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Determination of pollen viability of wild pomegranate accessions in the mid-hill zone of Himachal Pradesh

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ABSTRACT

Wild pomegranate, *Punica granatum* L is regarded as a biotype of cultivated pomegranate which resembles the latter in various aspects viz growth, flowering and fruit characters with high acidic taste. Not much work has been carried out on wild pomegranate as it is only confined to the mid-hill zone of western Himalayan region. Evaluation is the preliminary step for any breeding programme and the present study was carried out at the Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry with the aim of evaluating the genotypes for 2 consequent years (2013-14 and 2014-15) on the basis of pollen viability which plays an important role in pollination and fruit set. The freshly dehisced pollens from unopened buds were taken and viability test was conducted by using both erythrosine B (1%) and acetocarmine (2%) solution. The statistical analysis revealed that the viability of pollen grains ranged (polled) from 80.85 to 87.81 per cent and 83. 94 to 96.06 per cent through erythrosine B (1%) and acetocarmine (2%) respectively. Best pollen viability was observed in acetocarmine (2%). Plant # 8 of Mandi had maximum pollen viability (96.06%) among all the genotypes.

Keywords: Wild pomegranate; biotype; pollen viability; erythrosine; acetocarmine

INTRODUCTION

The pomegranate, *Punica granatum* L is one of the ancient edible fruit species originated in Mediterranean region close to Iran. Pomegranate is much more adaptive to a wide range of climates and soil conditions and grown in a large geographical region including Mediterranean region, Asia and in the United States particularly in the region of California (Holland et al 2009). Wild pomegranate commonly known as Daru or Darim in Himachal Pradesh is regarded as a biotype of the cultivated species and belongs to the family Punicaceae with somatic chromosome number 2n= 2x= 18 (Smith 1976). The plants are deciduous in nature and generally

grow wild in the sub-mountain track of Himachal Pradesh, Jammu and Kashmir and Uttarakhand at an altitude of 900-1800 m amsl. The TSS of the fruit varies from 11.0 to 15.5°B and titratable acidity ranges from 5.26 to 6.80 per cent (Bakshi et al 2014)). Higher acidity and TSS of the crop makes the same useful for the preparation of dried product Anardana which is preferred and fetches handsome price in the market. The flowers of pomegranate are large, showy with vibrant red orange color. Flowers are classified into male, hermaphrodite and intermediate based on flower morphology (Chaudhari and Desai 1993). The flower contains numerous anthers which are originated from the base of petals and protruding outside. Pollen grains are released some hours after flowering, even simultaneously or before flowering on the basis of cultivar and environmental conditions. Pollen grains are numerous and the number in each anther is about 16000-23000 and on average 270000 pollen grains have been counted from each flower. The size of pollen grain is about 16-25 micrometer which is distinct in different cultivars (Tabatabayi 2001, Vazifehshenas 2009). The pollen fertility plays a major role in pollination and effective fruit set of any crop. The pollen fertility of pomegranate varieties differs according to varieties and flower types also. Not much work has been carried out on the wild pomegranate and present study was carried out to estimate the percentage of viable pollen grains in each accession of the germplasm.

MATERIAL and METHODS

Wild pomegranate accessions were collected from four different districts of Himachal Pradesh viz Sirmour, Shimla, Solan and Mandi and planted in the farm of Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Solan, HP. Plant material for study was selected on the basis of regular flowering and fruiting behavior. Ten genotypes were selected from each district taken in a factorial randomized block design with three replications. Evaluation study was carried out for two consequent years (2013-14 and 2014-15) and data were recorded on various morphological traits viz plant height, girth, spread, leaf area, flower length and diameter, types of flower, fruit weight, length, diameter, aril weight, peel and rag weight, rind thickness, fruit TSS, acidity, sugars and yield per plant. Data were analyzed and expressed as pooled mean values.

Flower buds at balloon stage were collected and the anthers were detached and kept on a piece of butter paper under partial shade for dehiscence. The pollen grains thus collected were put in the glass vials. Freshly dehisced pollen grains were used for pollen studies.

Acetocarmine solution (2%) test

Pollen grains were stained in 2 per cent acetocarmine solution prepared by dissolving 2 grams of carmine powder in 45 ml of glacial acetic acid and final volume

made to 100 ml by adding distilled water. Solution was boiled for five minutes and filtered through Whatman # 1 filter paper. The pollen grains were dusted on glass slide and one to two drops of acetocarmine solution were put on these grains. After placing a cover slip over the stain it was left for five minutes for proper staining of pollen grains. Slides were observed under microscope. Deeply stained and normal looking pollen grains were considered to be viable whereas shriveled, lightly stained or colorless pollen grains were counted as non-viable. Three fields were observed; viable and non-viable pollen grains were counted in each field.

Erytrosine B (0.1%) test

Pollen grains were stained in 0.1 per cent erythrosine B solution. The unstained pollen grains were considered viable in this method. Stain solution (0.1%) was prepared by dissolving 100 mg of erythrosine B in distilled water to make a final solution of 100 ml. Staining procedure was same as in case of acetocarmine

RESULTS

The observations with respect to the accessed traits revealed that considerable variation persisted among the wild pomegranate germplasm and it varied according to different situations. The test conducted through erythrosine B (1%) showed that pollen viability ranged from 80.51 (Plant # 7 of Sirmour) to 88.69 per cent (Plant # 10 of Shimla) and 80.45 (Plant # 10 of Sirmour) to 88.16 per cent (Plant # 3 of Shimla) during 2013-14 and 2014-15 respectively (Table 1). Among the four districts Mandi had highest pollen viability during the year 2013-14 whereas during 2014-15 both Solan and Mandi districts had maximum value for the trait. From the pooled data it is evident that the viability varied from 80.85 to 87.81 per cent. Plant # 9 (83.86%) among the accessions of Sirmour, Plant # 3 (87.81%) among Shimla, Plant # 2 (86.56 %) among Solan and Plant # 7 (87.75 %) among Mandi were found having maximum pollen viability. Highest percentage of viable pollens was observed in Plant # 3 of Shimla (87.81%) and minimum in the Plant # 2 (80.85%) of Sirmour among the total germplasms. Plant # 3 of Shimla and Plant # 7 of Mandi district were statistically at par for the maximum pollen germination. Among the four districts the plants of Mandi district had higher mean (85.61%) pollen viability.

The viability test conducted through acetocarmine (2%) indicated that maximum pollen viability was recorded in the pollens from Plant # 8 of Mandi district (96.23%) and least in the pollens of Plant # 4 of Sirmour district (85.79) during 2013-14 and similar trendd was also seen during 2014-15 as well as for the pooled mean (Table 2). The polled mean revealed that Plant # 8 had maximum pollen viability (96.06%) whereas Plant # 4 had least (83.94%) among all the germplasms (D x P). Plant # 9 of Sirmour had maximum pollen viability among the plants of the same district

Table 1. Pollen viability of wild pomegranate genotypes (using 1% erythrosine B)

Plant #	2013-14					2014-15					Pooled				
	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean
1.	84.25	87.15	85.52	85.87	85.70	82.90	86.62	85.59	84.50	84.90	83.58	86.88	85.56	85.19	85.30
	(9.18)	(9.34)	(9.25)	(9.27)	(9.26)	(9.11)	(9.31)	(9.25)	(9.19)	(9.21)	(9.14)	(9.32)	(9.25)	(9.23)	(9.24)
2.	81.18	87.84	86.44	85.53	85.25	80.52	86.84	86.68	84.53	84.64	80.85	87.34	86.56	85.03	84.94
	(9.01)	(9.37)	(9.30)	(9.25)	(9.23)	(8.97)	(9.32)	(9.31)	(9.19)	(9.20)	(8.99)	(9.35)	(9.30)	(9.22)	(9.22)
3.	80.83	87.46	85.62	87.26	85.29	83.17	88.16	84.28	83.46	84.77	82.00	87.81	84.95	85.36	85.03
	(8.99)	(9.35)	(9.25)	(9.34)	(9.23)	(9.12)	(9.39)	(9.18)	(9.14)	(9.21)	(9.06)	(9.37)	(9.22)	(9.24)	(9.22)
4.	82.36	87.00	86.06	84.54	84.99	83.06	86.20	85.69	84.24	84.80	82.71	86.60	85.87	84.39	84.89
	(9.08)	(9.33)	(9.28)	(9.19)	(9.22)	(9.11)	(9.28)	(9.26)	(9.18)	(9.21)	(9.09)	(9.31)	(9.27)	(9.19)	(9.21)
5.	82.68	86.58	86.70	84.47	85.11	84.32	83.42	85.44	83.00	84.05	83.50	85.00	86.07	83.74	84.58
	(9.09)	(9.31)	(9.31)	(9.19)	(9.22)	(9.18)	(9.13)	(9.24)	(9.11)	(9.17)	(9.14)	(9.22)	(9.28)	(9.15)	(9.20)
6.	82.40	82.44	84.74	85.35	83.73	82.40	81.51	82.74	86.22	83.22	82.40	81.98	83.74	85.79	83.48
	(9.08)	(9.08)	(9.21)	(9.24)	(9.15)	(9.08)	(9.03)	(9.10)	(9.29)	(9.12)	(9.08)	(9.05)	(9.15)	(9.26)	(9.14)
7.	80.51	84.08	84.44	88.26	84.32	82.44	84.01	84.70	87.23	84.60	81.48	84.04	84.57	87.75	84.46
	(8.97)	(9.17)	(9.19)	(9.39)	(9.18)	(9.08)	(9.17)	(9.20)	(9.34)	(9.20)	(9.03)	(9.17)	(9.20)	(9.37)	(9.19)
8.	82.65	83.41	85.73	87.52	84.83	83.02	82.45	84.43	87.45	84.34	82.83	82.93	85.08	87.49	84.58
	(9.09)	(9.13)	(9.26)	(9.36)	(9.21)	(9.11)	(9.08)	(9.19)	(9.35)	(9.18)	(9.10)	(9.11)	(9.22)	(9.35)	(9.20)
9.	84.37	83.71	86.62	87.03	85.43	83.35	81.48	86.32	86.66	84.45	83.86	82.59	86.47	86.85	84.94
	(9.19)	(9.15)	(9.31)	(9.33)	(9.24)	(9.13)	(9.03)	(9.29)	(9.31)	(9.19)	(9.16)	(9.09)	(9.30)	(9.32)	(9.22)
10.	81.82	88.69	85.09	85.35	85.24	80.45	84.63	85.09	83.65	83.45	81.13	86.66	85.09	84.50	84.34
	(9.04)	(9.42)	(9.22)	(9.24)	(9.23)	(8.97)	(9.20)	(9.22)	(9.15)	(9.13)	(9.01)	(9.31)	(9.22)	(9.19)	(9.18)
Mean	82.31	85.84	85.70	86.12	84.99	82.56	84.53	85.10	85.10	84.32	82.43	85.18	85.40	85.61	84.65
	(9.07)	(9.26)	(9.26)	(9.28)	(9.21)	(9.09)	(9.19)	(9.22)	(9.22)	(9.18)	(9.08)	(9.23)	(9.24)	(9.25)	(9.20)
$CD_{0.05}$	D				0.02					0.04					0.03
	P				0.04					0.07					0.04
	D × P				0.08					0.14					0.08

Table 2. Pollen viability of wild pomegranate genotypes (using 2% acetocarmine)

Plant #	2013-14					2014-15					Pooled				
	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean
1.	91.90	90.66	91.18	95.12	92.22	91.53	90.16	92.22	93.34	91.81	91.72	90.41	91.70	94.23	92.02
	(9.59)	(9.52)	(9.55)	(9.75)	(9.60)	(9.57)	(9.49)	(9.60)	(9.66)	(9.58)	(9.58)	(9.51)	(9.58)	(9.71)	(9.59)
2.	90.42	92.28	91.57	93.69	91.99	93.65	91.96	92.33	92.95	92.73	92.04	92.12	91.95	93.32	92.36
	(9.51)	(9.61)	(9.57)	(9.68)	(9.59)	(9.68)	(9.59)	(9.61)	(9.64)	(9.63)	(9.59)	(9.60)	(9.59)	(9.66)	(9.61)
3.	90.98	94.19	90.98	93.16	92.33	90.84	93.32	91.64	92.24	92.01	90.91	93.75	91.31	92.70	92.17
	(9.54)	(9.70)	(9.54)	(9.65)	(9.61)	(9.53)	(9.66)	(9.57)	(9.60)	(9.59)	(9.53)	(9.68)	(9.56)	(9.63)	(9.60)
4.	85.79	91.32	95.36	91.10	90.89	82.09	87.98	94.59	91.83	89.12	83.94	89.65	94.97	91.46	90.01
	(9.26)	(9.56)	(9.76)	(9.54)	(9.53)	(9.06)	(9.38)	(9.73)	(9.58)	(9.44)	(9.16)	(9.47)	(9.75)	(9.56)	(9.48)
5.	90.29	92.33	94.89	90.38	91.97	89.29	91.96	94.19	90.04	91.37	89.79	92.15	94.54	90.21	91.67
	(9.50)	(9.61)	(9.74)	(9.51)	(9.59)	(9.45)	(9.59)	(9.70)	(9.49)	(9.56)	(9.48)	(9.60)	(9.72)	(9.50)	(9.57)
6.	90.77	91.04	93.79	94.25	92.46	93.40	93.97	92.89	93.32	93.40	92.09	92.51	93.34	93.79	92.93
	(9.53)	(9.54)	(9.68)	(9.71)	(9.61)	(9.66)	(9.69)	(9.64)	(9.66)	(9.66)	(9.60)	(9.62)	(9.66)	(9.68)	(9.64)
7.	91.26	94.12	94.99	90.58	92.74	92.26	93.84	92.66	90.25	92.25	91.76	93.98	93.82	90.42	92.49
	(9.55)	(9.70)	(9.75)	(9.52)	(9.63)	(9.61)	(9.69)	(9.63)	(9.50)	(9.60)	(9.58)	(9.69)	(9.69)	(9.51)	(9.62)
8.	93.99	92.08	94.09	96.23	94.10	95.09	91.08	92.42	95.89	93.62	94.54	91.58	93.25	96.06	93.86
	(9.69)	(9.60)	(9.70)	(9.81)	(9.70)	(9.75)	(9.54)	(9.61)	(9.79)	(9.67)	(9.72)	(9.57)	(9.66)	(9.80)	(9.69)
9.	94.12	92.32	94.68	92.02	93.28	95.48	91.25	94.61	91.86	93.30	94.80	91.79	94.64	91.94	93.29
	(9.70)	(9.61)	(9.73)	(9.59)	(9.66)	(9.77)	(9.55)	(9.73)	(9.58)	(9.66)	(9.74)	(9.58)	(9.73)	(9.59)	(9.66)
10.	89.91	89.79	92.59	90.48	90.69	86.87	90.50	92.98	90.91	90.32	88.39	90.15	92.79	90.69	90.50
	(9.48)	(9.48)	(9.62)	(9.51)	(9.52)	(9.32)	(9.51)	(9.64)	(9.53)	(9.50)	(9.40)	(9.49)	(9.63)	(9.52)	(9.51)
Mean	90.94	92.01	93.41	92.70	92.27	91.05	91.60	93.05	92.26	91.99	91.00	91.81	93.23	92.48	92.13
	(9.54)	(9.59)	(9.66)	(9.63)	(9.60)	(9.54)	(9.57)	(9.65)	(9.60)	(9.59)	(9.54)	(9.58)	(9.66)	(9.62)	(9.59)
$CD_{0.05}$	D				0.05					0.06					0.08
	P				0.08					0.09					0.07
	D × P				0.16					0.18					0.14

and Plant # 7 among plants of Shimla, Plant # 4 among the accessions of Solan and Plant # 8 among the plants of Mandi possessed maximum pollen viability. However from the mean district values it is clear that the plants of Solan district possessed highest (93.23%) pollen viability among the four districts.

DISCUSSION

Good pollen viability helps in better pollination and fertilization and results in good fruit set. As the crop bears functionally male and bisexual flowers among which the staminate (functionally male) flowers generally drop. It is therefore essential for the maximum bisexual flowers to set for a good crop. Maximum pollen viability was found 96.06 per cent and minimum 83.94 per cent by acetocarmine (2%) whereas highest pollen viability was registered as 87.81 per cent and least 80.85 per cent when tested by erythrosine one per cent. The results indicate that the pollens of wild pomegranate possessed good amount of viability which can affect the ultimate fruit set and the accessions having maximum viability can be used as pollinizers for commercial cultivation as well as can be used as parents in breeding programmes. Maximum pollen viability was obtained by using acetocarmine compared to erythrosine. The findings are in line with the findings of Sharma (2001) who recorded maximum pollen viability of 97.91 per cent in cv G-137 and 91.15 per cent in cv Mridula. The small variation may be due to the variation of genotypic constitution of cultivated pomegranate and the wild one or may be due to climatic conditions during both the years of study.

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