickness (mm)	-0.401	0.317*	-0.287	0.048	-0.356
Aril length (mm)	0.865*	-0.072	0.038	0.024	0.086
Aril width (mm)	0.907*	-0.120	-0.017	-0.009	0.088
Fruit weight (g)	0.947*	-0.051	-0.070	-0.048	-0.026
Fruit length (mm)	0.891*	-0.068	0.027	0.124	-0.150
Fruit width (mm)	0.890*	0.056	0.071	0.023	-0.144
Juice percentage	0.822*	-0.053	-0.120	-0.049	0.008
Crown length (mm)	0.054	0.078	-0.080	-0.734	0.399*
Eigen value	4.90	2.31	1.40	1.31	1.14
Variance (%)	32.73	15.44	9.36	8.73	7.60
Cumulative variance (%)	32.73	48.17	57.54	66.27	73.88

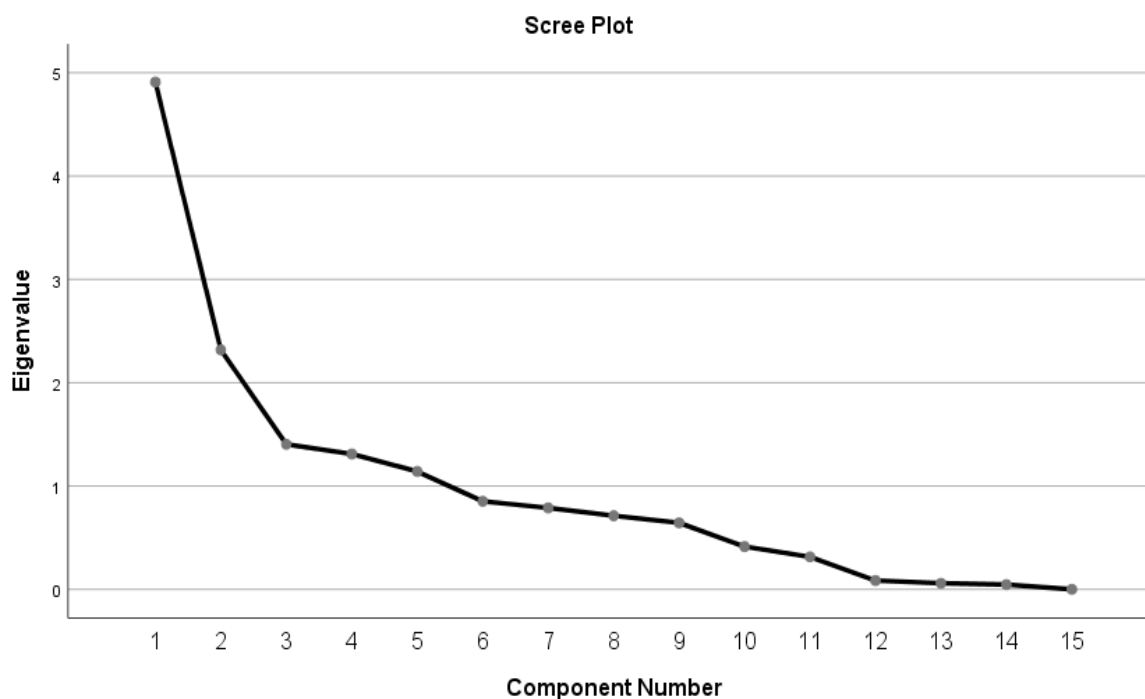


Fig. 1: Principal component analysis of different characters of wild pomegranate genotypes of Jammu region

Table 4.17. Principle component analysis of various biochemical components of wild pomegranate genotypes of Jammu region.

Characters	PC1
TSS ($^{\circ}$ Brix)	0.858
Titrateable acidity (%)	0.844
Reducing sugars (%)	0.941
Non reducing sugars (%)	0.937
Total sugars (%)	0.334
Ascorbic acid (mg/100g)	0.858
Eigen value	4.00
Variance (%)	66.71
Cumulative variance (%)	66.71

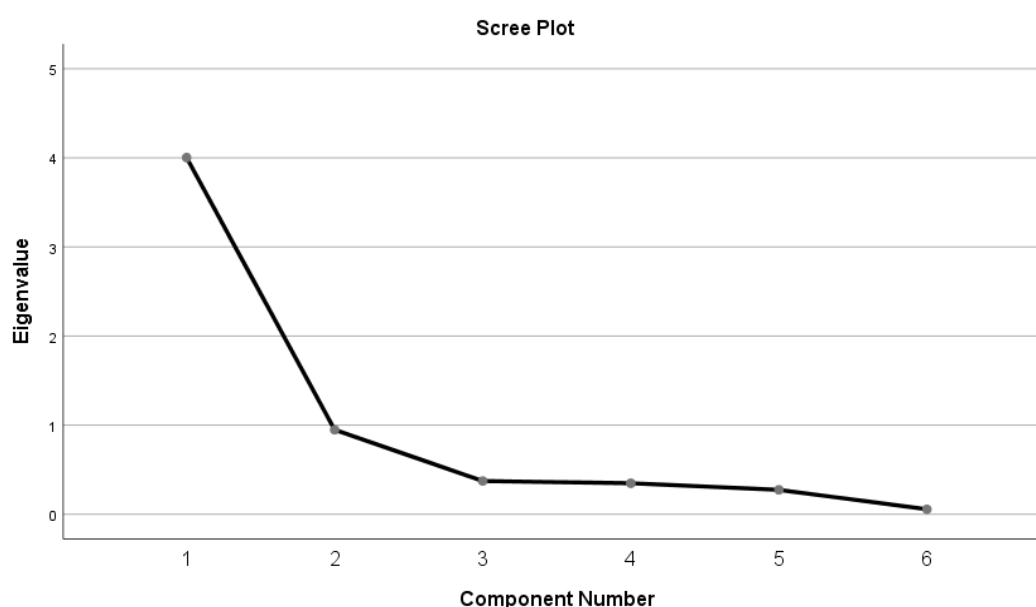


Fig. 2: Principal component of wild pomegranate genotypes based on biochemical characters

4.1.13 Cluster dendrogram

Cluster analysis of seventy wild pomegranate accession based on morphological and biochemical traits of fruits divided into two main clusters namely 1 and 2 with subclusters showed in fig-2. Clusters I comprised of thirty three genotypes and divided into two subclusters cluster 1A and B. Cluster 2nd consist of thirty seven genotypes and and further cluster 2 also divided into 2 subcluster.

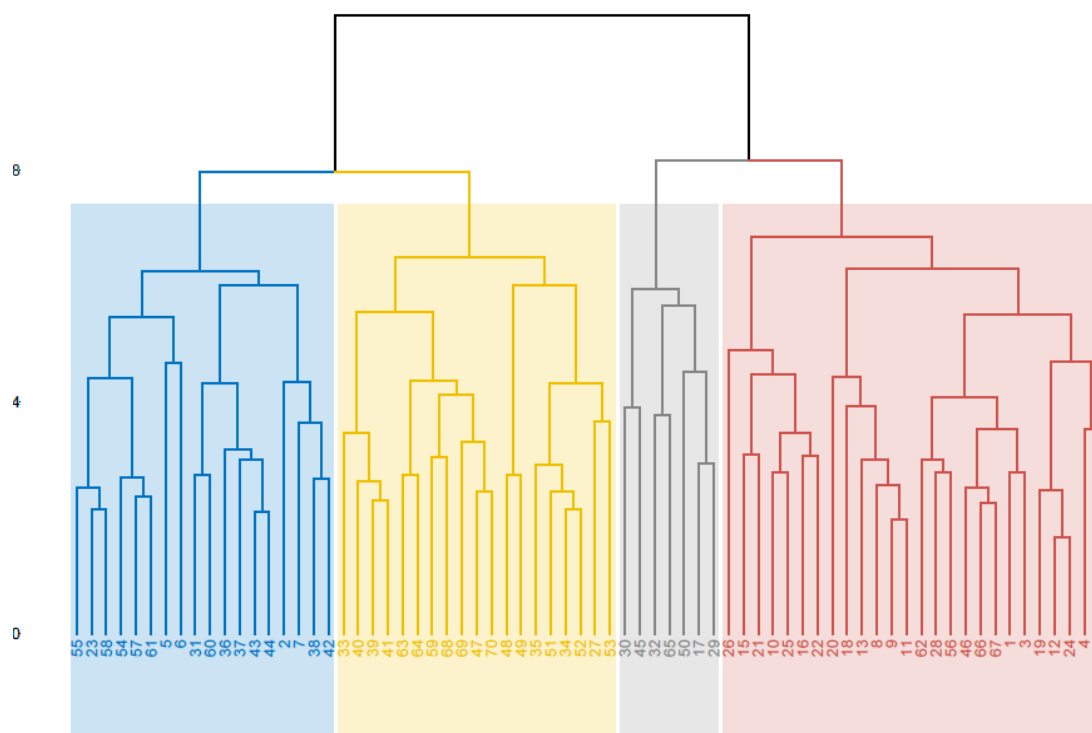


Fig. 3: Cluster dendrogram of seventy wild pomegranate genotypes on the basis of morphological and biochemical characters

4.2 Molecular characterization

4.2.1 SSR analysis

Twenty five SSR primer pairs were used to determine the degree of genetic variability among the 70 walnut genotypes. Only 12 of the 25 SSR primer pairs used to analyse genetic diversity revealed polymorphism. The list of markers, , major allele frequency, number of alleles amplified by each marker and their PIC value are all listed in the (Table 22). The primers produced an average of 3.30 alleles per locus, ranging from 1(**HvSSRT_69**) to 5 (**HvSSRT_104**) alleles. The Polymorphic Information Content (PIC) value of markers is used to measure marker informativeness and ranges from '0' (monomorphic) to '1' (very discriminative). The PIC values ranged from 0.00 (**HvSSRT_69**) to 0.59 (**PGCT093A**) across all genotypes, with an average value of 0.44. In addition to these parameters, major allele frequency values ranged from 0.303 (**Pom021**) to **0.60** (**HvSSRT_823**) with an average value of 0.47.

Table 4.18: List of amplified SSR primers along with major allele frequency, no of alleles scored and polymorphic information content.

S. No	Marker	Major alleles frequency	No. of alleles scored	Polymorphic information content
1	PGCT088	0.424	3	0.52
2	HvSSRT_317	0.454	4	0.48
3	HvSSRT_45	0.393	5	0.21
4	HvSSRT_104	0.454	5	0.14
5	HvSSRT_639	0.454	4	0.48
6	HvSSRT_823	0.606	4	0.33
7	HvSSRT_69	0.575	1	0.00
8	POM_AAC13	0.545	5	0.44
9	Pom006	0.424	3	0.52
10	Pom021	0.303	2	0.091
11	PGCT110	0.545	1	0.00
12	PGCT093A	0.545	3	0.59
	Maximum	0.606	5	0.59
	Minimum	0.393	1	0.00
	Average	0.479	3.30	0.44

4.2.1.2 Cluster analysis

To elucidate the genetic relationships among 70 wild pomegranate genotypes a dendrogram was generated by the Unweighted Pair Group Method based Arithmetic Average (UPGMA) method. The dendrogram revealed a high level of genetic diversity among all wild pomegranate accessions. The genetic relationship between the genotypes is depicted in the dendrogram (Fig 4.15) constructed from the matrix molecular data scored. Based on the dendrogram, the genotypes under study exhibited substantial spread with two major clusters, revealing significant genetic diversity of wild pomegranate genotypes of district Rajouri, Poonch and Udhampur of the Jammu region. The node distinguishing cluster I from Cluster II had a high bootstrap value indicating high variation between the genotypes in cluster I and II. The majority of the genotypes were grouped in cluster II (63 genotypes). Within the 2nd cluster, two sub groups were formed having 63 genotypes with three sub groups. The first cluster consisted of 7 genotypes with two sub groups is depicted in Fig. 4.15.

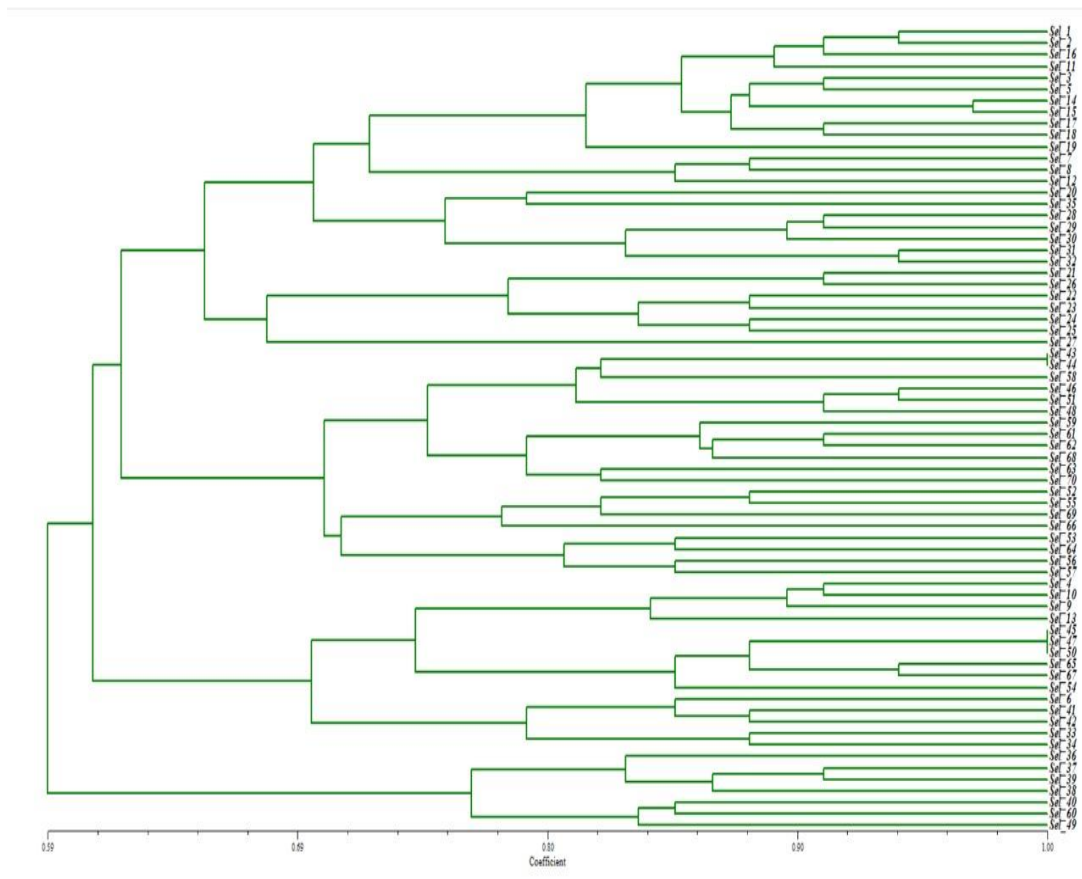


Fig. 4: Cluster analysis pattern of seventy wild pomegranate genotypes based on SSR data

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

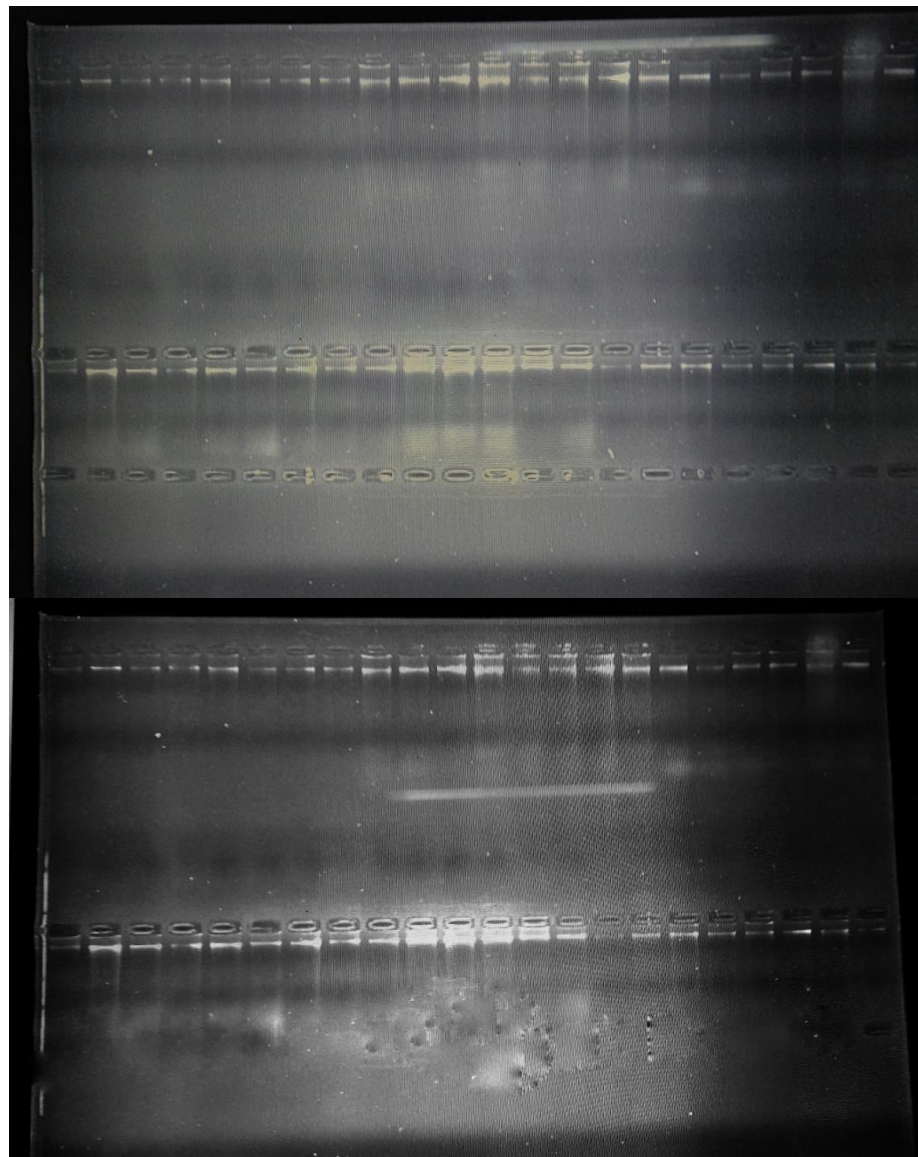


Plate 5: Quality check of DNA isolates of wild pomegranate genotypes

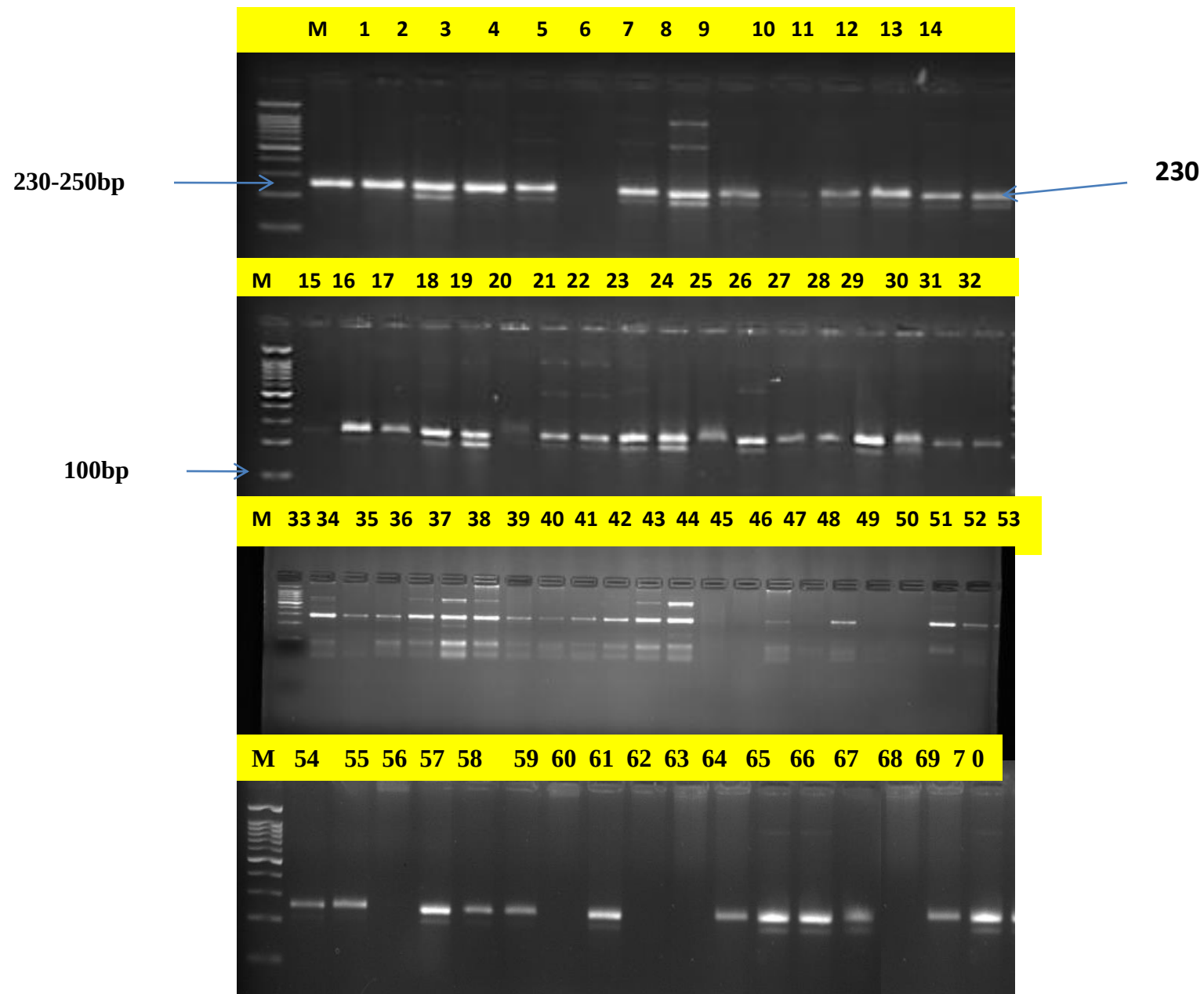


Plate 6: SSR based DNA based amplification pattern of wild pomegranate genotypes with Primer -HvSSRT_45, M depicts 100bp ladder

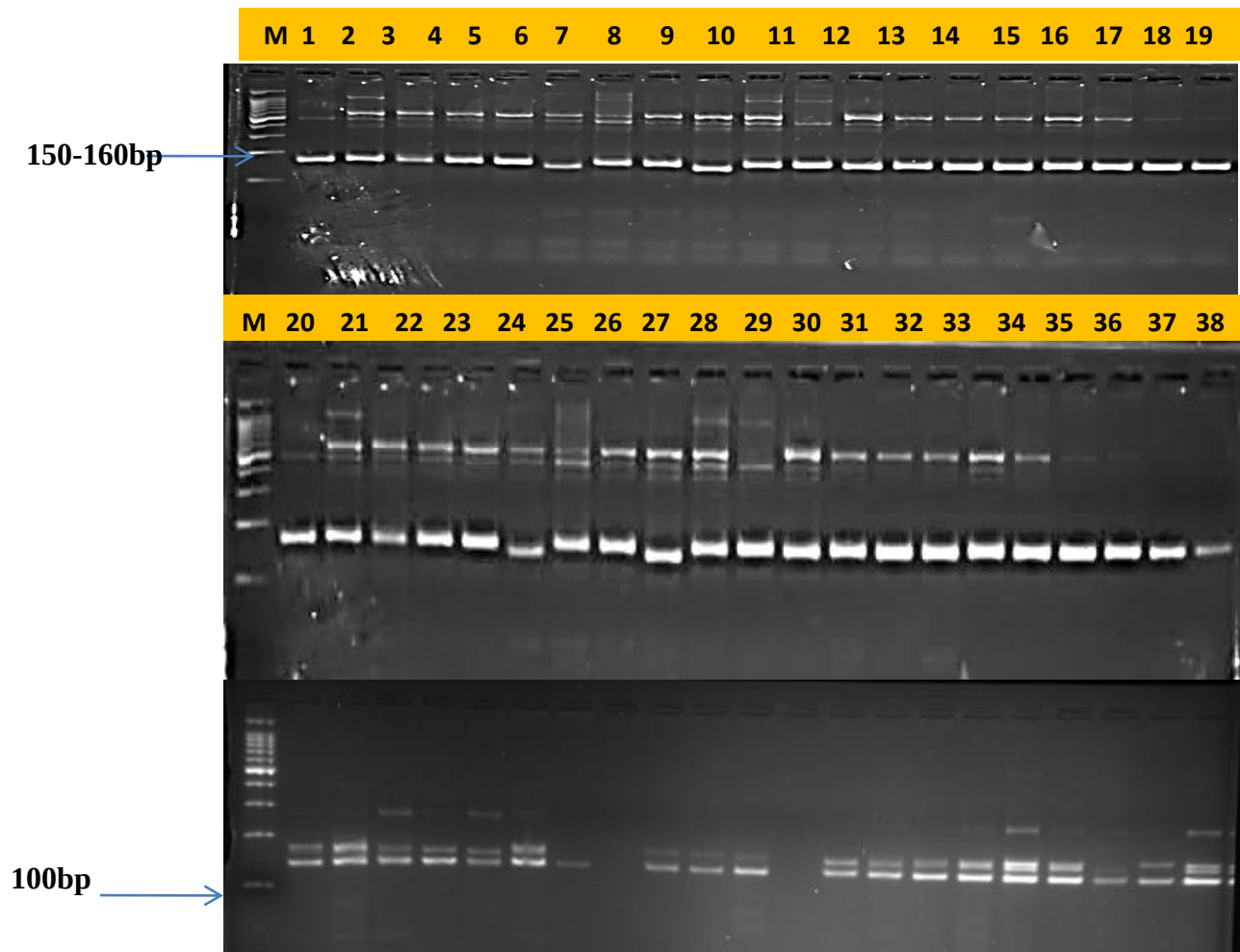


Plate 7: SSR based DNA amplification pattern of wild pomegranate genotypes with primer HvSSRT_104, M depicts 100bp ladder

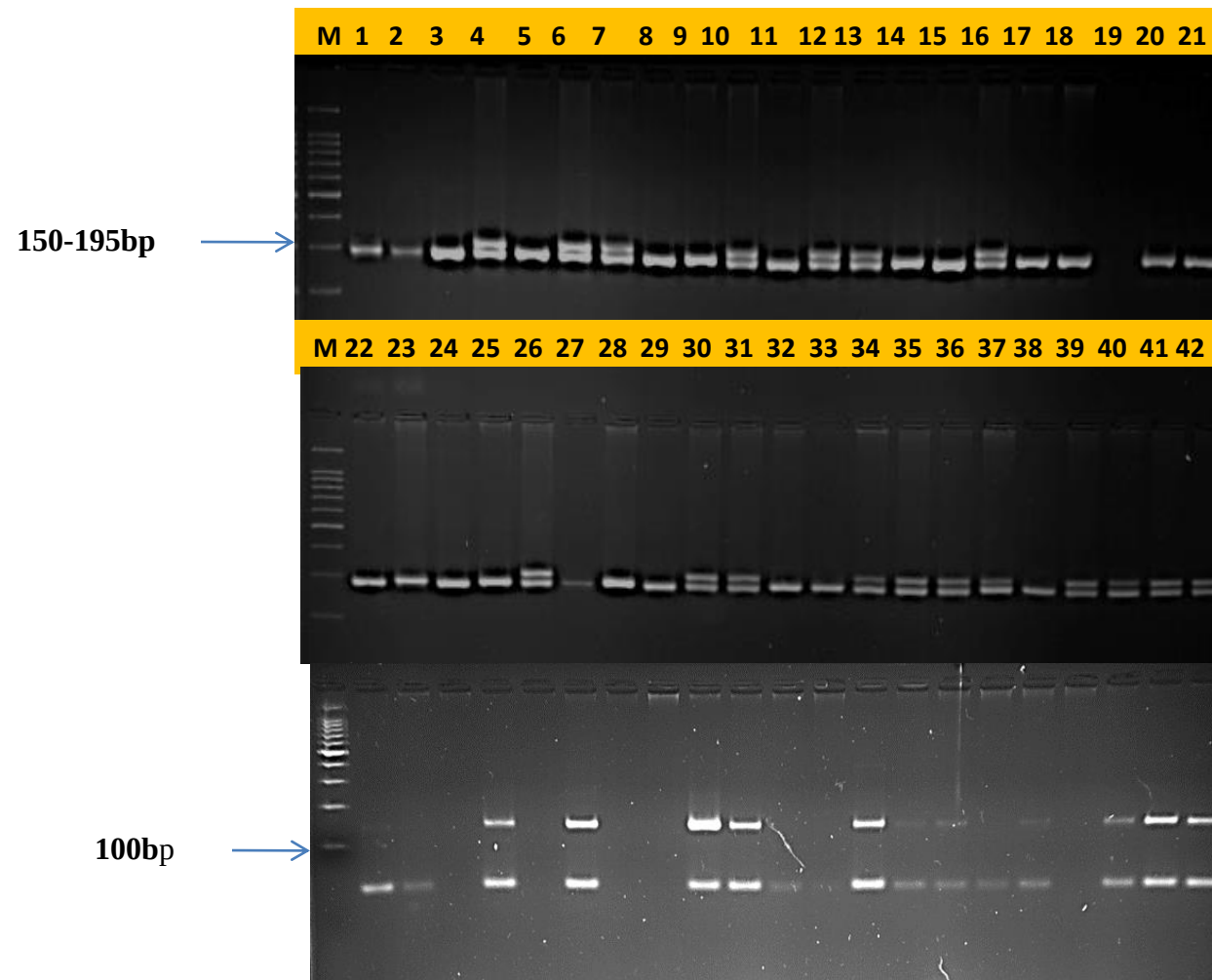


Plate 8: SSR based DNA amplification pattern of wild pomegranate genotypes with primer HvSSRT_639, M depicts 100bp ladder

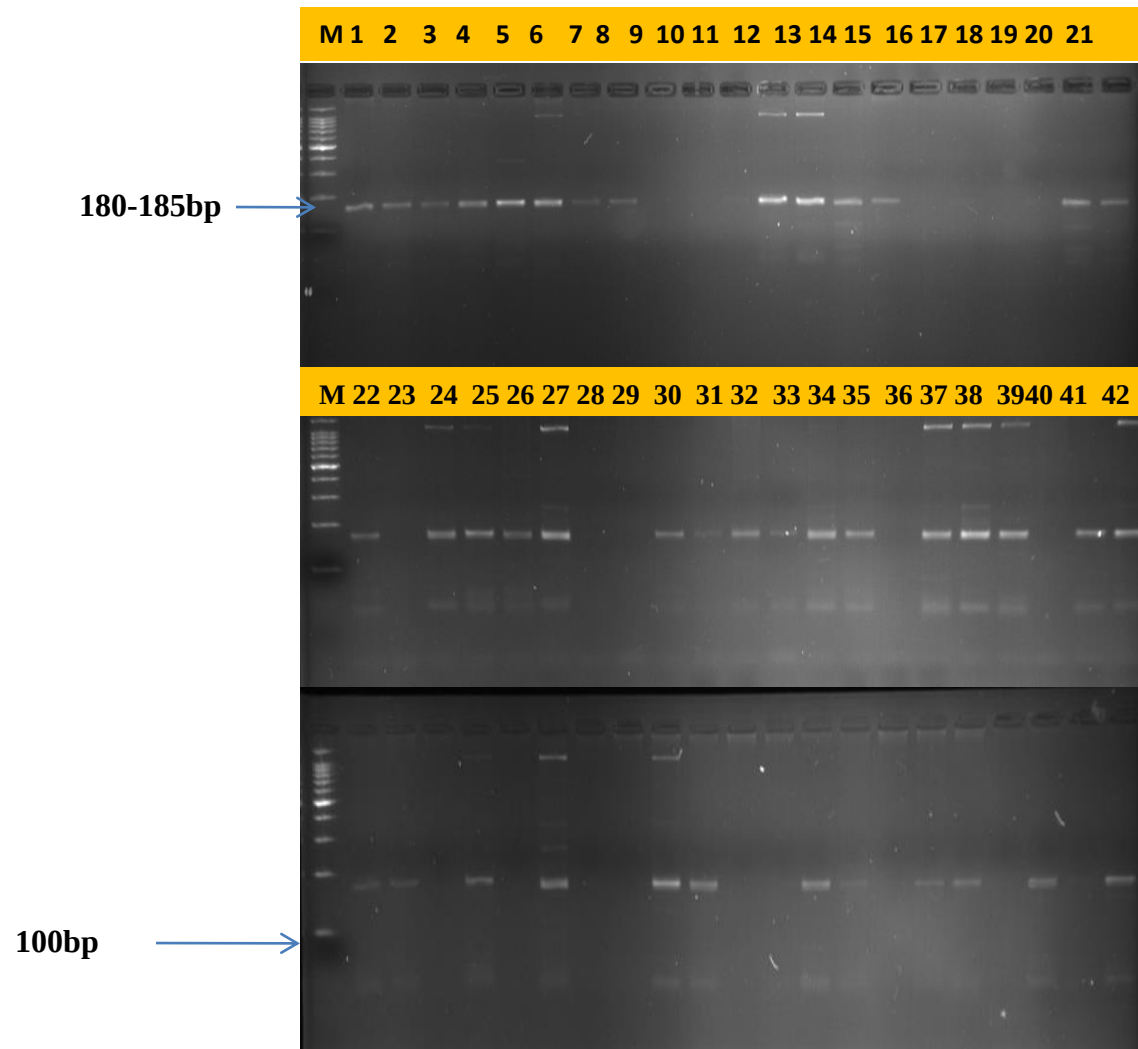


Plate 9: SSR based DNA amplification pattern of wild pomegranate genotypes with primer Pom006, M depicts 100bp ladder

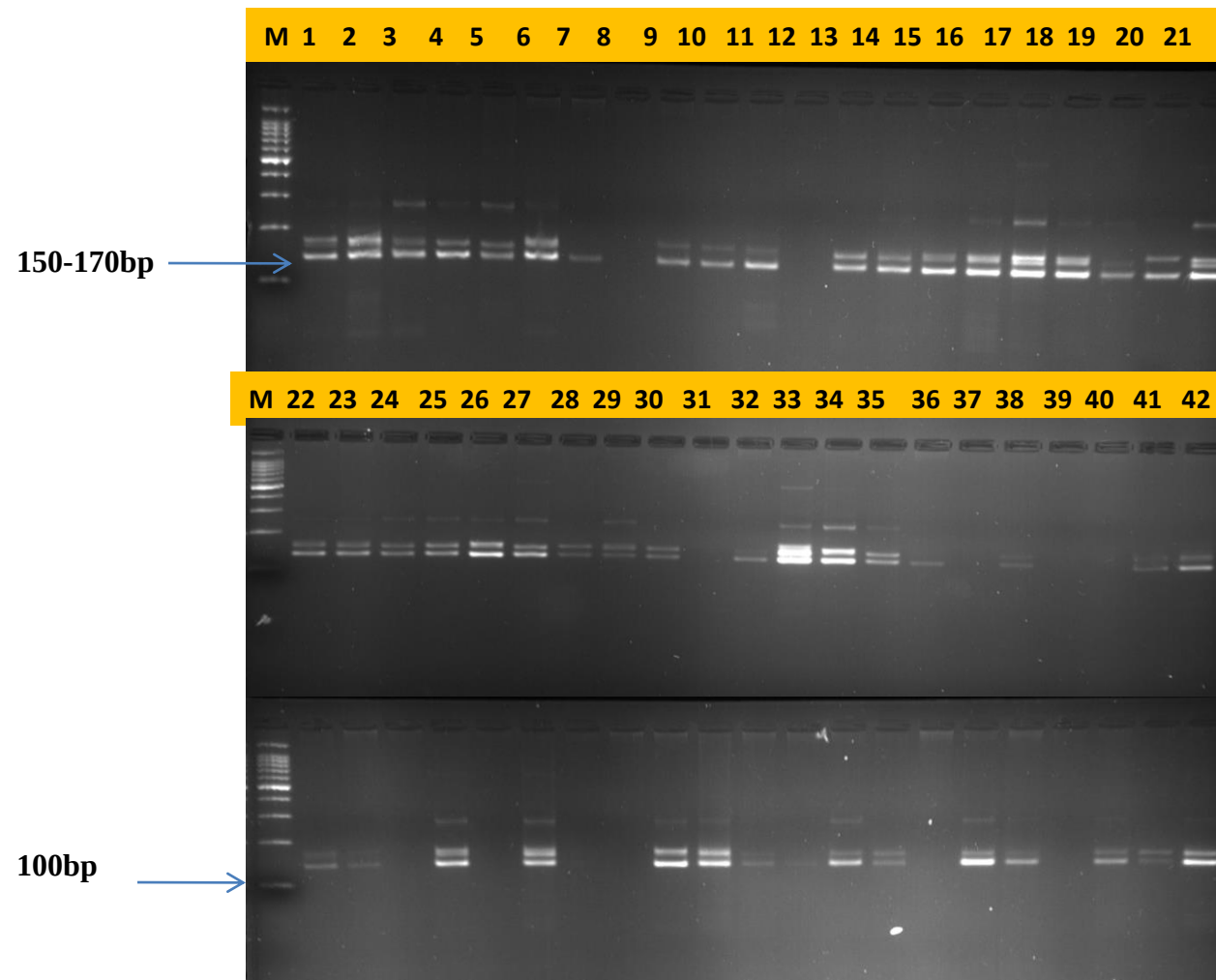


Plate 10: SSR based DNA amplification pattern of wild pomegranate genotypes with primer HvSSRT_823, M depicts 100 bp ladder

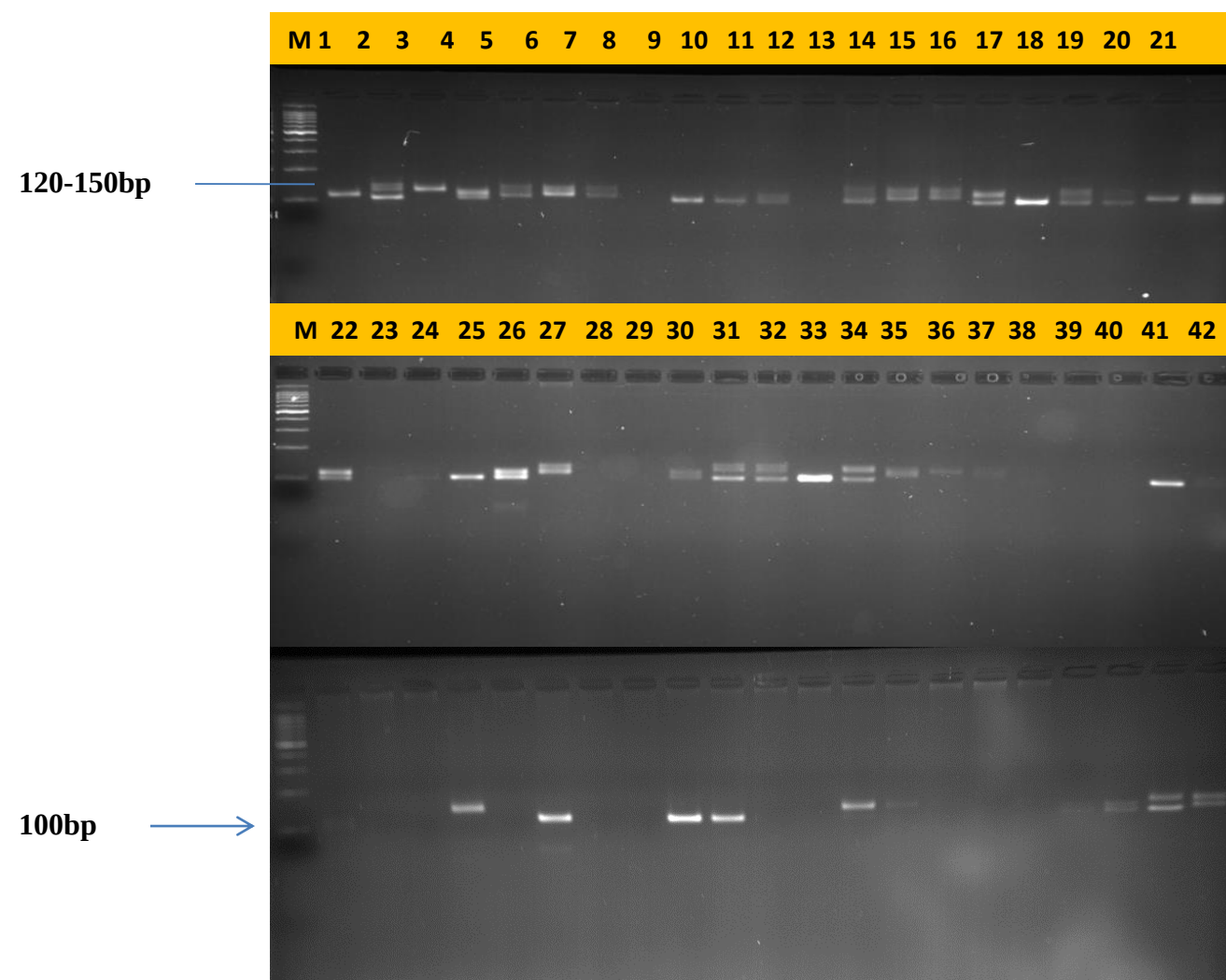


Plate 11: SSR based DNA amplification pattern of wild pomegranate genotypes with primer PGCT093A, M depicts 100bp ladder

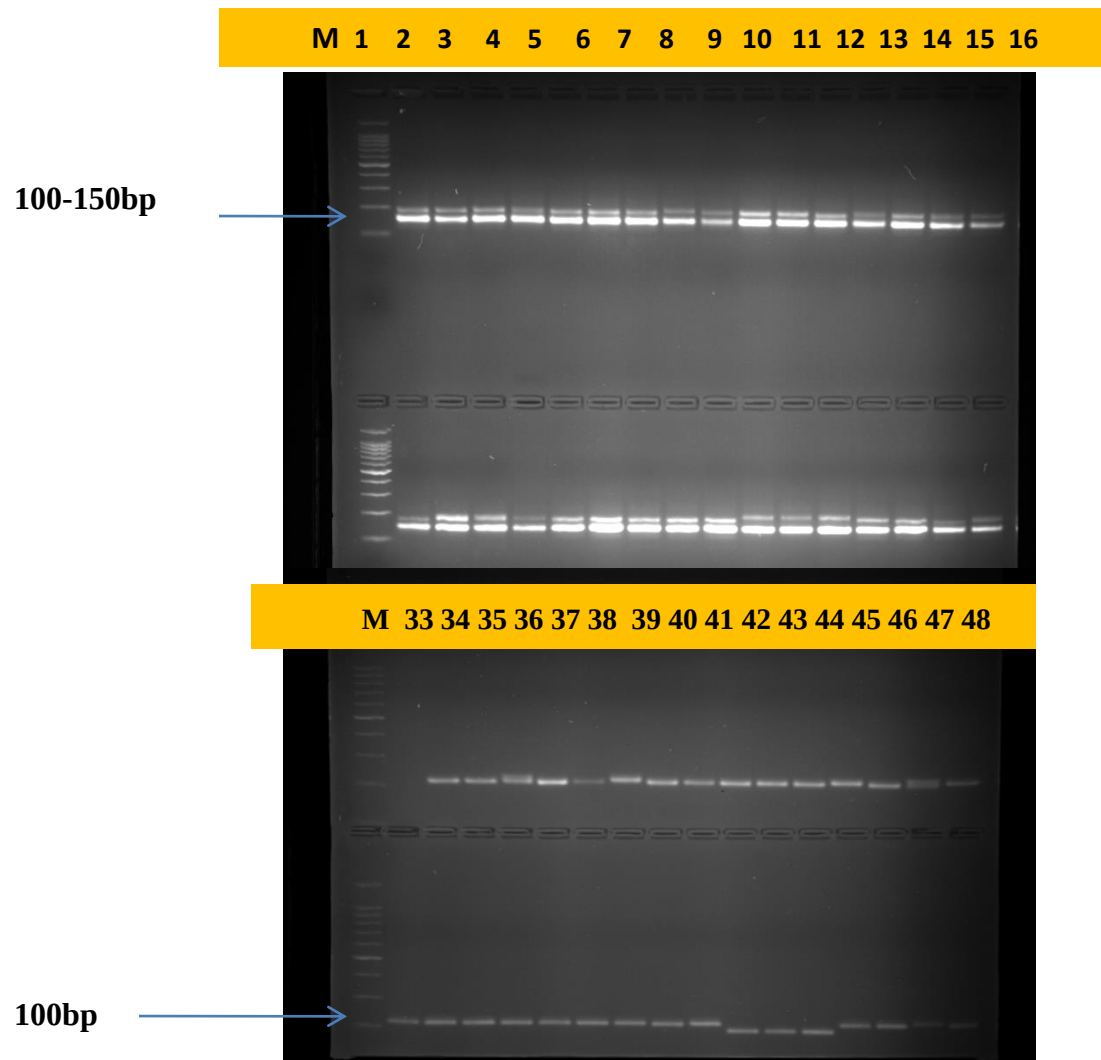


Plate 12: SSR based DNA amplification pattern of wild pomegranate genotypes with primer PGCT088, M depicts 100bp ladder



JWPR (Sel-5)



JWPP (Sel-36)



Plate 13: Best wild Pomegranate genotypes JWPR (Sel-5) and JWPP (Sel-36)



Planting of cuttings



Transplanting of cuttings in nursery



Plate 14: Best wild pomegranate accessions planted at KVK Rajouri

DISCUSSION

Although, pomegranate is originated from Iran but lots of genetic diversity was found in India and sub Himalayan region of Indian continent. So far the distribution of genetic diversity in the pomegranate, the gene pool based on morphological, biochemical parameters and molecular markers has not been well studied. The present study was carried out to characterize 70 wild pomegranate genotypes on the basis of morphological, biochemical parameters and molecular markers. The description of results obtained on various vegetative, biochemical and molecular characters are deliberated below in the light of available literatures and scientific knowledge.

Morphological characterization

Tree characters

A total of 70 wild pomegranate genotypes were characterized on the basis of plant height, growth habit, shoot thorniness, trunk type, leaf parameters like leaf length, leaf breadth, petiole length, fruit parameters like fruit shape, fruit weight, fruit length, fruit width, fruit peel thickness, aril percentage, aril colour and seed hardness. All 70 genotypes of wild pomegranate showed significant differences in their vegetative characters. Maximum plant height (5.55m) was recorded in JWPU (Sel-61) whereas the minimum plant height was observed in (2.05m) in JWPP (SEL-27). The variation in plant height might be due to some amount of genetic influence and the results of the present study are corroborated with Wani *et al.*, (2012) as they observed that the plant height in the range of 2.34 to 4.78 m, Dhiman *et al.* (2017) who recorded plant height range from 4.00 to 5.90m and Ashrifi *et al.* (2023) recorded plant height in wild pomegranate range from 2.5 to 6.5m. Tree growth habit was predominantly spreading (53 accessions), although other growth habit types were observed including upright (17) and these results are in conformity with khadivi *et al.* (2020) who reported that predominant growth habit in wild type pomegranate is spreading type.

Leaf characters

Three leaf shapes were observed: broad elliptic (26 genotypes), elliptic lanceolate (24 genotypes), and lanceolate (20 genotypes). Similar leaf shapes were reported in wild pomegranate accessions by Khadivi *et al.* (2020). Significant variation was recorded in the leaf length, leaf width, and petiole length across 70 wild pomegranate genotypes. The maximum leaf length (6.44 cm) was observed in JWPP (Sel-40), the greatest leaf width (3.23 cm) in JWPP (Sel-48), and the longest petiole (7.70 mm) in JWPU (Sel-62). These results align with the findings of Khadivi *et al.* (2020) and Ashrifi *et al.* (2022), who reported leaf length variation in wild pomegranate accessions ranging from 27.17 to 67.18 mm, and Parashuram *et al.* (2022), who observed a leaf length range from 4.22 to 5.99 cm.

Flowering characteristics

The timing of flowering is crucial for both effective characterization and the success of hybridization programs. The flowering patterns of genotypes are influenced by the current climatic conditions. The diversity in the initiation of flowering is likely attributed to factors such as topography, elevation, and the average temperature of the specific region where the wild pomegranate genotypes are found. Understanding and considering these environmental factors are essential for achieving successful outcomes in breeding programs and ensuring the optimal adaptation of genotypes to their growing conditions. In the present study flowering duration of 70 wild pomegranate genotypes showed variations in flowering characters out of 70 genotypes twenty seven genotypes initiated flowering from 3rd week of April and forty three genotypes showed flowering from 4th week of April and end of flowering from 2nd week of June to 3rd week of June. Maximum petal length (25.64mm), petal width (22.78mm), calyx length (36.44mm) and calyx width (15.87mm) was recorded in JWPP(Sel-36).

Fruit characteristics

Conducting qualitative and quantitative morphological analyses of pomegranate fruits serves as the foundation for comparing genotypes in the context of selection and breeding programs (Mars and Marrakchi, 1999). The outcomes of these studies reveal a significant level of diversity in attributes such as fruit, rind, aril, and seed weight, as well as the wood portion index in both wild and domestic

pomegranate accessions. This diversity is corroborated by the findings of Ercisli *et al.* (2007), Ozguven *et al.* (1997), Hasnaoui *et al.* (2011b), and Martinez *et al.* (2006), who demonstrated substantial variation in parameters like fruit size, rind thickness, aril weight and color, seed hardness and as well as juice contents, TSS, acidity, and ascorbic acid within wild accessions. The selection of genotypes is primarily based on the characteristics of the fruit. Examining fruit diversity is crucial for identifying superior wild pomegranate genotypes in breeding programs and variety development. External fruit quality is assessed through physical attributes, including fruit color, size, shape, and the absence of any visible defects. On the basis of qualitative characters three type of fruit shape were observed including thirty eight genotypes have round shape, eleven genotype have elliptic shape and twenty one genotype have oval shape. The market demand of round shape pomegranate fruits is always higher and most of the studied wild pomegranate fruits were round in shape which is beneficial for improving fruit shape of commercial accessions. Fruit shape and fruit color showed high variability within wild pomegranate genotype four different type of fruit color were observed thirty two genotype have reddish yellow color fruits, twenty five genotype have greenish yellow color fruits, thirteen genotypes have reddish green and three genotypes have green color fruits. Different fruit peel colors and fruit shape were reported in wild pomegranates from India (Parmar and Kaushal, 1982; Kher, 1999; Thakur *et al.*, 2011; Sharma and Thakur (2016), Khadivi *et al.* (2020) and Ashrafi *et al.* (2022) who reported variation in fruit color and shape of wild pomegranate. Fruit size significantly influences consumer preference in the fresh produce market, with weight being the primary factor considered during the selection process. Fruit weight ranged from 28.36 to 111.51g with an average of 61.52g. In several studies on wild pomegranates from India, the range of fruit weight has been reported as follows: 80.50–85.17 g (Parmar and Kaushal, 1982; Kher, 1999), 59.77–101.00 g (Pant, 1995), 55.10–83.50 g (Thakur *et al.*, 2011) and 85.64–131.60 g (Bakshi *et al.*, 2014). In addition, Singh and Kingsley (2008) reported an average fruit weight as 63.00 g. Similarly, Thakur *et al.* (2010) observed an average fruit weight as 64.00 g. Khadivi *et al.* (2020) reported that the fruit weight of wild pomegranates in Iran ranged from 19.20 to 185.00 g with an average of 59.89 g. Fruit length ranged from 37.14 to 50.74 mm and fruit width ranged from 29.72 to 55.65 mm. In several studies on wild pomegranates from India, the range of fruit length has been recorded as follows: 53.10–75.30mm (Pant, 1995) and 46.90–62.80mm (Thakur *et al.*, 2011).

In addition, several studies on wild pomegranates from India reported the range of fruit diameter as follows: 42–66mm (Parmar and Kaushal, 1982), 43.50–65.00mm (Pant, 1995), and 44.60–54.20mm (Thakur *et al.*, 2011). In another study, the average fruit length and fruit diameter have been reported as 58.90mm and 46.40 mm, respectively (Thakur *et al.*, 2010). Moreover, Sharma and Thakur (2016) recorded the average fruit length and fruit diameter of wild pomegranate fruits as 55.99mm and 43.26 mm, respectively. Genetic diversity and soil-climate conditions have an impact on fruit weight, as noted by Martinez *et al.* (2006). In this research, given that the genotypes were assessed across different geographic regions, differences in fruit weight could be attributed to both genetic factors and environmental conditions.

Rind thickness plays a critical role in the selection of fruit, distinguishing those suitable for processing, which typically have thinner peels, from those preferred for fresh consumption due to their thicker peels that ensure better resilience during transportation and storage (Khadivi *et al.*, 2018). Moreover, a thicker peel is advantageous for its potential to prevent peel cracking, offering a protective layer for the edible arils against pests and pathogens that might enter through such cracks (Jalilop, 2005). Variations in rind thickness within wild genotype is recorded and is ranged from 1.69 to 4.97mm similar results were obtained by Akram *et al.* (2015) who observed rind thickness ranged from 1.60 to 6.01mm and Ashrafi *et al.* (2022) recorded 1.41 to 5.44mm in wild pomegranate genotypes. Crown length ranged from 9.24 to 20.43mm and the result are in with Ashrafi *et al.* (2022).

Aril characteristics

Aril characteristics are one the most important criteria to attract the attention of consumers in pomegranate (Mena *et al.*, 2011). Aril length ranged from 2.25 to 7.35mm and aril width varied from 2.98 to 9.14 mm. In addition, fresh weight of 100 arils ranged between 6.12 and 16.18 g with an average of 11.15. In a study on wild pomegranates from India, Kher (1999) reported an average weight of 100 arils as 12.65 g. In another study, an average weight of 100 arils was reported as 15.34 g (Thakur *et al.*, 2010).

The seed softness is one of the important determining factors in the quality and type of pomegranate fruit consumption. In present study 54 genotypes have hard seed and 16 genotypes have semi hard seed type. The soft and semi-soft seeded

accessions are suitable in this important trait to be used for cultivation and in breeding programs for decreasing seed hardness in the commercial pomegranate cultivars. The softer the seeds of pomegranate fruit, the higher the quality and marketability (Mirjalili, 2002). A high range of diversity was observed among the accessions based on aril color including whitered (31), light yellow (10), red (13), medium pink (6), light pink (11) and pink (3). The genotypes showing pink or red arils are targets for pomegranate breeders (Zavala and Cozza, 2012). In a study on wild pomegranates from India, Parmar and Kaushal (1982) observed various colors for arils that ranged from pink to blood red. Furthermore, Bhat (2007) and Sharma and Thakur (2016) recorded pink color for arils, whereas Thakur *et al.* (2011) reported red-purple color for arils of wild pomegranate fruits.

Biochemical characteristics

Significant variations were observed in biochemical characters of wild pomegranate genotypes in terms of TSS, acidity, reducing sugars, non reducing sugars and ascorbic acid. High TSS content is highly desirable industrial traits in pomegranate fruit juice making as it associates with sweetness and flavor especially if it combined low juice acidity and tannin concentration (Shwartz *et al.*, 2009). The TSS ranged from 12.24 to 17.90°B with an average of 15.83°B. In several studies on wild pomegranates from India, the range of TSS has been reported as follows: 12.00-17.00% (Sharma and Sharma, 1990), 10.60-14.00% (Pant, 1995), 15.40-16.50% (Thakur *et al.*, 2011) and 9.30-27.00% (Khadivi *et al.*, 2020). In addition, Parmar and Kaushal (1982) and Singh and Kingsley (2008) reported an average of 15.50 and 16.40% for TSS in wild pomegranates, respectively. Moreover, an average of 20.08% TSS has been recorded by Thakur *et al.* (2010), Rawat *et al.*, (2012) observed 12°B TSS and 18.00% by Sharma and Thakur (2016) in wild pomegranates.

Acidity

Wild pomegranate fruit is valued for its high organic acid content, making it a popular choice as an acidulant in various culinary applications. In present study titrable acidity ranges from 4.17 to 6.91%. According to Kalyankar *et al.* (1952), citric acid is the predominant acid present in this fruit. Damirov and Shukybrov (1958) achieved a citric acid content as high as 9 percent from this fruit. Parmar and Kaushal (1982) reported a titratable acidity of 5.53 percent in wild pomegranate fruit. Studies

conducted in the Nauni area of Solan district (Sharma and Sharma, 1990) revealed a titratable acidity range of 4.6 to 8.7 percent in these fruits. Singh and Kingsley (2008) found the titratable acidity of wild pomegranate fruits to be 6.68 percent, higher than observations by Thakur *et al.* (2010) and Sharma and Thakur (2016), who recorded titratable acidity of 3.96 and 3.87 percent, respectively, in fruits from Sirmour and Mandi districts of Himachal Pradesh.

Sugars

Reducing sugars have aldehyde and keto groups in their structure, which let them take part in reduction reactions. Total sugars include both reducing and non-reducing sugars. Total sugars, reducing sugars and non reducing sugars in present study ranged from 14.27-8.18%, 9.72-5.58% and 2.60-4.55% respectively. Singh and Kingsley (2008) observed that wild pomegranate fruits from Solan district of Himachal Pradesh contained 8.3% reducing sugars and 9.1% total sugars. Meanwhile, Thakur *et al.* (2010) found that fruits from the Narag area of Sirmour district had 7.07% reducing sugars and 7.28% total sugars. In another study by Thakur *et al.* (2011), the range of reducing sugars was reported to be between 6.85% and 7.75%, and total sugars ranged from 7.85% to 8.55%. Bakshi *et al.* (2014) recorded 9.32 to 13.86% total sugar in anardana (Wild pomegranate) or Daru type from Dodda district of Jammu. Additionally, Sharma and Thakur (2016) discovered that the arils of wild pomegranate fruits from Mandi district contained 7.67% reducing sugars and 8.48% total sugars.

Ascorbic acid (mg/100g)

Wild pomegranate fruits are considered to be important sources of ascorbic acid which is also known as vitamin C. Ascorbic acid in the wild pomegranate genotypes range from 14.08 to 24.74mg/100g. Maximum ascorbic acid (24.74mg/100g) was recorded in JWPP (Sel-36) followed by 24.69mg/100g in JWPR (Sel-5). Singh and Kingsley (2008) reported a vitamin C content of 17.24 mg/100g in this fruit from the Solan district of Himachal Pradesh. However, Thakur *et al.* (2010) found the ascorbic acid content to be 21.08 mg/100g in the same fruit from the Narag area of Sirmour district. Thakur *et al.* (2011) reported a range of 16.83 to 21.14 mg/100g for the vitamin C content of wild pomegranate fruit, while Sharma and Thakur (2016) observed an average of 23.06 mg/100g.

Parameters of variability

Coefficient of variability

Analysis of genetic variability parameters in wild pomegranate genotypes demonstrated that phenotypic coefficients of variability were consistently larger in magnitude compared to genotypic coefficients across all traits. However, in the majority of cases, the differences were minimal, highlighting the significant influence of the environment on the expression of all studied traits. The variations in the coefficients of variability, which differed in magnitude from one character to another, further indicate the substantial diversity present within the genotypes studied. The phenotypic and genotypic coefficient of variability were found to be highest for petiole length (36.74.16 per cent and 36.65per cent respectively) which was followed by leaf width (34.16 per cent and 32.88 per cent respectively), fruit weight (32.69 per cent and 32.60 per cent), rind thickness (29.40 per cent and 29.39 per cent), 100 aril weight (27.56 per cent and 27.56per cent), aril length (26.97 per cent and 26.96 per cent), aril width (26.94 per cent and 26.93 per cent respectively), crown length (22.00 per cent and 21.98 per cent respectively). The findings of the current study align with those reported by Manohar *et al.* (1981), indicating that aril number, fruit number, fruit weight, rind weight, and acidity exhibit the highest genotypic coefficient of variation. Generally, phenotypic coefficient of variation was found to be higher than genotypic coefficient variation, suggesting that these traits are relatively stable and not significantly influenced by environmental factors. Moreover, Bist *et al.* (1994) noted the highest coefficient of variation in aril size (32.34%), followed by fruit weight (26.27%). Similarly, Meena *et al.* (2003) recorded the highest genotypic coefficient of variation for fruit weight, seed number, weight of 100 arils, and juice acidity.

Heritability

Traits exhibiting high heritability offer breeders the opportunity to select the best genotypes based on their phenotypic performance. Conversely, traits with medium heritability are significantly influenced by environmental factors and cannot be reliably selected based solely on their phenotypic expression. Traits with low heritability are associated with low genetic gains, indicating a predominance of non-additive gene action. Consequently, the improvement of these traits through selection

is challenging. Heritability in broad sense varied from 63.52 per cent in seed width to 93.83 per cent in fruit length. High heritability was observed for all twenty characters namely calyx width (99.99 per cent), 100 aril weight (99.97 per cent), fruit weight (99.95 per cent), aril length (99.90 per cent), calyx length (99.89 per cent), Total sugars (99.86 per cent) fruit length (93.83 per cent), non reducing sugar (99.84%), crown length (99.80), titratable acidity (99.75 per cent) fruit width (90.71 per cent), fruit length (90.67 per cent), TSS (99.50 per cent), ascorbic acid (99.19 per cent), rind thickness (99.00 per cent), leaf blade length (99.00), aril width (99.00), petiole length (99.56 per cent), reducing sugars (94.67) and leaf width (92.00). Mir *et al.* (2010) also observed high heritability (>90%) for the morphological traits, number of seeds per fruit, fruit volume, fruit weight, fruit diameter, aril weight and acidity. Heritability represents the magnitude of inheritance of vegetative characters. Heritability correlated to the genetic advance. Higher the heritability higher the genetic advance. So, heritability along with genetic advance is useful for the selection. Meena *et al.* (2003) stated that higher (GCV), heritability and genetic advance for the traits like fruit weight, seed weight, seed number, aril percentage. In other experiment higher heritability and higher genetic advance recorded for the characters fruit number, flowering duration and yield (Meena *et al.* 2004). Samadia and Pareek (2006) reported that fruit number, fruit yield and fruit weight had high heritability and genetic advance.

Correlation

The correlation coefficient quantifies the degree of a linear relationship between two variables. A correlation coefficient of 1 indicates a perfect positive correlation, while -1 denotes a perfect negative correlation. Correlation coefficients showed meaningful correlations between some characters. Genotypic correlation coefficients for all the characters were higher than phenotypic correlation coefficients. Fruit weight was highly significantly correlated with fruit length ($r=0.770$), fruit width (0.682), aril weight (0.293), aril weight (0.665), aril width (0.733) and juice per cent (0.574). Fruit length is positively and significantly correlated with fruit width (0.870), aril weight (0.428), aril length (0.654), aril width (0.714) and juice per cent (0.726). Fruit width was positively and highly significantly correlated with aril weight (0.624), aril length (0.706), aril width (0.740) and juice per cent (0.697). Aril weight was positively and significantly correlated with aril length (0.373), aril width (0.578) and

juice per cent (0.401). Aril length was positively and significantly correlated with aril width (0.938) and Juice per cent (0.5584). Aril width was positively and significantly correlated with juice per cent (0.612). The results are in line with Zamini *et al.*, (2013), (Karimi and Mirdehghan, 2013 Khadivi *et al.*, (2020) and Ashrifi *et al.* (2022) recorded similar results in pomegranate.

Principal Component Analysis

Principal Component Analysis (PCA) is a method utilized to highlight variation and reveal prominent patterns within a dataset. Its primary purpose is to facilitate the exploration and visualization of data by simplifying the complexity of high-dimensional datasets while preserving underlying trends and patterns. PCA achieves this by reducing the data into fewer dimensions, effectively serving as condensed summaries of the original features. For PCA, components with eigen values of more than 1.00 were retained to uphold reliability of the final output. Fourteen characters accounted for 73.88 per cent of the cumulative variance as the five main principal component. The first component accounted for 32.73% variance with maximum variation contribution by fruit weight (0.947), aril width (0.907), fruit length (0.891), fruit width (0.890), aril length (0.865) and juice per cent (0.822). The second component explained 15.44% diversity and recorded the highest contribution by petiole length (0.930), leaf width (0.466), petal length (0.379), rind thickness (0.317) and leaf length (0.276). Third component possessed 9.36% variance with the highest contribution of calyx width (0.720), petal length (0.516), leaf length (0.500) and leaf width (0.318). The fourth component recorded 8.73% variance with major contribution of calyx length (0.657), calyx width (0.385) and petiole length (0.122). The fifth component, explained 7.60% variability with major share of calyx length (0.589), leaf length (0.407) and crown length (0.399). Principal component analysis, of biochemical characters of wild pomegranate genotypes revealed maximum variations and two component shows Eigen value > 1.0 where PC1 possessed the highest Eigen value > 4.00. Six characters accounted for 88.21 per cent of the cumulative variance as the two main principal component. The first component accounted for 66.71% variance with maximum variation contributions by reducing sugars (0.941), non reducing sugars (0.937), TSS (0.858), ascorbic acid (0.858), titratable acidity (0.844) and total sugars (0.334). The second component explained

17.49% diversity and recorded the highest contribution by total sugars (0.824), reducing sugars (0.571), TSS (0.233) and titratable acidity (0.379).

The identified characteristics proved to be highly efficient in distinguishing and categorizing the examined genotypes. Moreover, these traits hold significant economic value and serve as practical tools for the selection of genotypes or the development of new cultivars with superior attributes. According to Khadivi-Khub *et al.* (2015), fruit-related traits play a crucial role in distinguishing and analyzing breeding materials when conducting morphological assessments of cultivated pomegranates.

Cluster analysis

Cluster analysis involves grouping similar observations into clusters based on the observed values of multiple variables for each individual. Cluster analysis of seventy wild pomegranate accession based on morphological and biochemical traits of fruits divided into two main clusters namely 1 and 2 with sub-clusters showed in fig2. Clusters I comprised of thirty three genotypes and divided into two sub-clusters cluster 1A and B. Cluster 2nd consist of thirty seven genotypes and further cluster 2 also divided into 2 sub cluster. The significant phenotypic variation observed underscores the value of such germplasm as crucial genetic resources for enhancing pomegranate cultivation. Consequently, it is essential to develop strategies aimed at preserving wild pomegranate germplasm and conserving these invaluable genetic resources.

Molecular characterization

For the efficient use of germplasm resources, it is important to know the amount of genetic variation and the genetic relations between genotypes of a population. This also helps to establish optimal plant breeding programs, to build the genetic map and to evaluate the QTLs (Yilmaz *et al.*, 2012). In the present study, SSR markers were used to study the genetic diversity, relationship and population structure of 70 wild pomegranate genotypes collected from three districts of Jammu Division i.e Rajouri, Poonch and Udhampur. The present study revealed efficient genetic information among the collected wild pomegranate genotypes. Twenty five SSR primer pairs were used to determine the degree of genetic variability among the 70 wild pomegranate genotypes. Only 12 of the 25 SSR primer pairs used to analyse

genetic diversity revealed polymorphism. The primers produced an average of 3.30 alleles per locus, ranging from 1 (HvSSRT_69) to 5 (HvSSRT_104) alleles. The Polymorphic Information Content (PIC) value of markers is used to measure marker informativeness and ranges from '0' (monomorphic) to '1' (very discriminative). The PIC values ranged from 0.00 (HvSSRT_69) to 0.59 (PGCT093A) across all genotypes, with an average of 0.44. In the same way, major allele frequency values ranged from 0.303 (Pom021) to 0.60 (HvSSRT_823) with an average of 0.47. The low level of cross pollination or high rate of asexual propagation in pomegranate could be the cause of low number of alleles per locus which is in accordance with the finding of Parvaresh *et al.* (2012) and Soriano *et al.*, 2011). The results of the present study proved that SSR markers could successfully be used in pomegranate germplasm characterization and molecular breeding (Pirseyedi *et al.*, 2010; Ebrahimi *et al.*, 2010; Koochi-Dehkordi *et al.*, 2007; Soriano *et al.*, 2011; Curro *et al.*, 2010). Average PIC value 0.44, indicating that selected markers could amplify high loci polymorphism in this research and this is in agreement with the results of Soriano *et al.* (2011), Pirseyedi *et al.* (2010) and Ebrahimi *et al.* (2010). Detected range of PIC, heterozygosity, allele frequency showed that genetic base of Pakistani wild and domesticated pomegranates is wide and there is potential to improve genotypes with wide gene base to uplift pomegranate industry by exploring new regions for its production through selective breeding programs. In this research work, high level of genetic diversity was recorded within pomegranate accessions of all 14 regions while this diversity was lower among accessions of different regions which is in accordance with the findings of Narzary *et al.* (2010). This might be due to reason that these regions have a high genetic overlap as a result of relatively high gene flow and this reasoning is strengthened by clonal propagation of pomegranate accessions. These findings are in accordance with Parvaresh *et al.* (2012) who revealed 91.52% of total genetic variation within regions and only 8.48% was estimated among regions. The selected SSR markers were proved high polymorphic in Pakistani pomegranate germplasm as it also proved by Noormohammadi *et al.* (2012) who stated that in combined data of three markers, SSR showed higher molecular diversity than two ISSR and RAPD markers in 36 Iranian pomegranate genotypes.

SUMMARY AND CONCLUSIONS

The present investigation entitled “**Morphological, Biochemical and Molecular Characterization of wild Pomegranate (*Punica granatum* L.) Genotypes of Jammu region**” was carried out to assess the magnitude of variability and to characterize walnut genotypes through morphological, bio-chemical and molecular characterization in order to select elite wild pomegranate genotypes. To exploit the available genetic variation, characterization and evaluation of the genetic potential and variability is must. For incremental improvement of of wild pomegranate, a diverse gene pool is essential. Characterization of genotype at the genetic level supplemented with phenotypic characters could be the first step towards efficient conservation, maintenance and utilization of existing genetic diversity. Assessment of diversity has traditionally been achieved through morphological characters, chemical composition and cytological characters. Usually, the easiest assessment of genetic variation is through systematic morphological or phenotypic measures. UPOV descriptor is an important means for the standardization of wild pomegranate characterization worldwide and provide an easy and rapid way to discriminate between wild pomegranate phenotypes.

Morphological characters are often limited in number and may not be reliable to discriminate between closely related wild pomegranate genotypes. Simple sequence repeat (SSR) markers have proven to be a very powerful tool to analyze genetic variability and relatedness at different hierarchical levels. As co-dominant and locus specific markers, SSR have been widely used as tools in genotype identification and population genetic studies in plants. Various scientists suggested the usefulness of this SSR resource as a powerful tool used for studying genetic variability, pedigree analysis, linkage mapping, genotype identification and for other purposes. Therefore, the present study was undertaken with the objectives to characterize wild pomegranate genotypes on the basis of morphological, bio-chemical and molecular markers. An effort was made to investigate the feasibility of using SSR markers for studying the genetic variation and to estimate genetic relationships among different wild pomegranate genotypes of Jammu region with respect to different morphological diversity analysis and molecular characterization using SSR technique. To achieve the

objectives, the observations were recorded on morphological features of each genotype as per the UPOV guidelines (UPOV, 2011). The 70 genotypes were analyzed for bio-chemical and for molecular characterization. For molecular characterization 25 simple sequence repeat (SSR) loci were used. The results obtained are briefly summarised and presented as under:

Among different tree character studied tree height ranged from 2.05 to 5.55m. Maximum tree height (5.55m) was recorded in JWPU (Sel-61) and minimum tree height (2.05m) was recorded in JWPR (Sel-27). Out of seventy genotypes spreading growth habit was observed in fifty three (75.71%) genotypes and upright growth habit was observed in seventeen (24.28%) genotypes. Trunk type was categorized as multi-trunk and single-trunk. Out of seventy genotypes multi-trunk was observed in sixty five genotypes (92.85%) and single trunk was observed in five (5.71%) genotypes.

Among leaf characters out of seventy wild pomegranate genotypes broad elliptic shape was observed in twenty six (37.14%) genotypes, elliptic lanceolate (34.28%) was observed in twenty four genotypes and twenty (28.57%) genotypes had lanceolate leaf shape. Significant variations were recorded in leaf blade length of wild pomegranate genotypes. Maximum leaf blade length (6.44cm) was recorded in JWPP (Sel-40) and minimum leaf blade length (3.45cm) was recorded in JWPR (Sel-2). Maximum leaf blade width (3.32cm) was recorded in JWPP (Sel-48) and minimum leaf blade width (1.02cm) was recorded in JWPR (Sel-9). Maximum petiole length (7.19mm) was recorded in JWPU (Sel-62) and minimum petiole length (2.06 mm) was recorded in JWPR (Sel-2).

Among flower characters wild pomegranate genotypes have flowering duration of within 47 to 54 days. Out of seventy wild pomegranate genotypes twenty seven genotypes showed their start of flowering 3rd week of April and forty three genotypes showed their start flowering from 4th week of April. Out of seventy wild pomegranate genotypes fifty nine showed end of flowering from 2nd week of June and eleven genotypes showed end of flowering from 3rd week of June. Calyx colour in the wild pomegranate genotypes ranged from orange and red. Out of seventy wild pomegranate genotypes forty five (64.28%) genotypes have orange colored calyx and twenty five (35.71%) genotypes have red colored calyx. Significant variation was recorded within petal length of wild pomegranate genotypes. Maximum petal length

(25.64mm) was recorded in JWPP (Sel-36) followed by (25.60mm) in JWPR (Sel-5). Minimum petal length (14.24mm) was recorded in JWPR (Sel-26). Maximum petal length (22.78mm) was recorded in JWPP (Sel-36) followed by (22.53mm) in JWPR (Sel-5) and minimum (12.34mm) was recorded in JWPR (Sel-26). Maximum petal length (36.44mm) was recorded in JWPR (Sel-36) followed by JWPR (Sel-5) and minimum calyx length (22.17mm) was recorded in JWPR (Sel-4). Maximum calyx width (15.92mm) was recorded in JWPR (Sel-5) followed by (15.87mm) in JWPP (Sel-36) and minimum (10.12mm) was recorded in JWPR (Sel-2).

Among fruit characters fruit shape of wild pomegranate genotypes varied from round, elliptical and oval. Out of seventy wild pomegranate genotypes thirty eight (54.28%) genotypes have round fruit shape, eleven genotypes (15.71%) have elliptical shape and twenty one (30%) have oval shaped fruit. Fruit color of wild pomegranate genotypes. Four types of fruit color were observed in wild pomegranate genotypes categorized into green, reddish green, greenish yellow and reddish yellow. Out of seventy wild pomegranate genotypes three (4.28%) genotypes have green fruits color, thirteen (18.57%) genotypes have reddish green color fruits, twenty five (35.71%) genotypes have greenish yellow color fruits and thirty two (45.71%) have reddish yellow color fruits. Maximum crown length (20.43mm) was recorded in JWPU (Sel-52) and minimum crown length (9.24mm) was recorded in JWPU (Sel-59). Rind thickness of wild pomegranate genotypes ranged from 1.69 to 4.97mm. Maximum rind thickness (4.97mm) was recorded in JWPR (Sel-6) and minimum rind thickness (1.69mm) was recorded in JWPR (Sel-18).

Fruit weight of seventy wild pomegranate genotype ranged from 28.36 to 111.51g. Maximum fruit weight (111.51g) was recorded in JWPP (Sel-36) followed by (110.08g) JWPR (Sel-5) and minimum fruit weight (28.35g) was recorded in JWPR (Sel-9). Fruit length of wild pomegranate genotypes ranged from 30.17 to 57.54mm and maximum fruit length (57.54mm) was recorded in JWPP (Sel-36) followed by (56.33mm) in JWPR (Sel-5) and minimum fruit length (30.17mm) was recorded in JWPR (Sel-9). Fruit width of wild pomegranate genotypes showed significant variation ranged from 29.72 to 55.65mm and maximum fruit width (55.65mm) was recorded in JWPP (Sel-36) followed by (55.30mm) in JWPR (Sel-5) while as minimum fruit width (29.72mm) was recorded in JWPR (Sel-9). Juice per cent shows significant variations within wild pomegranate genotypes. Maximum juice

percent (46.77%) was recorded in JWPP (Sel-36) followed by (46.34) in JWPP (Sel-5) and minimum juice (12.15%) percent was recorded in JWPR (Sel-18).

Among aril characters seed texture of wild pomegranate is categorized into hard, semi hard and soft. Out of seventy wild pomegranate genotypes fifty four (77.14%) have hard seed texture and sixteen (22.85%) genotypes have semi-hard genotypes. Number of arils in wild pomegranate genotypes ranges from 104.33 to 452.00. Maximum number of arils (452.00) was recorded in JWPP (Sel-36) followed by (394.00) in JWPR (Sel-5) and minimum number of arils (104.33) was recorded in JWPR (Sel-18). Weight of 100 arils in wild pomegranate genotypes ranged from 6.12g to 16.18g. Maximum aril weight (16.18g) was recorded in JWPP (Sel-36) followed by (15.13g) JWPR (Sel-5) and minimum aril weight (6.12g) was recorded in JWPR (Sel-18). Maximum aril length (7.35mm) was recorded in JWPP (Sel-36) followed by (6.44mm) JWPR (Sel-5) while as minimum aril length (2.25mm) was recorded in JWPR (Sel-18).) Aril width in wild pomegranate genotypes ranged from 2.98 to 9.14mm and maximum aril width (9.14mm) was recorded in JWPP (Sel-36) followed by (8.75mm) in JWPR (Sel-5) while as minimum aril width (2.98mm) was recorded in JWPR (Sel-18).

Among yield characters maximum number of fruits (88) was recorded in JWPR (Sel-13) and minimum number of fruits (16) was recorded in JWPP (Sel-35). Maximum yield (7.02kg/tree) was recorded in JWPP (Sel-36) followed by (6.27 kg/tree) in JWPR (Sel-5) and minimum yield (1.49 kg/tree) was recorded in JWPP (Sel-35).

Among biochemical characters maximum TSS (17.90°Brix) was recorded in JWPP (Sel-36) followed by (17.80°Brix) in JWPR (Sel-5) and minimum (12.24°Brix) was recorded in JMU-WP (Sel-26). Highest total sugar (14.47%) was recorded in JWPP (Sel-36) followed by (14.25%) in JWPR (Sel-5) while lowest (8.19%) total sugar was found in JWPR (Sel-26). Maximum reducing sugar (9.74%) in JWPP (Sel-36) followed by (9.71%) in JWPR (Sel-5) while minimum (5.58%) reducing sugar was found in JWPR (Sel-26). Maximum (4.73%) non reducing sugar recorded in JWPP (Sel-36) followed by (4.54%) in JWPR (Sel-5) and minimum (2.60%) reducing sugars was recorded in JWPR (Sel-26). Significant variation was recorded in titratable acidity of all genotypes whereas maximum (6.93%) titratable acidity recorded in

JWPP (Sel-36) followed by (6.86%) JWPR (Sel-5) and minimum titratable acidity (4.16%) was recorded in JWPR (Sel-26). Maximum (24.74%) ascorbic acid recorded in JWPP (Sel-36) followed by (24.69%) in JWPR (Sel-5) and minimum (14.08%) ascorbic acid was recorded in JWPR (Sel-26).

Estimates of genetic variability parameters for various characters in wild pomegranate genotypes revealed that the phenotypic coefficient of variability were higher in magnitude than the genotypic coefficient of variability for all the characters under study. The phenotypic and genotypic coefficient of variability were found to be highest for petiole length (36.74 % and 36.65 %) which was followed by leaf width (34.16 % and 32.88 %), fruit weight (32.69 % and 32.60 %), rind thickness (29.40 % and 29.39 %), 100 aril weight (27.56 % and 27.56 %), aril length (26.97 % and 26.96 %), aril width (26.94 % and 26.93 %), crown length (22.00 % and 21.98 %), non reducing sugars (18.77 % and 18.74 %), reducing sugars (16.91 % and 16.01 %), leaf length (16.14 % and 16.09 %), fruit width (15.98 % and 15.97 %) and fruit length (15.46 % and 15.41 %) whereas lowest phenotypic and genotypic coefficient of variability were observed in TSS (8.90 % and 8.88 %), respectively. Heritability in broad sense varied from 63.52 per cent in seed width to 93.83 per cent in fruit length. High heritability was observed for all twenty characters namely calyx width (99.99 %), 100 aril weight (99.97 %), fruit weight (99.95 %), aril length (99.90 %), calyx length (99.89 %), Total sugars (99.86 %) fruit length (93.83 %), non reducing sugar (99.84 %), crown length (99.80 %), titratable acidity (99.75 %), fruit width (90.71 %), fruit length (90.67 %), TSS (99.50 %), ascorbic acid (99.19 %), rind thickness (99.00 %), leaf blade length (99.00 %), aril width (99.00 %), petiole length (99.56 %), reducing sugars (94.67 %) and leaf width (92.00 %).

Genotypic correlation coefficients for all the characters were higher than phenotypic correlation coefficients. Fruit weight was and highly significantly correlated with fruit length ($r=0.770$), fruit width (0.682), aril weight (0.293), aril weight (0.665), aril width (0.733) and juice per cent (0.574). Fruit length is positively and significantly correlated with fruit width (0.870), aril weight (0.428), aril length (0.654), aril width (0.714) and juice per cent (0.726). Fruit width was positively and highly significantly correlated with aril weight (0.624), aril length (0.706), aril width (0.740) and juice per cent (0.697). Aril weight was positively and significantly correlated with aril length (0.373), aril width (0.578) and juice per cent (0.401). Aril

length was positively and significantly correlated with aril width (0.938) and Juice per cent (0.5584). Aril width was positively and significantly correlated with juice per cent (0.612).

Principal component analysis, of fourteen characters of wild pomegranate genotypes revealed maximum variations and five component shows Eigen value > 1.0 where PC1 possessed the highest Eigen value (4.90). Fourteen characters accounted for 73.88 per cent of the cumulative variance as the five main principal component. The first component accounted for 32.73% variance with maximum variation contribution by fruit weight (0.947), aril width (0.907), fruit length (0.891), fruit width (0.890), aril length (0.865) and juice per cent (0.822). The second component explained 15.44% diversity and recorded the highest contribution by petiole length (0.930), leaf width (0.466), petal length (0.379), rind thickness (0.317) and leaf length (0.276). Third component possessed 9.36% variance with the highest contribution of calyx width (0.720), petal length (0.516), leaf length (0.500) and leaf width (0.318). The fourth component recorded 8.73% variance with major contribution of calyx length (0.657), calyx width (0.385) and petiole length (0.122). The fifth component, explained 7.60% variability with major share of calyx length (0.589), leaf length (0.407) and crown length (0.399). Among biochemicals six characters accounted for 66.71 per cent of the cumulative variance as one main principal component. The component accounted for 66.71% variance with maximum variation contributions by reducing sugars (0.941), non reducing sugars (0.937), TSS (0.858), ascorbic acid (0.858), titratable acidity (0.844) and total sugars (0.334).

Cluster analysis of seventy wild pomegranate accession based on morphological and biochemical traits of fruits divided into two main clusters namely 1 and 2 with subclusters. Clusters I comprised of thirty three genotypes and divided into two subclusters cluster 1A and B. Cluster 2nd consist of thirty seven genotypes and and further cluster 2 also divided into 2 subcluster.

For molecular characterization twenty five SSR primer pairs were used to determine the degree of genetic variability among the 70 walnut genotypes. Only 12 of the 25 SSR primer pairs used to analyse genetic diversity revealed polymorphism. The PIC value, major allele frequency and polymorphic information content percentage of markers are all listed in the (Table 22). The primers produced an average

of 3.30 alleles per locus, ranging from 1(HvSSRT_69) to 5 (HvSSRT_104) alleles. The Polymorphic Information Content (PIC) value of markers is used to measure marker informativeness and ranges from '0' (monomorphic) to '1' (very discriminative). The PIC values ranged from 0.00 (HvSSRT_69) to 0.59 (PGCT093A) across all genotypes, with an average of 0.44. In the same way, major allele frequency values ranged from 0.303 (Pom021) to 0.60 (HvSSRT_823) with an average of 0.47. Based on the dendrogram, the genotypes under study exhibited substantial spread with two major clusters, revealing significant genetic diversity of wild pomegranate genotypes of district Rajouri, Poonch and Udhampur of the Jammu region. The node distinguishing cluster I from Cluster II had a high bootstrap value indicating high variation between the genotypes in cluster I and II. The majority of the genotypes were grouped in cluster II (63 genotypes). Within the 2nd cluster, two sub groups were formed having 63 genotypes with three sub groups. The first cluster consisted of 7 genotypes with two sub groups.

Conclusion

Based on the results it can be inferred that genotypes under study were found diverse in morphological (fruit characters, aril characters, yield efficiency), biochemical (TSS, titratable acidity, total sugars and ascorbic acid) and molecular characters. JWPR (Sel-5 and Sel-36) showed the best result among all the collected genotypes. Maximum fruit weight (110.01 and 111.51g), fruit length (56.53 and 57.54 mm), fruit width (55.30 and 55.91 mm), no of arils (394 and 452), 100 aril fresh weight (15.13 and 16.18g), aril width (6.44 and 7.27 mm), aril length (8.75 and 9.14 mm) and highest fruit yield (7.02 and 6.27 kg/bush) were recorded in wild pomegranate genotypes JWPR (Sel-5) and JWPP (Sel-36) respectively. Similarly biochemical characteristics were also recorded maximum in JWPR (Sel-5 and Sel- 36). Maximum TSS (17.90 and 17.80⁰ Brix, titratable acidity (6.84 and 6.91%) total sugar (14.27 and 14.16%), reducing sugar (9.66 and 9.72%), non reducing sugar (4.50 and 4.55%) and ascorbic acid (23.19 and 23.44mg/100g) respectively. The molecular characterization of seventy wild pomegranate genotypes was carried out with 25 microsatellite markers and 12 markers had shown polymorphism. Polymorphic information content ranged from PGCT093A (0.595) and HvSSRT_104 (0.14) with an average of 0.482. Dendrogram was generated using SSR data to determine pairwise distance between all possible pairs of wild genotypes, two main clusters were obtained with sub-

clusters. JWPR (Sel 5 and 36) that showed the excellent traits of interest and high genetic dissimilarity can be used for variety release or can be further used in breeding programmes to get segregates.

It is therefore recommended that these promising wild pomegranate genotypes may be used for further multiplication and breeding programmes in order to enhance the production, productivity and quality of wild pomegranate in the UT of J&K.

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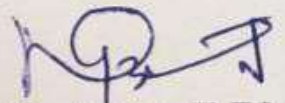
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CERTIFICATE-IV

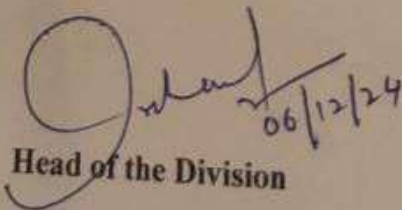
Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled "**MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF WILD POMEGRANATE (*Punica granatum* L.) GENOTYPES OF JAMMU REGION**" submitted by Ms. Safina Kosser, Registration No. J-19-D-370-A.



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