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# Genetic and correlation studies in fruit, seed and seedling characters in wild pomegranate (Daru)

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**Abstract**
Wild pomegranate is a well-known Mediterranean fruit that has been found in Himachal Pradesh as natural populations, primarily in well-drained mid-hill pasture meadows and cultivated fields, since ancient times. It thrives in the huge highland fringe areas of Himachal Pradesh and shares morphological characteristics with cultivated plants. Fruits of Daru were collected from different seed sources of Himachal Pradesh including five districts *viz*. Sirmour, Solan, Shimla, Mandi and Kullu. The morphological characters of seedlings were examined to find out the extent of genetic diversity. High heritability along with genetic gain were recorded in most of the traits and suitable for selection. Nursery traits were significantly correlated with each other.

**Keywords:** Daru, Genetic gain, genetic diversity, heritability, morphological characters

## Introduction
Understanding the diversity among and between tree populations is vital for determining priorities for the conservation and enhancement of tree genetic resources. The first stage in every improvement project is to determine the quantity, cause, and nature of variation existing in the species of interest. The genetic differences among trees is due to diverse environments in which trees develop, and the interaction between tree genotype and environment in which they grow. The performance of genotype that is superior in one environment might be inferior in another environment (de Jong 1990; Falconer and Mackay 1996) [4, 7]. Variations are necessary for adaptation and improvement, and the amount of variation determines the ability for breeding programmes to improve species. High genetic variation within and among populations has been demonstrated and this distribution of variation and evolutionary histories can lead to the recommendations of future breeding programmes (Namkoong, 1984) [14].
Any tree improvement programme begins with a field survey and selection, followed by the delimitation of seed sources capable of producing the best adapted trees. Quantitative features have been and continue to be the most important foundation for phenotypic selection in every breeding programme. The goal of tree breeders is to systematically exploit their genetic worth once a specific cycle of selection and improvement has produced superior and genetically diversified populations. There are only two species in the genus Punica *viz*: *Punica granatum* and *Punica protopunica* (ITIS, 2006) [8]. Wild pomegranate, a member of the Punicaceae family, is the best fruit species for bioactive components and have medicinal properties *viz*, fruit's rind is used to treat diarrhoea, dysentery etc. It is commonly referred to as "Daru" and its sun-dried seeds are referred as 'Anardana.' It is native to Turkey, Iran and spread to the Himalayas in northern India (Mars, 2000) [13], widely dispersed in the Himachal Pradesh. As the environment in Himachal Pradesh's mid hills is ideal for its cultivation. Fruit peel extracts are found to be suitable for applications in the food industry as they are an important source of phenolics, flavonoids and tannins occurring as natural ingredients (Viuda-Martos *et al*. 2011) [20]. 'Daru' - an economically important wild fruit species; is being studied in greater depth, owing to its distinctive development pattern and broad genetic variety.

## Materials and Methods
Present investigation was carried out during 2017-18 in the Department of Tree Improvement and Genetic Resources, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP). Seed study need to cover an extended vast stretch of species distribution hence ten seed sources of *Punica granatum* (Daru) were selected from different parts of Himachal Pradesh

with five trees from each seed source (Table 1). Fruit characteristics *viz*, fresh fruit weight, aril colour, 100 fresh and 100 dry seed weight were recorded. Germination percentage, energy and value were observed. Eight month old seedlings were evaluated for various morphological parameters i.e. seedling height, collar diameter, internodal length, no. of branches and no. of leaves per branch. The seed source selected were Narag and Neripul district Sirmour, Waknaghat and Sadhupul district Solan, Basantpur district Shimla, Sundernagar, Rewalsor and Aut district Mandi, Mohal and Banjar district Kullu of Himachal Pradesh. Trees were selected on the basis of various morphological traits *viz*., height, diameter, crown spread and tree form, free from diseases and insect pest attack. Colour chart of the Royal Horticulture Society, London was used to study variation in aril colour. Genotypic, phenotypic and environmental correlations were also estimated for all possible pairs.

**Table 1:** Seed sources of wild pomegranate (*Punica granatum* L.)

| District | Seed source | Altitude m (A.M.S.L.) | Latitude | Longitude |
|---|---|---|---|---|
| Sirmour | Narag | 1320 | 30.8170° N | 77.1881° E |
| | Neripul | 1148 | 31.0197° N | 77.3787° E |
| Solan | Waknaghat | 1500 | 31.0079° N, | 77.0881° E |
| | Sadhupul | 1180 | 30.9964° N, | 77.1622° E |
| Shimla | Basantpur | 1150 | 31.2081° N, | 77.1744° E |
| Mandi | Sundernagar | 900 | 31.5299° N, | 76.8889° E |
| | Rewalsor | 800 | 31.6322° N, | 76.8332° E |
| | Aut | 1050 | 31.7430° N, | 77.2082° E |
| Kullu | Mohal | 1220 | 31.9149° N, | 77.1169° E |
| | Banjar | 1250 | 31.6377° N, | 77.3441° E |

## Results

Population extended from subtropical foot hill of Himachal Pradesh ranging from latitude 30.8170° N to 31.9149° N. Within these growing limits a lot of variation was observed among seed sources. The fresh fruit weight showed wide variations among seed sources, with maximum value recorded for Neripul seed source (Table 2). Most depicted seed colour was yellow orange followed by red group and white group (Table 3). Maximum 100 fresh and 100 dry seed weight was recorded for Sadhupul and Sundernagar seed sources, respectively. Maximum germination percentage was reported from Sundernagar seed source. While, germination energy and value were recorded maximum in Basantpur seed source indicated fast germination rate (Table 4). Seedlings height, collar diameter and intermodal length was maximum from Sundernagar seed source. Correlation analysis as depicted in Table 7 revealed that germination percentage has significant positive correlation with all the nursery parameters.

## Discussion

In present study, fresh fruit weight varied from 12.66 g to 26.96 g in different seed sources. Parmar and Kaushal (1982) [16] and Kher (1999) [11] have reported a narrow range of weight as 80.50 g to 85.17g in this fruit. Pant (1995) [15] have reported average weight of this fruit in the range of 59.77 to 101.0 g from different geographical locations of HP.

The aril colour of fruit of this species is due to the presence of organic acids and anthocyanins that results in the formation of attractive pink-reddish tinge to its arils (Parmar and Kaushal (1982) [16]. In earlier studies by Bhat (2007) [1] and Sharma and Thakur (2016) [17] reported pink colour of arils of this fruit. Whereas, Thakur *et al*. (2011) [19] have recorded red purple colour of arils of mature wild pomegranate fruit procured from different geographical locations of HP.

The average weight of 100 fresh and 100 dry seed varied from 9.16 to 21.36 g and 3.00 to 3.53 g, respectively. Kher (1999) [11] has recorded average weight of 100 arils of wild pomegranate fruit as 12.65g. Thakur *et al*. (2011) [19] have reported a range of weight of arils per fruit as 33.00 to 48.70g from different districts of HP. Singh and Sethi (2003) [18] after screening seven pomegranate cultivars have reported that weight of 100 arils ranged between 21.73 to 29.41 g.

Seed germination values ranged from 20.31 to 70.46 percentage. While germination energy and germination value varied from 4.00 to 12.34% and 4.13 to 46.45%, respectively.

Morphological data (Table 5) of provenance trial of seed sources evaluated in nursery stage revealed significant variation among seed sources. Mean seedlings height ranged between 23.25 to 56.00 cm. The values for collar diameter varied between 1.25 to 3.56 mm. For internodal length range of 3.22 to 3.87 cm was observed. Number of branches showed variation between 5.03 to 9.43. The range for no. of leaves per branch observed was 5.80 to 8.43.

Maximum genotypic, phenotypic coefficient of variability (Table-6) and genetic gain were recorded for collar diameter (37.13%, 37.25% and 76.25%, respectively). Genetic estimates for germination per cent, seedling height and collar diameter revealed the maximum heritability value (broad sense) of 0.99 each, which means these characters were found to be under strong genetic control. While the genetic advance was obtained maximum for seedling height (25.94). Reduction in this environmental variance associated with test conditions enabled us to detect genetic variation in seed germination pattern that is known to exist in conifers such as white spruce (Caron *et al*. 1993; Krakowski and El-kassaby 2005) [2, 10], Sitka spruce (*Picea sitchensis* (Bong.) Carr) (Chaisurisri *et al*. 1992) [3] and Douglas-fir (El-kassaby *et al*. 1992) [6].

The results of present study are parallel to the findings of Jefferson (2015-18) in *Punica granatum* (Daru). The expression of a character is sum total of the contribution of so many other character and therefore, screening/selection should be done on the basis of components contributing towards that character. The biometrical tool for helping this is correlation which gives the nature and degree of association between various traits. So, the knowledge of association of different characters is the first hand information for any improvement programme (Table 7). Generally, highly significant linear relationships were found between germination variables and seedling growth characteristics at the age of eight month old seedlings. Germination per cent showed high significant correlation with collar diameter (r= 0.709) followed by seedling height (r= 0.692), internodal length (r=0.689), no. of branches (r=0.668) and no. of leaves per branch (r=0.665). Seedling height showed highly significant correlation with collar diameter (r= 0.992) followed by no. of leaves per branch (r=0.983), internodal length (r=0.982) and no. of branches (r=0.974). Collar diameter showed highly significant correlation with internodal length (r=0.987) followed by no. of branches (r=0.969) and no. of leaves per branch (r=0.959). Internodal length showed highly significant correlation with no. of branches (r=0.946) followed by no. of leaves per branch (r=0.942). Number of branches showed highly significant correlation with no. of

leaves per branch (r=0.951).

Results showed that the timing of seed germination influences the variation in the size of very young seedlings, which has been demonstrated in conifers (Dunlap and Barnett, 1983) [5]. Significant linear relationships were found between the average (family) means of all of the seed and eight months old seedling morphological characteristics. Results showed stronger correlations, like Lamhamedi *et al*. (2006) [12]. Similarly, Khalil (1981) [9] found a statistically significant regression for white spruce, where 1000-seed weight accounted for 22% of the variation in two-year old seedling height. He concluded that seed weight and annual height growth were genetically correlated and controlled pleiotropic ally or by linkage. Finally, it has been concluded that genetic variation persists among various seed sources. Hence, selection is made on the basis of the performance of the progenies providing a broad base for further breeding programmes.

**Table 2:** Variation in seed characteristics among seed sources

| Seed source | Fresh fruit weight (g) | 100 fresh seed weight | 100 dry seed weight |
|---|---|---|---|
| | Average mean values | | |
| Narag | 20.52 | 10.94 | 3.25 |
| Neripul | 26.96 | 12.67 | 3.41 |
| Waknaghat | 12.66 | 11.08 | 3.35 |
| Sadhupul | 18.20 | 21.36 | 3.36 |
| Basantpur | 13.34 | 10.84 | 3.32 |
| Sundernagar | 18.45 | 14.10 | 3.53 |
| Rewalsor | 15.81 | 9.16 | 3.51 |
| Aut | 14.94 | 16.22 | 3.29 |
| Mohal | 18.96 | 16.02 | 3.37 |
| Banjar | 16.86 | 12.74 | 3.00 |
| Range | 12.66-26.96 | 9.16-21.36 | 3.00-3.53 |
| CV | 23.28 | 26.51 | 4.44 |
| $CD_{0.05}$ | 6.11 | 0.74 | 0.28 |

**Table 3:** Germination parameters of various seed sources

| Seed source | Seed germination parameters | | | | |
|---|---|---|---|---|---|
| | Period taken to initiate germination | Period taken to complete germination | Germination (%) | Germination Energy (%) | Germination value |
| | Days | Days | | | |
| Narag | 19 | 25 | 37.30 | 6.33 | 14.47 |
| Neripul | 19 | 25 | 40.33 | 7.00 | 16.49 |
| Waknaghat | 16 | 25 | 43.66 | 7.66 | 17.62 |
| Sadhupul | 16 | 25 | 38.34 | 6.00 | 11.50 |
| Basantpur | 16 | 25 | 67.71 | 12.34 | 46.45 |
| Sundernagar | 12 | 25 | 70.46 | 10.67 | 30.68 |
| Rewalsor | 13 | 25 | 65.45 | 10.00 | 25.91 |
| Aut | 13 | 25 | 52.03 | 9.34 | 24.26 |
| Mohal | 15 | 25 | 30.01 | 4.34 | 6.95 |
| Banjar | 15 | 25 | 20.31 | 4.00 | 4.13 |
| Range | | | 20.31-70.46 | 4.00-12.34 | 4.13-46.45 |
| $CD_{0.05}$ | | | 0.08 | 0.57 | 0.12 |
| Mean | | | 46.56 | 7.76 | 19.84 |
| CV | | | 36.28 | 35.52 | 63.05 |

**Table 4:** Variation in seed colour (aril) among various seed sources

| Seed source | Tree number | | | | |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
| Narag | Red Group | White group | Red pink | Yellow | Red Group |
| Neripul | Yellow | White group | Yellow orange | Red pink | Red Group |
| Waknaghat | Red Group | White group | Yellow orange | Yellow orange | Red pink |
| Sadhupul | Red Group | Yellow orange | Red Group | Yellow orange | White group |
| Basantpur | Red Group | Yellow orange | Yellow orange | Red Group | Red Group |
| Sundernagar | White group | Red Group | Yellow orange | White group | Red Group |
| Rewalsor | White group | Yellow orange | Yellow orange | Red pink | Red Group |
| Aut | White group | Red Group | Yellow orange | White group N 155 A | Yellow orange 15 A |
| Mohal | Red pink | Red Group | Red Group | Yellow orange | Yellow orange |
| Banjar | White group | Yellow orange | Yellow orange | Red pink | Red Group |

**Table 5:** Variation in nursery performance at the age of 8 months

| Seed source | Seedling height (cm) | Collar diameter (mm) | Internodal length (cm) | Number of branches | Number of leaves per branch |
|---|---|---|---|---|---|
| | Average mean values | | | | |
| Narag | 32.25 | 1.76 | 3.34 | 6.26 | 6.93 |
| Neripul | 36.62 | 1.86 | 3.39 | 6.36 | 7.23 |
| Waknaghat | 55.01 | 3.34 | 3.86 | 9.87 | 8.43 |
| Sadhupul | 45.77 | 2.77 | 3.77 | 7.44 | 7.54 |
| Basantpur | 46.01 | 2.66 | 3.73 | 7.77 | 7.50 |
| Sundernagar | 56.00 | 3.56 | 3.87 | 9.43 | 8.13 |
| Rewalsor | 53.09 | 3.31 | 3.83 | 8.41 | 8.16 |
| Aut | 26.62 | 1.47 | 3.28 | 5.82 | 6.28 |
| Mohal | 23.98 | 1.30 | 3.23 | 5.60 | 5.98 |

| | | | | | |
|---|---|---|---|---|---|
| Banjar | 23.25 | 1.25 | 3.22 | 5.03 | 5.80 |
| Range | 23.25-56.00 | 1.25-3.56 | 3.22-3.87 | 5.03-9.87 | 5.80-8.43 |
| $CD_{0.05}$ | 0.98 | 0.05 | 0.08 | 0.48 | 0.73 |
| CV | 32.49 | 38.71 | 7.92 | 23.02 | 12.99 |

**Table 6:** Genetic Estimates for different characters of wild pomegranate

| Characters | Genotypic coefficient of variability (%) | Phenotypic coefficient of variability (%) | Heritability ($h^2$) | Genetic advance | Genetic gain (%) |
|---|---|---|---|---|---|
| Germination (%) | 20.21 | 20.24 | 0.99 | 19.39 | 41.64 |
| Seedling height (cm) | 31.64 | 31.82 | 0.99 | 25.94 | 64.81 |
| Collar diameter (mm) | 37.13 | 37.25 | 0.99 | 1.77 | 76.25 |
| Internodal length (cm) | 7.53 | 8.15 | 0.85 | 0.51 | 14.34 |
| Number of branches | 22.15 | 24.02 | 0.85 | 3.03 | 42.08 |
| Number of leaves per branch | 12.21 | 18.52 | 0.43 | 1.19 | 16.58 |

**Table 7:** Simple correlation measures

| Characters | Germination % | Seedling height (cm) | Collar diameter (mm) | Internodal length (cm) | Number of branches | Number of leaves per branch |
|---|---|---|---|---|---|---|
| Germination % | 1 | 0.692** | 0.709** | 0.689** | 0.668** | 0.665** |
| Seedling height (cm) | | 1 | 0.992** | 0.982** | 0.974** | 0.983** |
| Collar diameter (mm) | | | 1 | 0.987** | 0.969** | 0.959** |
| Internodal length (cm) | | | | 1 | 0.946** | 0.942** |
| Number of branches | | | | | 1 | 0.951** |
| Number of leaves per branch | | | | | | 1 |

## Conclusion

Thus, study indicated that variability exists in seed morphological and seedling characters of *Punica granatum*, collected from various seed sources of Himachal Pradesh and the 100 dry seed weight can be exploited as a parameter for predicting germination and seedling growth rate both in nursery and field conditions. It has been concluded that germination values influenced the seedling performances. It has also been observed during the survey of various seed sources that there was less incidence of insect pest attack in wild pomegranate which offers selection and hybridization for improvement in commercially cultivated *Punica granatum* cultivars. The results obtained in the present study comprise of seed source; for which the variability on morphological traits and fruit quality have been found to exist both between the seed source and within the seed sources. This information emphasises the wild pomegranate improvement programs as combination of individual tree selections especially for fruit characters and traits are a step forward to explore improved genotype in the wild pomegranate populations.

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