Biological Forum – An International Journal 13(3a): 523-527(2021)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Pollen Behavior of Andromonoecious Flowers of wild Pomegranate (*Punica granatum* L.) in North – Western Himalayas

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(Received 09 July 2021, Accepted 22 September, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The research was carried out at two sites *viz*; Tatool (S_1) district Solan, Narag (S_2) district Sirmour, Himachal Pradesh. The major challenge of the presented study was that this species consists of three types of flowers and pollen viability should be measured of male and hermaphrodite flowers separately. In this study, three types of flowers *viz*; male, intermediate, and hermaphrodite were observed in wild pomegranate. Two-way ANOVA and the factorial experimental design were used for analysis. Percentage of hermaphrodite flowers founded maximum 50.66 % at Tatool and 52% at Narag. Pollen viability of freshly dehisced pollen grains of hermaphrodite and male flowers was examined in 2% acetocarmine solution and the highest mean value observed for hermaphrodite flowers was 85.98 % and 84.02 % among both the sites and in all genotypes respectively. The significant results were obtained for pollen viability in selected genotypes and their interaction site × tree, tree × concentration, site × concentration, and in site × tree × concentration. These showed that hermaphrodite flowers pollen has more pollen viability than male flowers. Therefore, if we use the pollen of hermaphrodite flowers it may give more fruit set success than pollen of male flowers.

Keywords: pollen viability, acetocarmine, pomegranate, andromonoecious flower.

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family Lythraceae. There are two subspecies of species granatum; the *Punica granatum* ssp. *chlorocarpa* is indigenous to Trans-Caucasus region and the *Punica granatum* ssp. *porphyrocarpa* is a native of Central Asia (Sharma *et al*., 2009). According to Pliny, the name *Punica* was given by the Romans, referring to the city of Cartago, in Tunis (Punic, Phoenician, Carthaginian), from where the best pomegranate (from Latin "*pome*" which means apple and "*granate*" meaning many-seeded) arrived in Europe. The genera *Punica* contains two species *Punica granatum* L. and *Punica protopunica* Balf. (Guerrero – Solano *et al*., 2020). Initially, *Punica granatum* L. was known as *Malum punicum*, the apple of Cartago, but later, Carl Linnaeus (1707–1778) chose the current name, with a specific epithet of granatum, which means granular (Zeynalova *et al*., 2017). Wild Pomegranate (*Punica granatum* L.), Vern. Daru is an edible fruit and capable of growing in different agro-climatic conditions of tropical to subtropical land (Levin, 2006 and Jalikop, 2007). It is considered as a prototype of a cultivated one and resembles a cultivated pomegranate for various morphological characters. In India wild pomegranate is found only in the Western Himalayan zone (Narzary *et al*., 2009). This Himalayan variety has resistance to the deadly bacterial blight but these are not high yielding whereas, cultivated varieties are high yielding but susceptible to bacterial blight resulted in yield losses up to 60-80% (Sharma *et al*., 2015). It is highly valued for its nutrition and medicinal purposes (Aziz *et al*., 2020). Generally, pomegranate is propagated through seed and vegetative methods such as softwood, semi-hardwood, and hardwood cutting, layering, and grafting. (Koteswara-Rao *et al*., 2021). Research conducted at the Indian Institute of Horticulture Research revealed that not only the wild types but also the hybrids of both cultivated and wild types are found to be resistant against bacterial disease (Jalikop, 2005). In addition to this, the commercial market value of pomegranate fruit is further limited by physiological disorders like rind cracking, chilling injury, husk scald, and excessive weight loss (Caleb *et al*., 2012).

Wild pomegranate fruits have less fruit cracking and low attack of anarbutterfly because of its thick rind, breeding for these characters gaining importance (Jalikop *et al*., 2010). In pomegranate; usually, three types of flowers occur *viz*., pin, thrum, and homostylous (Singh *et al.*, 2006), they vary in their viability and germination. Detailed studies on flower types, pollination, pollen viability, and pollen germination are prerequisites to increase wild pomegranate production. In the present study variations in pollen viability of male and hermaphrodite was examined simultaneously. Therefore, the presented study can be used by plant breeders to develop highly productive, disease resistant hybrids of cultivated and wild type pomegranates. Also, there is a growing demand for good quality fruits both for fresh use and for making anardana, which are the dried arils of wild pomegranate with a distinct sour flavour and are mostly consumed in culinary preparations (Murtaza and Ahmad 2017).

MATERIALS AND METHODS

The investigation was conducted at the Department of Tree Improvement and Genetic Resources, College of Forestry, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. Two sites were selected for the study of flower types and collection of pollen *viz;* Tatool (S_1) district Solan and Narag (S_2) district Sirmour in HP. At each site, morphologically best five trees of wild pomegranate were randomly selected. Two-way ANOVA and factorial design were used for analysis of the observed data.

Analysis of variance (ANOVA): Variance and data were analysed by using the following model as suggested by Panse and Sukhatma (1967).

$Y_{ij} = \mu + gi + bj + eij$

i = 1, 2,..............g

j = 1, 2,..............r

where,

Y_{ij} = phenotypic observation of i^{th} entry and j^{th} replication

μ = general mean of the population

g_i = effect of i^{th} genotype

b_j = effect of j^{th} replication, and

e_{ij} = error component

Source of variation	d.f	Sum of Square	Mean of squares	Expectation
Replication	(r-1)	$1/g\ \Sigma\ jy\ i^2$ – C.F.	M_r	
Genotype	(g-1)	$1/r\ \Sigma\ iy\ i^2$ – C.F.	M_g	$\sigma^2 e + r\,\sigma^2 g$
Error	(r-1) (g-1)	By substraction	M_e	$\sigma^2 e$
Total	**(rg-1)**			

where,

r = Number of replications

g = Number of genotypes

M_r = Mean sum of squares due to replications

M_t = Mean sum of squares due to genotypes

M_e = Mean sum of squares due to error.

Critical difference (CD): The critical difference (CD) was calculated as under:

CD = $SE_d \times t_{0.05}$ error degree of freedom

where,

SE_d = Standard error of difference calculated as:

$SE_d = \sqrt{2M_e / r}$

$t_{0\cdot05}$ error degree of freedom = t value at 5 per cent level of significance.

Flower types: Thirty flowers were marked on each tree to examine the sex ratio and the type of flowers during the flowering phase. At anthesis, flowers were observed to determine the flower types. Flowers were divided into three groups *viz*., hermaphrodite, medium, and male flowers on the basis of the length of the style relative to the staminal column.

Pollen study: In each tree five branches were selected and ten flowers of different sex were marked on these selected branches to collect pollen and to observe their viability.

Pollen viability: Pollen viability was assessed from freshly collected pollen and stored pollen. Slides were made for hermaphroditic and male flowers separately. The pollen spread on a clean glass slide with a hairbrush and a drop of acetocarmine solution added to it. The prepared slides were left for 5-10 minutes and examined under a microscope. Deeply stained and normal-looking pollens were counted as viable whereas, wrinkles and weakly stained as non-viable.

RESULT AND DISCUSSIONS

Flower types: Flowers were divided into three groups *viz*., hermaphrodite, medium, and male flowers on the basis of the length of the style relative to the staminal column. Among the three types of observed flowers, only the Male and Hermaphrodite flowers (Fig. 1) were counted at both the sites because the percentage of intermediate types of flowers was very less or negligible. Maximum percentage was observed of Hermaphrodite flowers among trees and between both the sites (Table 1).

T_4S_1 and T_1S_1 had a maximum mean percentage (53.33%) of hermaphrodite flowers and T_4S_1 had a minimum percentage of male flowers. The same trend was observed for all selected trees. In Narag, S_2T_2 had a maximum proportion (56.67%) of hermaphrodite flowers and a minimum proportion of male flowers (20.0%). These traits represented a significant variation in the reproductive biology of this species, indicating their importance in breeding programs. The Mishra *et al.,* (2016) examined a maximum proportion of hermaphrodites (65.67%) and a minimum proportion of male flowers (23.00%) in wild pomegranate of the Mandi district. Kumar *et al*., (2020) investigated two stylous (heterostylous) flowers in pomegranate hermaphrodite and functionally male flowers on the same tree. They termed this situation as functional and romonoecy. The study was also supported by Singh *et al*., (2019), who reported two types of male and hermaphrodite flowers in wild pomegranate. Seday *et al.,* (2013) examined male (A-type- unfertile) and hermaphrodite flowers (B- type -fertile) on the same plants of pomegranate cultivars and also evaluated the effects of the pollens of different types of flowers on fruit set.

Fig. 1. Variation in flower types in pomegranate.

Table 1: Observed percentage of Male and hermaphrodite flowers in wild pomegranate.

Genotypes/Trees	Total flowers	Hermaphrodite (%)	Intermediate (%)	Male (%)
Tatool (S_1) T_1	30	53.33	26.67	20.00
T_2	30	50.00	30.00	20.00
T_3	30	46.67	30.00	23.00
T_4	30	53.33	30.00	16.67
T_5	30	50.00	30.00	20.00
	Mean	**50.66**	**29.33**	**20.01**
Narag (S_2) T_1	30	50.00	30.00	20.00
T_2	30	56.67	23.33	20.00
T_3	30	50.00	26.67	23.33
T_4	30	53.33	23.33	23.33
T_5	30	50.00	30.00	20.00
	Mean	**52.00**	**26.67**	**21.33**

Pollen viability: Brightly colored and normal-looking pollen were counted as viable pollen, wrinkled and lightly colored as non-viable, and viability was expressed in percentage (Fig. 2). The results observed for percent pollen viability were significant for locations, trees, types of flowers, and their interactions. A higher percentage of pollen viability (Table 2) (75.02) was recorded in pollens of Narag (S_2) trees compared to Tatool (73.83%) (S_1). Among all the selected trees, the maximum percentage of mean pollen viability was recorded in T_5 (79.76%) and the minimum in T_1 (70.03%). The highest percentage of pollen viability (85.00 %) was observed in pollens of hermaphrodite flowers and lowest in pollens of male flowers (63.85 %). In the site × tree interaction, the maximum mean percentage viability in Tatool (site1) was observed in S_1T_5 (79.50) while the minimum in S_1T_1 (69.08).

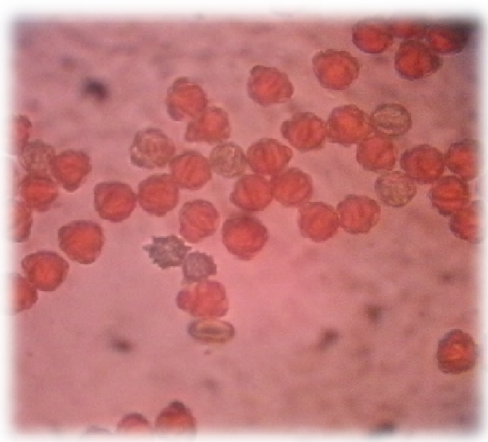

Fig. 2. Pollen viability in 2 % acetocarmine solution.

At Narag (site 2), the maximum mean percent pollen viability was recorded in S_2T_5 (80.02), which was comparable to S_2T_4, but the minimum was recorded in S_2T_3 (70.62%). For the site × flower interaction, the maximum mean viability percentage was observed for hermaphrodite flowers at both the locations; 84.02% at site 1 and 85.98% at site 2, while the minimum percentage recorded for male flowers at both the sites, i.e., 63.65% at site 1 and 64.05% at site 2. In tree × flower interaction, the highest pollen viability was recorded in T_5 for hermaphrodite flowers (90.33 %) and in T_5 (69.18 %) for the male flower, while the minimum percentage of viability for hermaphrodite flowers was recorded in T_2 (79.83%) and for male flowers in T_1 (58.48%). In case of the tree × location × flower interaction, the maximum percentage of pollen viability for hermaphrodite (91 %) and male (68 %) flowers was recorded in S_1T_5, but the minimum value was recorded in S_1T_1 for both types of the flowers, i.e., (79.33) for hermaphrodite flowers and (58.83) for male flowers. At Narag, the maximum percentage of pollen viability for hermaphrodite flowers was observed in S_2T_5 (91.50), while it was minimal in S_2T_2 (79.50). These results show that the pollen viability was observed highest for pollens collected from hermaphrodite flowers and minimal for male flowers among selected trees and between both the sites. These results help in better planning of breeding systems, developments of productive-disease resistant cross hybrid with the use of pollens collected from hermaphrodite flowers. The obtained results are in concurrence with the findings of Mishra *et al.*, (2016); Sharma *et al*., (2021), who reported pollen viability (83.94 % to 96.06 %) of wild pomegranate genotypes and of peach (97.78%) in 2 percent acetocarmine solution respectively. Pollen viability of pomegranate pollens in acetocarmine, ranged from 86.38 to 97.81 percent whereas, in tetrazolium and erythrosin B from 88.27- 95.13 and 83.34 to 92.15 percent respectively, reported by Dinesh *et al*. (2017). Sinha *et al*., (2017) investigated pollen viability of cultivated pomegranate germplasm accessions up to 97.93% in 2 % acetocarmine solutions. Significant differences for pollen viability of cultivars and flower types (Hermaphrodite and male) examined by Seday *et al*., (2013), who reported that, rate of viable pollen of hermaphrodite flowers varied between 64.35-29.44 %. Aksoy and Dalkilic (2019) showed pollen viability up to 84.7% in hermaphrodite flowers of Efenar 35 cultivar of pomegranate and pollen germination 71.2% hermaphrodite flowers of Kamilbey 35 cultivars.

Table 2: Pollen viability (%) in pomegranate flowers.

Tree No.	Tatool (S_1)			Narag (S_2)			Type of flower		Mean
	Herpahrodite flower	Male flower	Mean	Herpahrodite flower	Male flower	Mean	Herpahrodite flower	Male flower	
T_1	79.33	58.83	**69.08**	83.83	58.13	**70.98**	81.58	58.48	**70.03**
T_2	80.17	66.87	**73.52**	79.50	68.50	**74.00**	79.83	67.68	**73.76**
T_3	84.40	61.20	**72.80**	85.40	55.83	**70.62**	84.90	58.52	**71.71**
T_4	85.20	63.33	**74.27**	91.50	67.43	**79.47**	88.35	65.38	**76.87**
T_5	91.00	68.00	**79.50**	89.67	70.37	**80.02**	90.33	69.18	**79.76**
Mean	**84.02**	**63.65**	**73.83**	**85.98**	**64.05**	**75.02**	**85.00**	**63.85**	

CD Site	Tree	Flower	Site x Tree	Site × Flower	Tree × Flower	Site × Tree × Flower
0.38	**0.60**	**0.38**	**0.85**	**0.54**	**0.85**	**1.2**

CONCLUSION

Fruit set and quality production effected by the proportion of fertile flowers (hermaphrodites), pollen viability, and pollen germination. When we collect pollen from hermaphroditic flowers for pollination, it increases the success rate of reproduction. Since very few studies have been conducted on wild pomegranate, it can be used by the breeder as a guide for the development of disease-resistant cultivated pomegranate hybrids and productive strains of wild access. We observed the maximum percentage of hermaphrodite flowers at both selected locations. Pollen viability was observed highest for hermaphrodite flowers than for male flowers among all selected trees and between both the sites. These selected locations can be used as a seed and pollen source by researchers for future research and by farmers to increase anardana production.

FUTURE SCOPE

The findings of this article can be used as a reference to develop hybrids of wild and cultivated pomegranate. Also, can be used to obtain better fruit set with pollens of the hermaphrodite flowers.

Acknowledgement. Special thanks to Dr. Tara Gupta (Major advisor) and to my advisory committee members for their guidance and support throughout the course of study.
Conflict of Interest. None.

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How to cite this article: Dhiman, J., Gupta, T. and Gupta, R.K. (2021). Pollen Behavior of Andromonoecious Flowers of wild Pomegranate *(Punica granatum* L.) in North – Western Himalayas. *Biological Forum – An International Journal*, *13*(3a): 523-527.