Genetic variability and divergence studies in quantitative traits of Wild Pomegranate (*Punica granatum* L)

Abstract:

The mean performance and variability in quantitative traits of *Punica granatum* seed sources collected from five different districts comprising of ten seed sources. Two seed sources were considered from each district viz; Narag and Neripul from Sirmour district, Waknaghat and Sadhupul from Solan district, Sundernagar and Rewalsar from Mandi district, Mohal and Banjar from Kullu district, Basantpur and Sunni from Shimla district of Himachal Pradesh, India, were evaluated during the year 2018-19. The quantitative traits viz., morphological and seedling characteristics of seed sources varied significantly among the different districts and within the districts. The phenotypic coefficient of variation (PCV) for all the characters was slightly higher than genotypic coefficient of variation (GCV), which signified the presence of environmental influence to some degree in the phenotypic expression of characters. Collar diameter had the highest PCV (76.83) and GCV (26.67). Genetic advance was recorded as maximum for tree diameter per plant (5.57), whereas genetic advance as percent of mean was highest for tree height (4.86). Highest heritability (H^2 , 99.77) was coupled with higher genetic advance (5.57) estimated for tree diameter per plant, which indicated that the character is controlled by additive genes and therefore further improvement could brought by selection. Tree diameter was significantly and positively associated with most of the characters except number of branches per plant, at both genotypic and phenotypic levels; therefore, these are important prerequisites to formulate a successful improvement program. Cluster analysis was performed and the seed sources were grouped into three clusters. The results showed significant variance among different morphological characters, traits namely tree height (m), tree diameter (cm), crown spread E-W (m), crown spread N-S (m), fruit weight (g), leaf length(cm), internodal length(cm), collar diameter (mm), number of branches per plant and leaf petiole (cm)

Key words: Correlation, Coefficient of variations, genetic variability, genetic advance, heritability, strawberry, *Punica granatum* L, Wild Pomegranate

1. Introduction:

Knowledge of genetic variability of a species is essential for the initiation of tree-breeding programs, as it affects not only the composition of group variation but also the evolutionary potentialities of the group concerned. (Kaushik et al., 2007). Introduction and evaluation of provenances and seed sources is a critical aspect in forestry research. Considerable genetic variation in quantitative traits can be expected at the level of provenance and seed sources and this variation may be useful as a source of future genetic selection in tree improvement programs, particularly selection of seed sources having desirable characteristics. The wild pomegranate (Punica granatum L.) also known as daru, dalim or dadima, is a deciduous agro-forestry and horticulture cash crop. It is thought to have originated in South West Asia, most likely in Iran and some neighboring countries (De Candolle, 1967). Pomegranate is cultivated in Central Asia and to some extent in the USA (California), Russia, China, and Japan for fruit production, and it is also grown as an ornamental tree in East Asia (Mars, 1996; Ercisli et al., 2007). Pomegranate raisins (anardana) are dried arils of wild pomegranates that are manually separated from the rind and septa of the fruit and sun- or air-dried. Pomegranate raisins have a distinct sour or tart flavor, and are commercially available in many West and East Asian countries, where they are consumed in large quantities (Jaiswal et al., 2010). It often grows as shrub or small tree. In India, it is found in vast tracts of hill slopes of Himachal Pradesh, Jammu & Kashmir and Uttarakhand at altitudes ranging from 900 to 1800 m amsl. In Himachal Pradesh, it is distributed in some pockets of Solan, Sirmour, Mandi, Shimla, Kullu and Chamba districts (Bhrot, 1998). This species provides a good source of income for villagers. Seeds with aril is sun dried and commercially marketed as Anardana which is a good source of vitamin C, sugar and iron. The powdered flower buds are used in Bronchitis. Fruit rind, juice of leaves, and young fruits are used for the treatment of gastrointestinal disorders. The fruit rind, root, stem bark, and leaves are good sources of tannin, The pomegranate and its usage and deeply embedded in human history, and utilization is found in many ancient human cultures as food and as a medical remedy. Despite this, pomegranate cultivation has been relegated to the status of a minor crop. Keeping in view the economic importance of this species present study was conducted to identify the best seed source and to explore the variation in quantitative traits in *Punica granatum* seed sources collected from five districts of Himachal Pradesh of India.

2. Materials and Methods

2.1 Data Description:

A survey was conducted in five districts of Himachal Pradesh to explore the natural population of *Punica granatum* and geographic coordinates recorded for each seed source are presented in Table 1.

Table 1: Geograpical coordinates of seed sources

Seed Source	**District**	**Latitude(N)**	**Longitude (E)**
Basantpur	Shimla	31.2545°	77.3758°
Sunni	Shimla	31.1190°	77.6426°
Narag	Sirmour	30.8170°	77.1880°
Neripul	Sirmour	31.0370°	77.3671°
Waknaghat	Solan	31.0079°	77.0881°
Sadhupul	Solan	30.9964°	77.1622°
Mohal	Kullu	31.9149°	77.1169°
Banjar	Kullu	31.6377°	77.3441°
Rewalsar	Mandi	31.6322°	76.8332°
Sundernagar	Mandi	31.5299°	76.8889°
Basantpur	Shimla	31.2545°	77.3758°

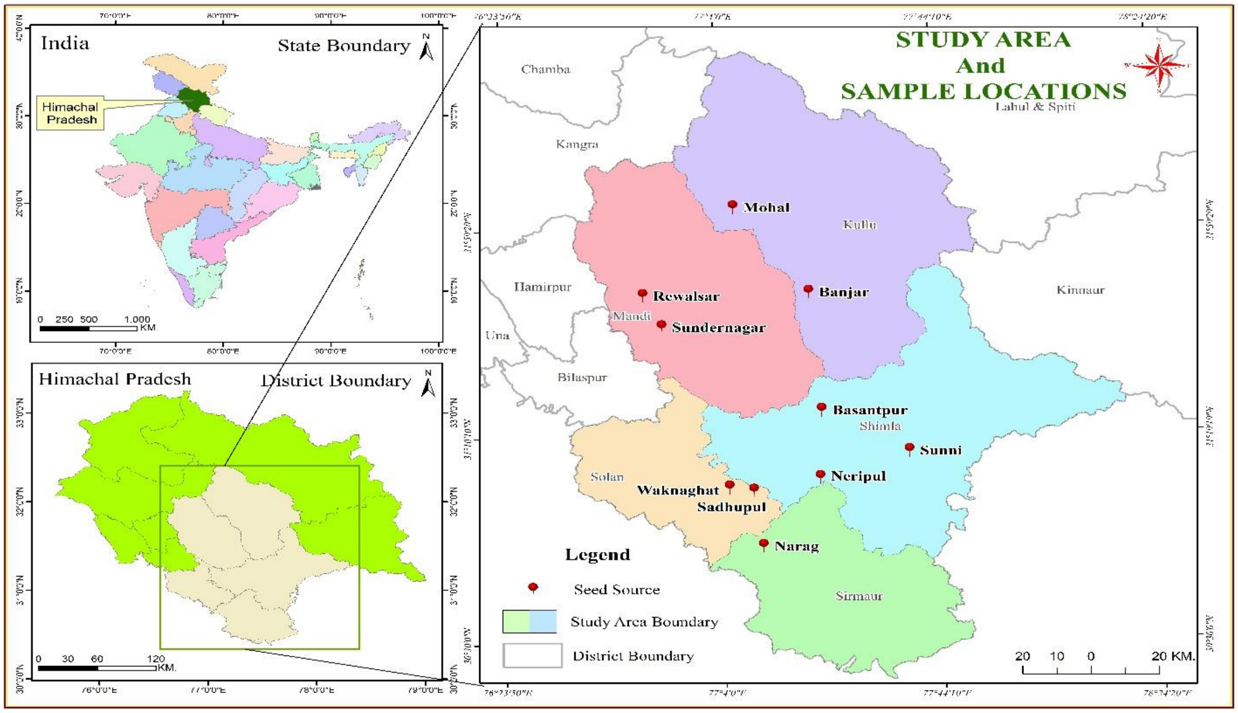


Fig. 1: Study area and Sample locations

The data on various morphological characteristics viz. tree height (m), tree diameter (cm), crown spread E-W (m), crown spread N-S (m), fruit weight (g), leaf length (cm), internodal length (cm) were collected from each seed source and nursery were raised for each seed source and Collar diameter (mm), Number of branches per plant and Leaf petiole (cm) were recorded. Descriptive statistics of each characteristics has been presented in Table 2.

Table 2: Descriptive statistics for characteristics of *Punica granatum*:

Characters	**Mean**	**Max**	**Min**	**Coefficient of variation**
Tree height	6.75	8.04	4.36	23.43
Tree diameter	12.31	14.26	9.91	13.57
Crown Spread E-W	4.39	5.04	3.52	13.29
Crown Spread N-S	6.23	6.74	5.56	7.33
Leaf length	4.72	5.30	3.93	12.03
Fruit Weight	25.58	27.71	24.06	5.46
Number of branches	7.03	8.82	6.27	15.58
Collar diameter	2.13	2.94	1.76	23.83
Leaf petiole	0.45	0.48	0.39	7.88
Internodal length	3.47	3.74	3.34	4.91

2.2 **Statistical Methods:**

2.2.1 Genetic Estimates

Genetic estimates i.e. phenotypic variance (Vp), genotypic variance (Vg), environment variance (Ve), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), environment coefficient of variation (ECV), broad sense heritability, genetic advance and genetic gain were computed for each character to study the variation among seed sources. Genotypic and phenotypic correlation describes the degree of relationship between characters, which may facilitate the figuring out of a further criterion for selection in breeding program. The improvement program should pay attention to correlated quantitative characters because improving one character can lead to simultaneous correlated changes in the other.

***Genotypic Variance* (Vg)** = $\frac{\text{MSg} - \text{MSe}}{\text{r}}$ where MSg = Mean sum of squares due to seed source, MSe = Mean sum of squares due to error and r = replication

Phenotypic Variance (Vp) = Vg + Ve; where Ve (Environment Variance) = MSe;

Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\mathrm{Vp}}}{\mathrm{Mean}} \times 100$

Genotypic coefficient of variation (GCV) = $\frac{\sqrt{\mathrm{Vg}}}{\mathrm{Mean}} \times 100$

Broad Sense Heritability (H^2) = Vg/Vp;

Genetic advance (GA) = $k \times V_p \times H^2$; where k =selection criteria;

Genetic gain (GG) = $\frac{\mathrm{GA}}{\mathrm{Mean}} \times 100$

2.2.2 Cluster Analysis

When certain multivariate populations are found to be heterogeneous and there is a need to find out which subsets of populations are most alike and which are least alike. The cluster analysis was done by Mahalanobis. D^2 statistic as suggested by Rao (1952).

3. Results and Discussion

Intraspecific variation research among the seed sources has set a strong base for putting off many plantation barriers in addition to enhancing plantation programs. In the present investigation, the variation in seed and pod characters of *Albizia procera* tree differed significantly among different seed sources of Uttrakhand (Meenakshi *et al.* 2023)

Cluster Analysis: Based on the performance of various characteristics, the clustering pattern of different seed sources of *Punica granatum* has been presented in Table 3. All the seed sources were grouped into 3 clusters. Maximum (4) number of seed sources i.e Rewalsar, Sundernagar, Sunni, and Mohal were accommodated in cluster II followed by three seed sources in cluster I and cluster III respectively.

Table 3: Clustering membership.

Cluster	**No. of Seed sources**	**Seed source**
I	3	Narag, Neripul, Sadhupul
II	4	Rewalsar, Sundernagar, Sunni, Mohal
III	3	Waknaghat, Basantpur, Banjar

Cluster means for different characters to assess the considerable amount of variation which shows the existence of diversity among seed sources. The cluster means of various characters are presented in Table 4. The maximum height was recorded in cluster II (6.95 m) and minimum height was recorded in cluster III (6.51 m). Maximum diameter was recorded in cluster I (13.12 cm) and the minimum diameter was observed in cluster II (11.91 cm). Maximum crown spread E-W was recorded in cluster I (4.62 m) and minimum crown spread E-W was observed in cluster II (4.24 m). Maximum crown spread N-S was recorded in cluster I (6.47 m) and minimum crown spread N-S was observed in cluster II (6.06 m). Maximum fruit weight was observed in cluster II (26.43 g) and the minimum was observed in cluster I (24.36 g). Maximum leaf length was recorded in cluster II (5.07 cm) and minimum leaf length was observed in cluster III (4.57 cm). The maximum internodal length was recorded in cluster II (3.57 cm) and minimum internodal length was observed in cluster I (3.35 cm). The maximum collar diameter was recorded in cluster II (2.30 mm) and the minimum collar diameter was observed in cluster I (1.81 mm). A maximum number of branches was recorded in cluster II (7.76) and a minimum number of branches was observed in cluster I (6.35). Maximum leaf petiole was recorded in cluster I (0.47 cm) and minimum leaf petiole were observed in cluster III (0.43 cm).

Table 4: Cluster means.

Characters	**Cluster**		
	I	**II**	**III**
Tree height (m)	6.73	**6.95**	6.51
Tree diameter (cm)	**13.12**	11.91	12.06
Crown spread E-W (m)	**4.62**	4.24	4.37
Crown spread N-S (m)	**6.47**	6.06	6.20
Fruit weight (g)	24.36	**26.43**	25.65
Leaf length (cm)	4.58	**5.07**	4.57
Internodal length (cm)	3.35	**3.57**	3.43
Collar diameter (mm)	1.81	**2.30**	1.82
Number of branches	6.35	**7.76**	6.77

Leaf petiole (cm)	**0.47**	0.45	0.43

Average inter-cluster distance values are presented in Table 5. The inter-cluster distance D^2 value was highest between cluster I and cluster II (18.73) and the lowest inter-cluster distance D^2 value was observed in cluster II and cluster III (8.98). This indicates that seed sources included in clusters I and II have wide genetic diversity and could be used in a hybridization programme aimed at direct selection for characters or improvement of seed sources. Characters like tree height, tree diameter, and crown spread had more contribution towards genetic divergence, hence these characters are major determinants of genetic diversity in the present set of seed sources.

Table 5: Average inter-cluster distance (D^2)

Clusters	**I**	**II**	**III**
I		18.73	10.18
II	18.73		8.98
III	10.18	8.98	

Table 6: Estimates of genetic parameters for morphological characters in Wild pomegranate

Characters	GV	EV	PV	GCV	PCV	H	GA	GG
Tree height	2.37	0.01	2.38	22.79	22.84	0.99	4.86	71.98
Tree diameter	2.71	0.01	2.72	13.38	13.40	0.99	5.57	45.22
Crown Spread E-W	0.33	0.01	0.34	13.01	13.21	0.97	0.65	14.87
Crown Spread N-S	0.20	0.01	0.21	7.12	7.30	0.95	0.39	6.19
Leaf length	0.30	0.01	0.31	11.59	11.79	0.97	0.60	12.66
Fruit Weight	1.21	2.36	3.57	4.30	7.39	0.34	0.84	3.30
Number of branches	0.22	2.36	2.58	6.62	22.83	0.08	0.04	0.53
Collar Diameter	0.32	2.36	2.68	26.67	76.83	0.12	0.08	3.76

Leaf petiole	0.22	2.36	2.58	10.36	35.36	0.09	0.04	8.55
internodal length	0.29	2.36	2.65	15.44	46.91	0.11	0.06	1.84

PCV: Phenotypic coefficient of variation, GCV: genotypic coefficient of variation, h^2 : narrow sense heritability, H^2 : broad sense heritability, GA: genetic advance,

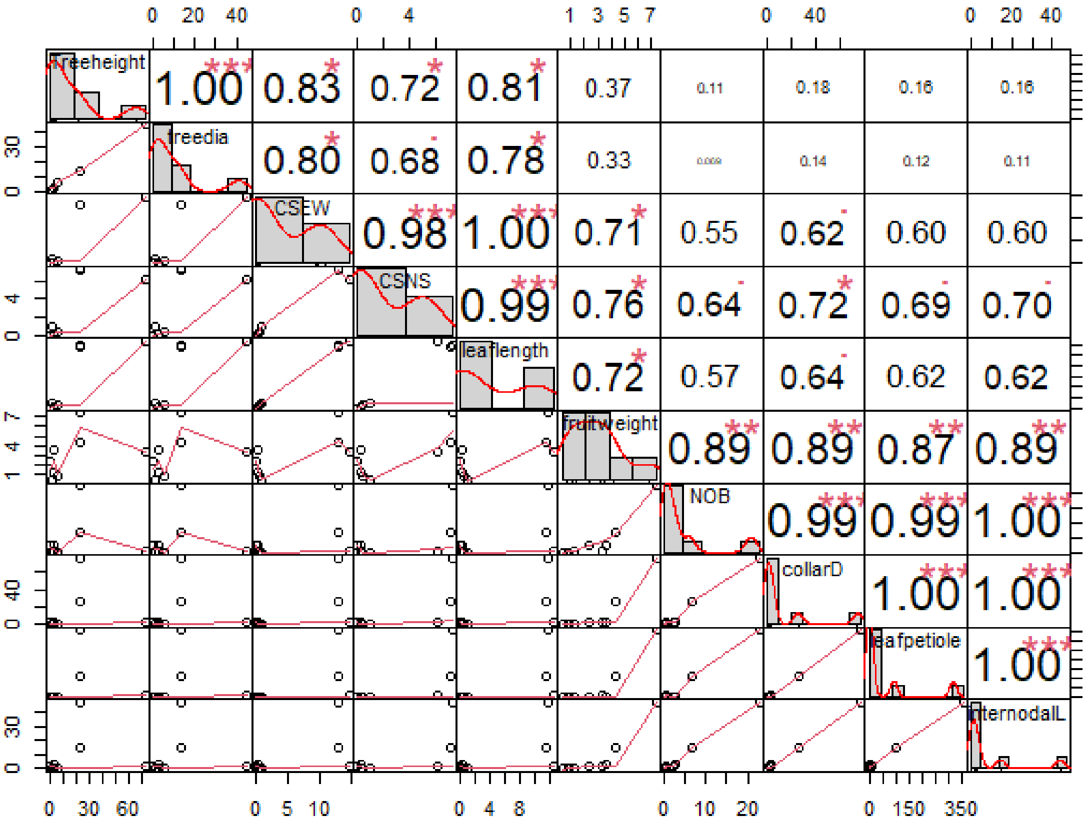
Table 7: Analysis of variances for different morphological characters in Wild pomegranate.

Source of variation	**d.f**	**Mean Sum of Squares**				
		Tree height (m)	**Tree diameter (cm)**	**Crown spread E-W (m)**	**Crown spread N-S (m)**	**Leaf Length (cm)**
Replication	2	0.93	0.46	1.11	0.46	0.32
Seed sources	9	7.12	8.15	0.99	0.60	0.91
Between districts	4	15.02	16.72	2.04	1.25	1.95
SS within district 1	1	0.95	1.41	0.09	0.09	0.10
SS within district 2	1	1.25	1.66	0.51	0.25	0.08
SS within district 3	1	1.34	1.28	0.03	0.00	0.14
SS within district 4	1	0.11	1.19	0.00	0.03	0.03
SS within district 5	1	0.41	0.88	0.10	0.04	0.02
Error	18	0.01	0.06	0.01	0.03	0.01
Total	29	2.21	2.59	0.38	0.23	0.31

Table 8: Analysis of variances for different morphological characters in Wild pomegranate.

Source of variation	d.f	Mean Sum of Squares				
		Fruit weight (g)	**Leaf petiole (cm)**	**Collar diameter (mm)**	**Internodal length (cm)**	**Number of branches**
Replication	2	50.25	0.001	45.65	0.01	0.52
Seed sources	9	5.99	0.002	1.22	0.08	3.37
Between districts	4	11.75	0.01	1.34	0.17	7.23
SS within district 1	1	0.24	0.002	1.82	0.02	0.001
SS within district 2	1	0.50	0.002	1.87	0.04	0.002
SS within district 3	1	0.35	0.001	0.41	0.03	0.003
SS within district 4	1	5.72	0.003	1.38	0.02	0.40
SS within district 5	1	0.14	0.001	0.12	0.01	1.03
Error	18	2.36	0.007	3.35	0.01	0.02
Total	29	6.79	0.04	5.60	0.02	1.09

Table 9: Correlation coefficient between different morphological characters of Wild pomegranate



0.05* **0.01**** **0.001*****

Conclusion:

All Seed sources were grouped into 3 clusters and maximum inter cluster distance was between cluster I and II and minimum inter cluster distance was recorded in cluster II and III. Maximum number of seed sources (four) were accommodated in Cluster II, whereas three seed sources were accommodated in cluster I and Cluster III. The present study illustrated the existence of wide ranges of variations for most of the characters among the wild pomegranate, which provides opportunities for genetic gain through selection. Tree height and tree diameter had high heritability along with high genetic advance.

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