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Morphological and Floral Studies in Wild Pomegranate (Daru)

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ABSTRACT: The current study was conducted at ten seed sources throughout different districts of Himachal Pradesh in the Department of Tree Improvement and Genetic Resources, Dr. Y.S. Parmar University of Horticulture & Forestry Nauni, Solan (H.P.), between 2016 and 2018. Being important wild fruit species and extent of variation present in Himachal Pradesh the present study was formulated. The study's goal was to document the morphological and floral variability in Wild Pomegranate (*Punica granatum* L.). We looked at floral qualities such as flower appearance, flower type, pollen vectors, and pollen viability, as well as leaf morphometric traits such as leaf length, leaf breadth, area, and leaf petiole length, and leaf morphological traits such as leaf shape and colour. The degree of genetic variation in Himachal Pradesh was demonstrated by the substantial variance in leaf morphometric, floral, and pollen traits detected within seed sources.

Keywords: Floral, morphometric, seed source, wild pomegranate, Daru.

INTRODUCTION

One of the earliest known edible wild fruits is the wild pomegranate (*Punica granatum* L.), or "Daru," which may grow in a variety of agro-climatic situations from tropical to subtropical areas (Jalikop, 2007). It originally originated in Turkey and Iran, and it later spread to the Himalayas in Northern India (Mars, 2000). In Northern India, a wild pomegranate variety known as "Daru" grows spontaneously. It has narrow petals, friable seeds, and hardy fruits. Ambe-bahar (flowering in January-February), mrig-bahar (flowering in June-July), and hasth-bahar are the three separate waves or flowering seasons (September-October flowering). The inflorescence is a dichasial cyme with urcerate, campanulate, and tubular hermaphrodite, staminate, and intermediate flowers, respectively. Hermaphrodite flowers can have pin-type or thrum-type blooms. Seasonally and in terms of variation, the sex ratio fluctuates. In pomegranates, self- and cross-pollination take place. Protogyny is the cause of cross-pollination in the pomegranate. Pomegranates bloom in the spring in North India, but they bloom all year round in Central and South India. On newly formed branches from the same year, typically on spurs or short branches, flowering takes place roughly a month following bud break. The majority of the flowers are produced in clusters of two to three, either terminally or auxiliary, and the inflorescence is thought to be a cyme (Nath and Randhawa 1959). Full bloom lasts for about a month, and fruit set happens in around three or four different stages (El Sese, 1988). The production of "Anardana," in which juice sacs (arils) are dried in the sun for 10 to 15 days before being sold as a condiment, is a significant use of wild fruits in Northern India. Fruit selection is a crucial strategy in pomegranate breeding projects because the wild pomegranate is often cultivated through seeds, which tend to produce

heterozygosity and variability (Jalikop and Kumar 1990). Small and slick in texture, "daru" leaves also possess therapeutic qualities. Its leaves are used to cure stomach ache, sleeplessness, diarrhoea, and jaundice. However, it is regrettable that all commercial pomegranate cultivars in India are prone to serious ailments and insect pests, particularly bacterial blight and wilt diseases (Jadhav and Sharma 2007). When compared to commercially grown pomegranates, wild pomegranates have been discovered to be more resistant to pest insect attacks. With the use of hybridization and other breeding tools, this can be exploited for breeding programmes that transfer resistance genes from wild species to commercial cultivars. It is vital to review the existing state of knowledge on the floral biology of wild pomegranate as well as leaf characteristics because a complete grasp of floral biology is a necessary prerequisite for any crop development programme (Babu *et al*., 2009a). Therefore, it is essential to start the study project on the reproductive side, keeping in mind the vast potential for improvement and breeding of wild pomegranate (Daru), on the basis of its reproductive characteristics, fruit variations, and also the socio-economic value.

MATERIAL AND METHODS

Current study was carried out during 2017 in the Department of Tree Improvement and Genetic Resources, College of Forestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. Seed sources included Narag (S_1) and Neripul (S_2) district Sirmour, Waknaghat (S_3) and Sadhupul (S_4) district Solan, Basantpur (S_5) district Shimla, Sundernagar (S_6), Rewalsor (S_7) and Aut (S_8) district Mandi, Mohal (S_9) and Banjar (S_{10}) district Kullu were selected (Table 1). On the basis of their height, diameter, appropriateness, and general appeal, five medium-sized trees in the same age group were chosen. On each tree, nine flowers were marked to analyse the sex ratio, floral type before anthesis, and classification into three groups: hermaphrodite, intermediate, and male flowers.

Table 1: Seed sources of wild pomegranate.

District	Seed source	Code	Altitude m (a.m.s.l.)	Latitude	Longitude
Sirmour	Narag	S_1	1320	30.8170° N	77.1881° E
	Neripul	S_2	1148	31.0197° N	77.3787° E
Solan	Waknaghat	S_3	1500	31.0079° N	77.0881° E
	Sadhupul	S_4	1180	30.9964° N	77.1622° E
Shimla	Basantpur	S_5	1150	31.2081° N	77.1744° E
Mandi	Sundernagar	S_6	900	31.5299° N	76.8889° E
	Rewalsor	S_7	800	31.6322° N	76.8332° E
	Aut	S_8	1050	31.7430° N	77.2082° E
Kullu	Mohal	S_9	1220	31.9149° N	77.1169° E
	Banjar	S_{10}	1250	31.6377° N	77.3441° E

Leaf Characteristics

For the purpose of examining the morphological variables relating to the trees producing various shapes, leaf samples from each of the chosen plants were collected. The leaves were collected from middle branches' terminal section. The traits of the leaf include:

Leaf size (cm). With the aid of a scale, the length and width of each leaf were measured from the tip of the apex to the base.

Leaf area (cm^2). Leaves were collected from individual trees. Leaf area was worked out using the digital leaf area meter (LICOR-model 3100 A).

Petiole length (cm). The average value of the leaf petiole from three replications of selected trees was calculated using hand scale measurements.

Leaf shape. By consulting the Standard Cyclopedia of Horticulture, leaf form was observed (Bailey, 1963). Oblanceolate, lanceolate, oblong, elliptical lanceolate, and broad elliptical leaf shapes have all been recorded for this species. But there are just two common leaf shapes, lanceolate and elliptical, which were seen in all variations.

Leaf colour. July through August of 2017 saw the best increase of the leaf's colour. Leaf colour was assessed using the Standard Cyclopedia of Horticulture as a guide (Bailey, 1963).

Floral Biology

Flower size (cm). The flower length and width of fifteen (hermaphrodite) flowers from each tree were measured.

Flower type

To explore the sex ratio and flower type at the pre-anthesis stage, nine blooms were tagged on each tree. The flowers were studied once they had opened to be divided into three groups: hermaphrodite, intermediate, and male blooms. The measurements were made based on the style's length in respect to the staminal column. The flower was thought to be hermaphrodite if its style approached the staminal column. The structure and function of a carpel and a pistil were explored in cases where the style is clearly below the staminal column.

When the styles are simple and restricted to the calyx cup, a flower is said to be male or female depending on whether and roecium and gynoecium are present and serving a functional purpose.

Pollen Studies

To determine the viability and germination rate of pollen, research was conducted as follows;

Pollen vectors. Each seed source's pollen vector content was noted both visually and by imaging.

Pollen collection. From July through August 2017, pollen was collected by picking flowers with butter paper. The gathered pollen grains were placed in vials and sealed containers for later use in the pollen viability investigation.

Pollen viability (%). Hermaphrodite and male newly obtained flowers' pollen viability was investigated by making slides in 1 per centacetocarmine solution and seeing them under a microscope. Under three replications, pollen grains that were deeply stained and otherwise normal-looking were tallied as viable pollen, whereas pollen that was shrivelled and weakly stained was recorded as non-viable and reported as a percentage. For the pollen of hermaphrodite and male flowers, the vitality of the pollen was determined independently.

RESULTS AND CONCLUSION

A. Leaf Characteristics

For leaf morphometric characteristics, data presented in Table 2 showed that Banjar (S_{10}) had maximum leaf length of 5.98 cm which was statistically at par with Mohal (S_9) *i.e.* 5.83 cm. Aut (S_8) had maximum leaf breadth of 1.72 cm followed by Waknaghat (S_3) with value 1.67 cm. Results for leaf area showed significant variation among seed sources with Sadhupul (S_4) having maximum leaf area of 12.66 cm^2 which is statistically at par with Neripul (S_2), Banjaar (S_{10}), Basantpur (S_5) and Mohal (S_9) with 12.65 cm^2, 12.62 cm^2, 12.36 cm^2, 12.34 cm^2 respectively. The mean value from Table 2 revealed that Mohal (S_9) had maximum leaf petiole length of 0.52 cm followed by Banjar (S_{10}) with 0.50 cm.

Table 2: Variation in mean values for leaf morphometric characters in wild pomegranate.

Seed Source	leaf length (cm)	leaf breadth (cm)	leaf area (cm^2)	leaf petiole length (cm)
S_1	3.80	1.23	9.13	0.37
S_2	5.00	1.28	12.65	0.48
S_3	5.21	1.67	10.91	0.47
S_4	5.29	1.53	12.66	0.45
S_5	5.73	1.61	12.36	0.49
S_6	4.98	1.47	11.17	0.42
S_7	3.96	1.28	10.52	0.46
S_8	4.48	1.72	9.90	0.44
S_9	5.83	1.24	12.34	0.52
S_{10}	5.98	1.32	12.62	0.50
Range	(3.52-6.95)	(1.01-1.89)	(7.41-15.43)	(0.32-0.61)
$CD_{.05}$	0.16	0.05	0.53	0.02

The maximum leaf length, leaf breadth and leaf area was observed from the leaf samples collected from middle portion terminal part of the tree. This revealed significant variation in leaf morphometeric characters. Similar findings have been computed by Pratap (1997) and Bist *et al*. (2001). At par can be used as a model for choosing individual trees and can be used for active selection. Maximum leaf area was also noted, measuring 15.43 cm^2. Wani *et al.* (2012) provided the parallel findings to these findings. He calculated the leaf area of genotypes of wild pomegranates that ranged from 4.48 cm^2 to 14.04 cm^2. According to the research, plants produce many leaf types as they grow. The first few true leaves that appear are often smaller, simpler, and anatomically distinct from leaves that appear later in development. Present findings are also consistent with reports of Esau (1965) and Byrne *et al.* (2001) as they have explained the change in shape and size of successive leaves on a plant on the basis of physiological changes associated with increasing age of plant along with the interaction between shoot apical meristem and developing leaf primordial, under a variety of environmental factors. Verwijst and Wen (1996) found supporting results in *Salix* species, they observe the leaf length, leaf width ratio also changes with leaf size which varied between different types of shoots. The present findings are supported by the results of Ferris *et al*. (2001); Taylor *et al*. (2001) who have reported the role of elevated CO_2 in promoting individual leaf size.

For the leaf's morphological characteristic, or shape, elliptical, lanceolate, and oblong shapes were noted. The majority of the leaves in all seed sources were oval in shape. In seed sources *viz.*, Narag (S_1), Sundernagar (S_6), Aut (S_8), and Mohal (S_9), oblong leaf shape was observed, whereas in Sadhupul seed source lanceolate leaf shape was found in majority of the trees. Useful variations are presented in Table 3.

Table 3: Variation in leaf shape among different seed sources of wild pomegranate.

Seed Source	Tree Number				
	T_1	T_2	T_3	T_4	T_5
S_1	Elliptical	Lanceolate	Elliptical	Elliptical	Oblong
S_2	Elliptical	Elliptical	Lanceolate	Lanceolate	Elliptical
S_3	Lanceolate	Lanceolate	Elliptical	Elliptical	Elliptical
S_4	Lanceolate	Elliptical	Lanceolate	Lanceolate	Elliptical
S_5	Lanceolate	Elliptical	Lanceolate	Elliptical	Elliptical
S_6	Lanceolate	Elliptical	Oblong	Elliptical	Elliptical
S_7	Lanceolate	Elliptical	Elliptical	Lanceolate	Elliptical
S_8	Lanceolate	Oblong	Lanceolate	Elliptical	Elliptical
S_9	Elliptical	Elliptical	Oblong	Lanceolate	Elliptical
S_{10}	Elliptical	Elliptical	Elliptical	Lanceolate	Elliptical

Leaf colour was observed with Green group (N137 A, N138 B, 138 A, N138 B, 143 B and 143 C) and Yellow green group (143 A and 147 A). Yellow green group leaves were detected in seed sources Neripul (S_2) and Mohal (S_9), while the bulk of the remaining seed sources had green group leaves. Useful variations are presented in Table 4 (Fig. 1).

Table 4: Variation in leaf colour among different seed sources of wild pomegranate.

Seed Source	Tree Number				
	T_1	T_2	T_3	T_4	T_5
S_1	Green group N137 A	Green group N138 B	Green group 138 A	Green group N138 B	Green group N137 A
S_2	Green group N137 A	Green group 143 B	Green group 143 C	Yellow green group 147 A	Green group N137 A
S_3	Green group 138 A	Green group N138 B	Green group N138 B	Green group 138 A	Green group N137 A
S_4	Green group 138 A	Green group 138 A	Green group N138 B	Green group N137 A	Green group N138 B
S_5	Green group N137 A	Green group 147 A	Green group 143 B	Yellow green group 147 A	Green group 143 B
S_6	Green group 143 A	Yellow green group 147 A	Green group 143 B	Green group 143 A	Green group 143 A
S_7	Green group N138 B	Green group 138 A	Green group N138 B	Green group N138 B	Green group 138 A
S_8	Green group 143 B	Yellow green group 143 A	Green group 143 B	Green group 143 B	Yellow green group 143 A
S_9	Yellow green group 143 A	Green group N137 A	Green group N137 A	Yellow green group 147 A	Green group N137 A
S_{10}	Green group 143 B	Green group 143 B	Green group 143 C	Green group 143 B	Green group 143 C

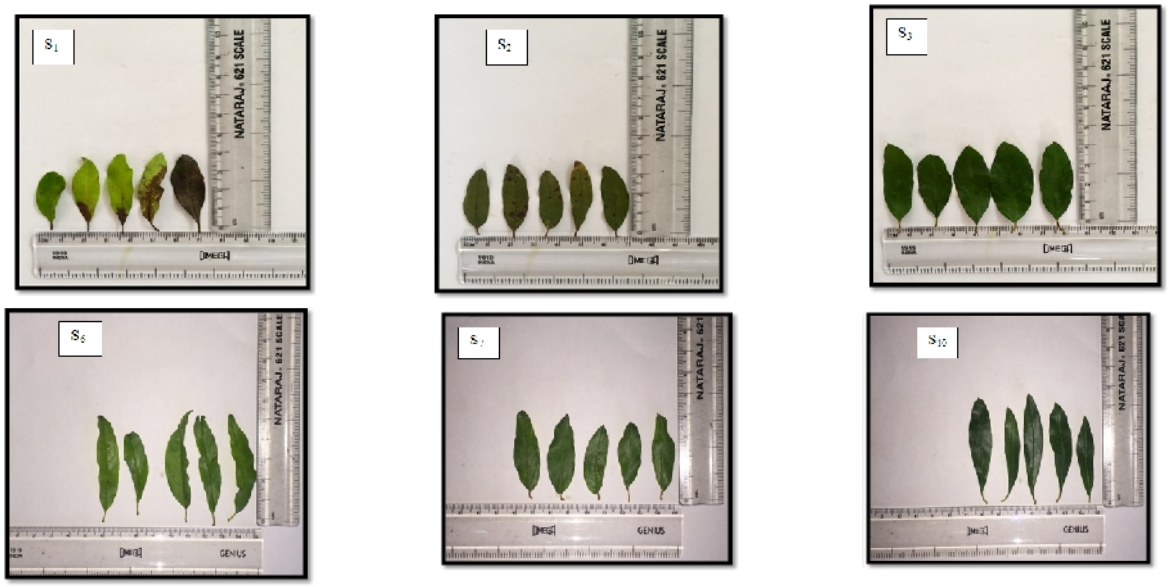

Fig. 1. Variation in leaves among various seed sources in wild pomegranate.

In the current study, lanceolate shapes with acute apexes were seen and documented as leaf morphological features, in particular the varied leaf shapes. These findings are consistent with research done on this species in Nepal by Joshi and Joshi (2001) and Lama *et al.* (2001). Pomegranate leaves are whole, lanceolate to widely oblanceolate, and elliptical lanceolate, according to their calculations. Singh (2012) documented the pomegranate germplasm's leaf shape and leaf tip in a desert ecosystem in India, where the leaf shape was found to be acute, cuspidate, mucronate and obtuse.

B. Floral Characteristics

Flower appearance. In this species, the flower buds typically emerge in groups of three buds in the leaf axils or as a single flower on the shoot. While the other two blooms in the cluster likely to be either male or intermediate, the center flower, which is considerably larger and is said to be hermaphrodite with pin condition. It was noted that the appearance of single and clustered flowers varied from tree to tree within the seed source during the blossoming season. Five and six sepal lobes were observed (Fig. 2).

Five sepal lobes Six sepal lobes

Fig. 2. Variation in Sepal lobes in wild pomegranate.

Flower type. All three flower kinds—hermaphrodite, intermediate, and male—were noted for various seed sources, and it was discovered that the per cent proportion of hermaphrodite flower types varied between seed sources. The flower types were studied in all the seed sources and the maximum percentage was observed for hermaphrodite flower accounting for 54.00 per cent in Waknaghat (S_3) followed by intermediate flowers (30.81%) in Aut (S_8) and male flower (23.45%) in Narag (S_1) (Table 5). The results are consistent with the findings of Nath and Randhawa (1959a), Nalawadi *et al*. (1973) and Singh *et al*. (1978) for pomegranate cultivars. The maximum percentage for hermaphrodite flowers was observed in Waknaghat (S_3) 54.00 followed by Neripul (S_2) 53.00. Maximum percentage for intermediate flowers was observed in Aut (S_8) 30.81 followed by Sadhupul (S_4) 30.78. Maximum percentage for male flowers was recorded in Narag (S_1) 23.45 followed by 39 Banjar (S_{10}) 21.11.

Table 5: Percentage of type of flower in wild pomegranate (*Punica granatum* L.).

Seed Source	Hermaphrodite (%)	Intermediate (%)	Male (%)
S_1	46.34	30.21	23.45
S_2	53.00	26.00	21.00
S_3	54.00	27.66	18.34
S_4	52.00	30.78	17.22
S_5	52.89	28.11	19.00
S_6	51.03	28.97	20.00
S_7	50.67	30.33	19.00
S_8	48.81	30.81	18.00
S_9	49.78	30.22	20.00
S_{10}	50.01	28.88	21.11
Mean	50.85	29.19	19.71

When choosing a seed source, especially for fruit quality and fruit yield, knowledge of reproductive biology and pollination dynamics is crucial. The environment and locale of the seed source in this case have an impact on the vegetative and reproductive growth of this species. As the cymose inflorescence decides the total number of hermaphrodite flowers which ultimately turn up as a fruit. Such variations were also reported by Lawrence (1951); Watson and Dallwitz (1992). As they stated, male flowers have a well-defined shape, while hermaphrodite blooms have a base shape. Similar findings on the existence of

functionally unisexual male flowers on pomegranate cultivars have been reported by Nath and Randhawa (1959c), who distinguish them from hermaphrodite or perfect flowers by their long styles that clearly protrude through the staminal column.

C. Pollen Studies

The main primary pollinating agent observed at all the seed sources was honey bee (*Apiscerena)*, black ants and bumble bees in this species (Fig. 3).

Fig. 3. Flowers of wild pomegranate and its pollinators.

Pollen viability (%). The results obtained in Table 6 showed that there was significant difference with regard to the viability percentage among seed sources for hermaphrodite and male flowers. Results for pollen studies revealed that maximum pollen viability for hermaphrodite flower (76.75%) and male flower (68.45%) was registered for Waknaghat (S_3) seed source followed by Basantpur (S_5) (75.18 %) for hermaphrodite flower, Sundernagar (S_6) *i.e.* 66.78 per cent for male flower.

The abundance of hermaphrodite and male flowers govern the pollen quality which is a combination of pollen viability and pollen germination. The results obtained from present findings on pollen viability from two types of flowers have been discussed on percent basis in Table 6.

Table 6: Pollen viability percentage in wild pomegranate flowers (*Punica granatum* L.).

Seed Source	Hermaphrodite flower (%)	Male flower (%)
S_1	70.65	66.01
S_2	70.81	60.23
S_3	76.75	68.45
S_4	69.43	64.45
S_5	75.18	63.21
S_6	74.15	66.78
S_7	72.31	64.10
S_8	66.81	59.23
S_9	65.34	58.03
S_{10}	67.67	61.23
$CD_{0.05}$	0.36	0.56

The maximum average percentage of pollen viability (76.75%) was recorded in hermaphrodite flowers followed by male flowers (68.45%) from Waknaghat (S_3) seed source. Lowest pollen viability (65.34%) from hermaphrodite flowers was recorded from Mohal (S_9) seed source. It is discovered that each seed source differs greatly from the others, which is a marker of variations in out-crossing processes and fruit setting. The viability of the male flowers from the Waknaghat seed source was lower (68.45%). The variation in pollen viability on these lines are in agreement with study of Gozlekci (1997); Nath and Randhawa (1959b); Josan *et al*. (1979) and Sharma and Bist (2005) in pomegranate species and cultivars.

Complex factors, including age, nutrient status, and environmental conditions at the time of pollen generation, govern the amount of pollen generated by any species (Stanley and Linskens 1974). Large amounts of pollen produced by chosen genotypes are preferred for use in tree breeding. The majority of the methods entail keeping flowers separate, causing dehiscence, and gathering released pollen for storage and potential use in breeding. The current studies were conducted with the intention of directly extracting huge amounts of pollen for use in a breeding programme in the future. The method used for pollen collecting is in line with the methods recommended by Stanley and Linksens (1974); Jett *et al*. (1993); Kopp *et al*. (2002).

CONCLUSION

The current study found that there is variation in morphological traits for seed source, both within and within seed sources. The wild pomegranate improvement activities are highlighted as a step toward looking at enhanced genotype in the wild pomegranate populations using a combination of individual tree selections.

FUTURE SCOPE

This study helps identify the best genotypes in Himachal Pradesh in terms of morphological and floral traits. For use in pharmacological and medical research, its leaves can be extracted. The male parent's pollen viability and the female parent's total number of hermaphrodite flowers are of utmost importance from the perspective of crop enhancement through hybridization.

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Conflict of interest. None.

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