Hybridization studies on pomegranate (*Punica granatum* L) cultivars and wild germplasm accessions

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ABSTRACT

Five cultivars namely Ganesh, G-137, Kandhari Kabuli, Bhagwa and Mridula and two wild pomegranate (Daru) germplasm accessions namely NT-1 and MH-1 were used for pollen and hybridization studies. Pollen viability was assessed by acetocarmine stain (2%), tetrazolium stain (1%) and erythrosin B stain (0.1%). The pollen viability in acetocarmine ranged from 86.38 to 97.81 per cent whereas in tetrazolium and erythrosin B from 88.27 to 95.13 and 83.34 to 92.15 per cent respectively. Maximum pollen germination was obtained in 10 per cent sucrose solution after 72 hours with 42.34 per cent germination and minimum (2.17%) in 20 per cent sucrose solution at 72 hours. Some pomegranate cultivars/wild germplasm accessions showed no germination after 24, 48 and 72 hours in different concentrations of sucrose. Amongst all the cross combinations attempted the highest (79.09%) fruit set was observed in Bhagwa x NT-1 and minimum in MH-1 x G-137 (57.76 %). Fruit retention ranged from 10.34 to 30.09 per cent in all the cross combinations. Seed germination from hybrid seeds ranged from 0.00 to 19.43 per cent in all cross combinations.

Keywords: Germplasm accessions; *Punica granatum*; hybridization,; pollen viability; in vitro germination

INTRODUCTION

Pomegranate (*Punica granatum* L), a member of family Punicaceae is a favourite table fruit of the tropical and subtropical regions. It is very much liked for its cool refreshing juice and valued for its medicinal properties. The clonal degeneration of adapted varietal lot, absence of varieties suited to local climatic conditions and devastating outbreak of bacterial blight necessitates the breeding of varieties resistant to the same. Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv *punicae* has become an increasingly serious threat for pomegranate growers in the Indian subcontinent. So far there is no known source of gene present imparting resistance to this disease except wild pomegranate (*P granatum* ssp *protopunica*) Daru and dwarf ornamental pomegranate variety Nana which are hardy in nature and do not exhibit any symptoms of the disease (Jalikop et al 2006). The search for resistant varieties forms the basis for a successful management of this disease in the future globally.

The information on pollen characters viz pollen viability and pollen germination is a prerequisite for carrying out a meaningful crop improvement programme particularly when a trait of interest is to be incorporated from a wild source to cultivated forms. Keeping in view the above mentioned facts the present investigations were carried out involving Ganesh, G-137, Mridula, Kandhari Kabuli, Bhagwa and wild pomegranate germplasm accessions (NT-1 and MH-1) possessing bacterial blight resistance with a view to determine the extent of cross compatibility to achieve successful crossing between different cultivars.

MATERIAL and METHODS

The present investigations were carried out in pomegranate blocks of the Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh and the research farm of Horticultural Research Station, Kandaghat, Solan, Himachal Pradesh. The cultivars undertaken for study were Ganesh, G-137, Mridula, Kandhari Kabuli,

Bhagwa and wild pomegranate germplasm accessions (NT-1 and MH-1).

Pollen studies: Pollen viability was ascertained by using 3 different stains viz acetocarmine (2%), tetrazolium (1%) and erythrosin B (0.1%). In case of acetocarmine (2%) and tetrazolium (1%) deeply stained and normal looking pollen grains were considered to be viable whereas shrivelled, lightly stained or colourless pollen grains as non-viable. In erythrosin B (0.1%) the unstained pollen grains were considered viable and stained as non-viable. Pollen germination was assessed using hanging drop method in different concentrations (10, 15 and 20%) of sucrose. The pollen tube growth was assessed for each cultivar under microscope after 24, 48 and 72 hours. The pollen grains having pollen tube at least two times longer than the pollen size were considered to be germinated.

Hybridization studies: For emasculation/crossing about to open healthy flower buds which were marked by the appearance of cracks at the apex of bud were selected and emasculated with the help of forceps. The remaining opened and immature flower buds were removed from the branch. The emasculated flowers showing stigma receptivity (shiny appearance and showing signs of wetness) were pollinated with desired pollen parent in each cultivar. The cross combinations involving Ganesh x NT-1, Ganesh x MH-1, G-137 x NT-1, G-137 x MH-1, Mridula x NT-1, Mridula x MH-1, Kandhari Hansi x NT-1, Kandhari Hansi x MH-1, Bhagwa x NT-1 and Bhagwa x MH-1 including their reciprocals were attempted.

The number of fruits set in each cross combination was recorded after six weeks of pollination and percentage was worked out as per the formula given below:

$$\text{Fruit set (\%)} = \frac{\text{Number of fruits developed}}{\text{Total number of flowers}} \times 100$$

The fruits retained on the plant were recorded one week before harvesting of fruits.

Hybrid seed germination: Seeds were extracted from hybrid fruits after harvesting. The freshly extracted seeds from each cross combination were subjected to germination in pots for subsequent raising of hybrid seedlings.

The germination percentage was recorded after 15 days of sowing of seeds by using following formula:

$$\text{Seed germination (\%)} = \frac{\text{Seeds germinated}}{\text{Total number of seeds}} \times 100$$

The hybrid seedlings were transplanted and lined out in nursery beds when they attained a height of 4-6 inches.

Different stages of procedure for hybridization in pomegranate are given in Plate 1 and developing hybrid fruits of different cross combinations in Plate 2.

Statistical analysis: The data were analyzed in completely randomized design (pollen germination and pollen viability) and randomized block design (fruit set and fruit retention) as per the procedures given by Panse and Sukhatme (1985).

RESULTS and DISCUSSION

Pollen studies: The stainability test though not a sure test of pollen viability (Porlingh 1956) is employed widely as an index of pollen viability. In the present studies pollen viability was tested with acetocarmine (2%), tetrazolium (1%) and erythrosin B (0.1%) and it ranged between 83.34 to 97.81 per cent in all the three stains. Mean pollen viability in all cultivars was lowest (86.49%) in erythrosin B (Table 1). Acetocarmine dissolves the cell wall and stains the protein (chromatic material) content of pollen grains red coloured (Stanley and Linskens 1974) whereas tetrazolium salt is reduced by the enzyme named succinic dehydrogenase which is an essential enzyme of Kreb's Cycle. In tetrazolium viable pollen grains show red colour due to accumulation of formazan (Prakash et al 2010). Since protein (chromatin material) may be present in every pollen but succinic dehydrogenase is present only in those pollen grains where metabolic activity is going on therefore the percentage of viability through acetocarmine is higher because protein containing metabolically dead pollen grains are also counted as viable (Sharma 2001).

The observations on pollen viability are in agreement with the findings recorded by Ali et al (1998), Sharma and Bist (2003), Prakash et al (2010) and

Table 1. Pollen viability of pomegranate cultivars/wild germplasm accessions with different stains

Cultivar	Acetocarmine (2 %)	Tetrazolium (1 %)	Erythrosin B (0.1 %)
Ganesh	95.84 (9.79)	93.89 (9.69)	85.95 (9.27)
G-137	94.63 (9.73)	95.13 (9.75)	83.90 (9.16)
Kandhari Kabuli	97.81 (9.89)	93.52 (9.67)	92.15 (9.60)
Bhagwa	90.52 (9.51)	91.09 (9.54)	85.76 (9.26)
Mridula	93.74 (9.68)	90.10 (9.49)	90.37 (9.51)
NT-1	86.38 (9.29)	92.07 (9.60)	83.95 (9.16)
MH-1	88.79 (9.42)	88.27 (9.40)	83.34 (9.13)
Mean	92.53 (9.62)	92.01 (9.59)	86.49 (9.30)
$CD_{0.05}$	0.14	0.16	0.28

Figures in parentheses are square root transformed values

Kumar (2012). However Gozlekci and Kaynak (2000) recorded somewhat lower pollen viability (30 to 65%) in different pomegranate cultivars. The variation in pollen viability may be attributed to the genetic constitution of the cultivars.

In vitro pollen germination: The observations on pollen germination of various cultivars/wild germplasm accessions in 10, 15 and 20 per cent sucrose solution at room temperature were recorded after 24, 48 and 72 hours. The recorded data revealed that average pollen germination was found maximum (27.57%) after 72 hours in 10 per cent sucrose and minimum average (1.86%) was recorded in 20 per cent concentration at 24 hours.

The pollen germination is considered more reliable and more convenient method to assess the level of viability compared to staining tests (Griggs et al 1953, Oberle and Watson 1953, Werner and Chang 1981). The pollen germination percentage in the present investigations as assessed by germination in different concentrations of sucrose and pollen germination was ideal in 10 and 15 per cent of sucrose solution at 24, 48 and 72 hours however pollen germination was very low in 20 per cent sucrose solution. Similar observations have been made by Nath and Randhawa (1959) and Sharma and Bist (2003). The variation in pollen germination percentage may be attributed to the genotypic constitution of pollen in different cultivars.

The germination of pollen was found to be maximum in 10 per cent of sucrose after 72 hours followed by 15 per cent and the least in 20 per cent (Table 2). However Wetzstein et al (2011) reported 74 to 79 per cent germination in different pomegranate cultivars. They reported that significant variation in germination percentage was due to the temperature differences during pollen germination, collection time, method of collection, pollen storage, pollen density and composition of germination medium (Kwack 1965, Singh et al 1961, Stanley and Linkens 1974, Visser 1955, Zienlinski and Olez 1963) and genetic constitution of pollen in different cultivars. Similar observations have been recorded by Nath and Randhawa (1959), Sharma and Bist (2003) and Kumar (2012).

Hybridization studies: All the pomegranate cultivars/ wild germplasm accessions in which hybridization was done were found to be cross-compatible. Fruit set in different cross combinations ranged from 57.76 to 79.09 per cent (Table 3). The fruit set through artificial cross pollination varied with different cross combinations. Similar results have been obtained by Josan et al (1979), Karale et al (1993) after attempting different crosses in pomegranate. Kumar (2012) attempted crosses between soft- and hard-seeded pomegranate cultivars. Fruit set in the different cross combinations ranged from 37.60 to 80.95 per cent. However Sharma and Bist (2003) reported maximum fruit set up to 90 per cent by artificial cross pollination in different cultivars.

Fruit retention varied from 10.34 to 30.09 per cent (Table 3). This significant variation was observed due to adverse climatic conditions resulting in fruit cracking and attack of bacterial blight disease during the hybridization programme. Kumar (2012) achieved similar results while attempting inter-varietal crosses in pomegranate with fruit retention ranging from 8.14 to 29.60 per cent.

Hybrid seed germination: Seed germination in different cross combinations ranged from 0.00 to 19.43

Table 2. In vitro pollen germination of pomegranate cultivars/wild germplasm accessions in different concentrations of sucrose at different time intervals

Cultivar	Pollen germination in different concentrations of sucrose and time intervals											
	After 24 h*				After 48 h**				After 72 h**			
	10%	15%	20%	Mean	10%	15%	20%	Mean	10%	15%	20%	Mean
Ganesh	15.99 (3.99)	25.93 (5.09)	3.93 (1.98)	15.28 (3.69)	20.41 (26.83)	28.93 (32.52)	6.69 (14.94)	18.68 (24.76)	23.02 (28.65)	33.25 (35.20)	9.23 (17.60)	21.83 (27.15)
G-137	16.65 (4.08)	18.45 (4.28)	0.00 (0.00)	11.70 (2.79)	27.37 (31.50)	23.55 (29.01)	0.00 (0.00)	16.97 (20.17)	30.01 (33.21)	30.16 (33.29)	0.00 (0.00)	20.05 (22.16)
Mridula	11.89 (3.44)	9.72 (3.11)	2.60 (1.61)	8.07 (2.72)	15.27 (22.98)	16.37 (23.85)	4.66 (12.39)	12.10 (19.74)	17.19 (24.18)	21.49 (27.60)	5.66 (13.53)	14.78 (21.77)
Kandhari Kabuli	22.84 (4.78)	15.02 (3.87)	4.08 (2.01)	13.98 (3.55)	28.28 (32.13)	24.02 (29.33)	7.23 (15.57)	19.84 (25.68)	34.97 (36.25)	26.92 (31.18)	9.69 (18.13)	23.86 (28.52)
Bhagwa	27.19 (5.21)	15.81 (3.97)	2.43 (1.55)	15.14 (3.58)	36.78 (37.33)	29.72 (33.03)	3.59 (10.79)	23.37 (27.05)	42.34 (40.58)	37.09 (37.51)	3.95 (11.32)	27.79 (29.80)
NT-1	14.03 (3.74)	7.93 (2.82)	0.00 (0.00)	7.32 (2.18)	21.20 (27.37)	15.17 (22.91)	0.00 (0.00)	12.12 (16.76)	25.01 (29.95)	16.13 (23.55)	0.00 (0.00)	13.71 (17.83)
MH-1	9.35 (3.05)	19.56 (4.42)	0.00 (0.00)	9.64 (2.49)	16.65 (24.06)	26.74 (31.13)	0.00 (0.00)	14.46 (18.40)	20.48 (26.85)	27.49 (31.60)	2.17 (8.472)	16.71 (22.31)
Mean	16.85 (4.04)	16.06 (3.99)	1.86 (1.02)		23.71 (28.89)	23.50 (28.83)	3.17 (7.67)		27.57 (31.42)	27.50 (31.38)	4.39 (9.87)	

*Figures in parentheses are square root transformed values, **Figures in parentheses are arc sine transformed values

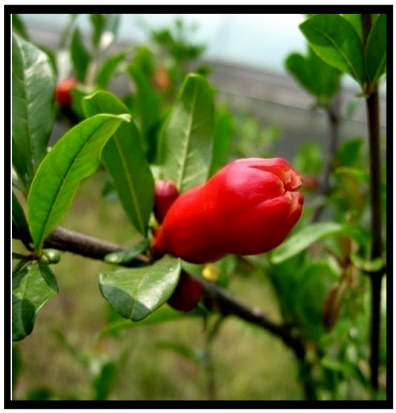
Table 3. Fruit set and retention under artificial cross pollination

Cross combination	Fruit set (%)*	Fruit retention (%)**
Ganesh x MH-1	72.99 (58.72)	27.45 (5.23)
Ganesh x NT-1	73.14 (58.83)	26.64 (5.16)
Mridula x MH-1	65.86 (54.25)	18.53 (4.30)
Mridula x NT-1	66.03 (54.36)	26.22 (5.12)
Bhagwa x MH-1	76.47 (61.01)	26.89 (5.18)
Bhagwa x NT-1	79.09 (62.80)	29.42 (5.42)
G-137 x MH-1	67.72 (55.42)	20.03 (4.47)
G-137 x NT-1	68.83 (56.08)	24.08 (4.91)
Kandhari Kabuli x MH-1	76.95 (61.40)	30.09 (5.48)
Kandhari Kabuli x NT-1	77.51 (61.70)	29.56 (5.44)
MH-1 x Ganesh	61.37 (51.58)	15.16 (3.89)
NT-1 x Ganesh	74.35 (59.99)	17.62 (4.20)
MH-1 x Mridula	65.66 (54.14)	10.34 (3.21)
NT-1 x Mridula	61.56 (51.69)	17.23 (4.15)
MH-1 x Bhagwa	69.81 (56.77)	27.56 (5.25)
NT-1 x Bhagwa	72.87 (58.85)	29.08 (5.39)
MH-1 x G-137	57.76 (49.52)	14.53 (3.81)
NT-1 x G-137	59.38 (50.47)	13.52 (3.67)
MH-1 x Kandhari Kabuli	62.50 (52.25)	24.87 (4.99)
NT-1 x Kandhari Kabuli	64.75 (53.60)	22.95 (4.79)
$CD_{0.05}$	4.06	0.32

*Figures in parentheses are arc sine transformed values, **Figures in parenthesis are square root transformed values

Table 4. Hybrid seed/seedlings obtained from different cross combinations in pomegranate

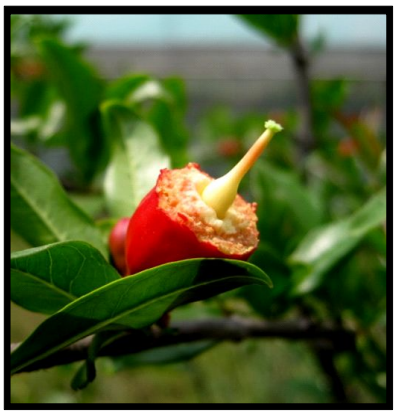
Cross combination	Hybrid seeds obtained/sown	Seed germination (%)	Seedlings obtained
Ganesh x MH-1	240	17.50	18
Ganesh x NT-1	170	17.64	14
Mridula x MH-1	250	15.20	20
Mridula x NT-1	170	15.88	21
Bhagwa x MH-1	230	15.21	18
Bhagwa x NT-1	225	15.11	14
G-137 x MH-1	245	13.06	18
G-137 x NT-1	315	12.69	18
Kandhari Kabuli x MH-1	230	17.39	12
Kandhari Kabuli x NT-1	220	16.36	19
MH-1 x Ganesh	230	0.00	0
NT-1 x Ganesh	225	4.54	0
MH-1 x Mridula	220	0.00	0
NT-1 x Mridula	210	6.67	0
MH-1 x Bhagwa	180	17.22	09
NT-1 x Bhagwa	175	8.57	07
MH-1 x G-137	220	8.18	10
NT-1 x G-137	230	6.96	09
MH-1 x Kandhari Kabuli	175	14.29	17
NT-1 x Kandhari Kabuli	175	19.43	23



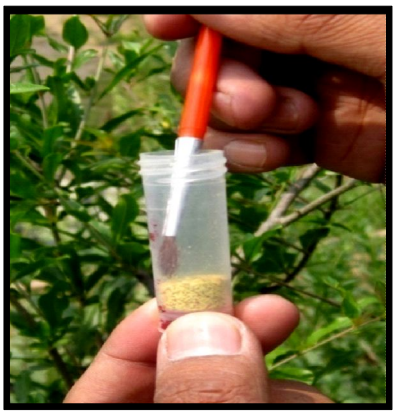
a. Selection of appropriate bud for emasculation

b. Removal of calyx cup

c. Removal of stamens



d. Emasculated flower



e. Stored pollen grains in vial

f. Pollen application with Camel's hair brush

Plate 1. Different stages of procedure for hybridization in pomegranate

Plate 2a. Developing hybrid fruits of different cross combinations

Ganesh x MH-1 | Mridula x MH-1 | Kandhari Kabuli x NT-1

MH-1 x Ganesh | MH-1 x Mridula | NT-1 x Kandhari Kabuli

Plate 2b. Developing hybrid fruits of different cross combinations

Kandhari Kabuli x MH-1 | MH-1 x Kandhari Kabuli | NT-1 x Bhagwa

MH-1 x G-137 | G-137 x MH-1 | Bhagwa x NT-1

Plate 2c. Developing hybrid fruits of different cross combinations

per cent (Table 4). The seed germination obtained from different cross combinations varied significantly. Jalikop (2003) observed that seed germination of hybrid seeds obtained from different cross combinations ranged from 0.12 to 38.82 per cent . The findings of current investigations are contrary to the findings of Kumar (2012) who reported 75.12 to 93.53 per cent seed germination among the different inter-varietal crosses.

On the whole the total of 247 hybrid seedlings so obtained in the present study were maintained in polyhouse conditions for their subsequent screening and evaluation against the bacterial blight of pomegranate through artificial inoculation of disease causing bacteria.

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