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In vitro pollen germination potential of wild pomegranate (*Punica granatum* L.) genotypes in Western Himalayan region

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Abstract

An experiment was conducted at Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan during 2013-15 in order to evaluate the germination potential of pollen grains of wild pomegranate genotypes collected from different districts of Himachal Pradesh. Freshly dehisced pollen grains were collected and tested in different media containing varied amount of sucrose. Maximum pollen germination was observed in the genotypes collected from Shimla district and least in Mandi district. Among all the media used for germination, 15 percent sucrose with 10 ppm boric acid solution proved its superiority over the rest. Increasing the sucrose amount from 10 to 15 per cent in the germinability.

Keywords: Wild pomegranate, pollen germination, germination media, sucrose, boron

Introduction

Wild pomegranate (*Punica granatum* L.) is considered as a proto type of cultivated one and resembles cultivated pomegranate for various morphological characters. In India pomegranate is found wild only in Western Himalayan zone (Narzary *et al.*, 2009)^[6] and ample amount of genetic diversity of the types was reported in that very region. (Mishra *et al.* 2017)^[3]. The species is popular in the native area due to its sour aril which is used as anardana in that very area. Apart from the aril, other parts like rind, bark and younger leaves are used for various medicinal and industrial purposes. Besides the pomological characters, the crop has another significant trait which creates a lot of interest among the pomegranate breeders. The wild pomegranate was found to have resistance against the deadly pomegranate bacterial blight disease. Research carried out at 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 the bacterial disease (Jelicop, 2005)^[2]. Further research is needed in the field of gene transmission both in the form of conventional or nonconventional methods as the gene of resistance is linked with the gene of acidity.

Before exploitation of any plant species in any breeding programme, apart from the pomological traits, the details of flower types, pollination, pollen viability and pollen germination should be well studied. Pollen viability and germination are directly correlated with fruit set and yield. The wild pomegranate plants growing in western Himalayan region commonly have three different flower types (Mishra *et al.* 2017)^[3] and abundant pollen grains with the pollen viability ranging from 83.94 per cent to 96.06 per cent (Mishra *et al.* 2016)^[4]. The present study was conducted in order to evaluate the germination ability of the wild pomegranate pollen grains and also the variation in the germination percentage in different nutrient media.

Materials and Methods

The investigation was carried out in the pomegranate block (Kalaghat) of Department of Fruit Science, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan during the year 2013-2015. The plant materials included for studies were wild pomegranate (Daru) plants collected from four different districts (Sirmour, Shimla, Solan, Mandi) of HP and planted in

the university farm at Kalaghat. Plants from each of four districts were selected as experimental materials on the basis of their regular fruiting and flowering behavior. The experiment was formulated in factorial randomized block design with 10 genotypes (plant types) from each districts replicated thrice.

The freshly dehisced pollen grains were used for germination test. Different concentrations of sucrose solution were prepared. The different solutions used were

- a) 10 per cent sucrose solution
- b) 15 per cent sucrose solution
- c) 15 per cent sucrose solution +10 ppm boric acid
- d) 20 per cent sucrose solution

The pollen grains were placed on cavity slides containing a hanging drop of sucrose solution and pollen tube growth was assessed in each cultivar under microscope. The pollen grains which had pollen tube at least two times larger than pollen size were considered to be germinated.

Results and Discussions

The pooled mean of both the years of results revealed that, there were significant variations among the wild germplasm with respect to the germinability of the pollen grain. The *in vitro* pollen germination varied from 36.80 per cent (Mandi) to 44.01 per cent (Shimla) in 10 per cent sucrose medium, whereas 41.82 per cent (Mandi) to 58.32 per cent (Shimla) in the medium having 15 per cent sucrose. The wild types collected from district Shimla had maximum germination

percentage among all, irrespective of germination medium. However, by increasing the sucrose amount nearly 5 percent in the germination media, the percentage of pollen germination increased. The wild types collected from the district Shimla may have the genetic character for higher germinability of the pollen than the other genotypes. But the germination percentage decreased in all the genotypes when the sucrose concentration increased up to 20 percent.

Germination test was performed by using 10 per cent and 15 per cent sucrose solution with and without the addition of boric acid. Both the years of observations revealed that there were significant differences present among the genotypes collected from different districts of Himachal Pradesh. Pollen germination (pooled district mean) was recorded maximum for Shimla district (62.20%) and minimum for Sirmour (56.55%) in the medium containing 15 per cent sucrose and 10 ppm boric acid (table 3). The interaction value even showed higher germination percentage of 66.61 (plant no. 6 of Shimla). It was observed that the addition of boric acid along with sucrose increases the germination percentage of the pollen grain than the lone sucrose medium. Bist (1994)^[1] also observed higher pollen germination of pomegranate cultivars in 12 per cent sucrose + 10 ppm boric acid solution. Boron has a regulatory role in pollen germination and pollen tube growth in many plants. In the boron deficient medium, the acidic pectin accumulated at the tip of the pollen tube whereas it is generally weakly distributed in entire length in the standard medium (Wang et al. 2003)^[6].

Table 1: Pollen germination (Sucrose 10%) of wild pomegranate genotypes

Plant	at 2013-14						2	014-2015	;		Pooled					
no.	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	
1	39.72	37.93	35.68	38.47	37.95	33.95	36.30	35.48	38.50	36.06	36.84	37.12	35.58	38.49	37.01	
1.	(39.07)	(38.02)	(36.68)	(38.33)	(38.03)	(35.64)	(37.05)	(36.56)	(38.35)	(36.90)	(37.35)	(37.53)	(36.62)	(38.34)	(37.46)	
2.	45.12	42.63	38.12	37.74	40.90	43.47	43.89	35.79	36.73	39.97	44.30	43.26	36.95	37.23	40.44	
	(42.20)	(40.76)	(38.13)	(37.90)	(39.75)	(41.25)	(41.49)	(36.73)	(37.31)	(39.19)	(41.73)	(41.13)	(37.43)	(37.60)	(39.47)	
2	42.71	45.63	37.07	37.69	40.77	42.57	43.96	36.40	38.03	40.24	42.64	44.80	36.73	37.86	40.51	
5.	(40.81)	(42.49)	(37.50)	(37.88)	(39.67)	(40.73)	(41.53)	(37.11)	(38.07)	(39.36)	(40.77)	(42.01)	(37.30)	(37.97)	(39.51)	
4	44.01	48.48	38.85	36.77	42.03	43.31	48.50	37.68	36.11	41.40	43.66	48.49	38.26	36.44	41.71	
4.	(41.56)	(44.13)	(38.56)	(37.33)	(40.40)	(41.16)	(44.14)	(37.87)	(36.91)	(40.02)	(41.36)	(44.14)	(38.21)	(37.12)	(40.21)	
5	45.37	46.67	37.83	36.02	41.47	44.30	41.00	36.27	35.98	39.39	44.83	43.83	37.05	36.00	40.43	
5.	(42.34)	(43.09)	(37.96)	(36.88)	(40.07)	(41.73)	(39.82)	(37.03)	(36.86)	(38.86)	(42.03)	(41.45)	(37.49)	(36.87)	(39.46)	
ć	44.48	40.33	38.51	37.63	40.24	45.48	34.64	40.51	38.30	39.73	44.98	37.49	39.51	37.97	39.99	
0.	(41.83)	(39.42)	(38.36)	(37.84)	(39.36)	(42.41)	(36.06)	(39.53)	(38.23)	(39.06)	(42.12)	(37.74)	(38.94)	(38.04)	(39.21)	
7	43.67	45.81	37.75	37.33	41.14	44.00	44.81	34.42	38.67	40.48	43.84	45.31	36.09	38.00	40.81	
7.	(41.36)	(42.60)	(37.91)	(37.66)	(39.88)	(41.56)	(42.02)	(35.91)	(38.45)	(39.49)	(41.46)	(42.31)	(36.91)	(38.06)	(39.68)	
0	42.88	46.26	37.72	37.75	41.15	42.72	45.92	36.72	38.09	40.86	42.80	46.09	37.22	37.92	41.01	
0.	(40.91)	(42.85)	(37.89)	(37.91)	(39.89)	(40.81)	(42.66)	(37.29)	(38.11)	(39.72)	(40.86)	(42.76)	(37.59)	(38.01)	(39.80)	
0	45.45	48.00	38.21	34.93	41.65	44.79	46.34	39.21	35.80	41.53	45.12	47.17	38.71	35.37	41.59	
9.	(42.39)	(43.86)	(38.18)	(36.23)	(40.17)	(42.01)	(42.90)	(38.77)	(36.75)	(40.11)	(42.20)	(43.38)	(38.48)	(36.49)	(40.14)	
10	45.57	47.35	37.82	31.61	40.59	45.91	45.68	39.15	33.94	41.17	45.74	46.52	38.49	32.77	40.88	
10.	(42.46)	(43.48)	(37.95)	(34.21)	(39.53)	(42.65)	(42.52)	(38.74)	(35.63)	(39.89)	(42.56)	(43.00)	(38.34)	(34.92)	(39.71)	
Moon	43.90	44.91	37.76	36.60	40.79	43.05	43.11	37.16	37.01	40.08	43.47	44.01	37.46	36.80	40.44	
Wiean	(41.49)	(42.07)	(37.91)	(37.22)	(39.67)	(40.99)	(41.02)	(37.55)	(37.47)	(39.26)	(41.24)	(41.54)	(37.73)	(37.34)	(39.47)	
CD0.05	D				0.33					0.53					0.35	
	Р				0.52					0.83					0.55	
	D×P				1.03					1.67					1.09	

Table 2: Pollen germination (Sucross	e 15%) of wild pomegranate genotypes
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Plant	nt 2013-14						2	014-2015	;		Pooled					
no.	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	
1	52.80	45.84	49.80	41.87	47.58	54.92	47.85	45.92	41.77	47.62	53.86	46.85	47.86	41.82	47.60	
1.	(46.60)	(42.61)	(44.88)	(40.32)	(43.61)	(47.83)	(43.77)	(42.66)	(40.26)	(43.63)	(47.21)	(43.19)	(43.77)	(40.29)	(43.62)	
2	48.36	46.46	46.86	50.79	48.12	49.86	43.02	46.85	46.82	46.64	49.11	44.74	46.86	48.80	47.38	
Ζ.	(44.06)	(42.97)	(43.20)	(45.45)	(43.92)	(44.92)	(40.99)	(43.20)	(43.18)	(43.07)	(44.49)	(41.98)	(43.20)	(44.32)	(43.50)	
2	49.88	55.37	52.54	51.63	52.35	44.91	49.88	41.91	54.89	47.90	47.39	52.63	47.22	53.26	50.13	
5.	(44.93)	(48.09)	(46.46)	(45.94)	(46.35)	(42.08)	(44.93)	(40.34)	(47.81)	(43.79)	(43.51)	(46.51)	(43.40)	(46.87)	(45.07)	
4	45.76	53.45	43.73	42.84	46.45	54.87	53.92	48.85	52.55	52.55	50.31	53.68	46.29	47.70	49.50	
4.	(42.57)	(46.98)	(41.40)	(40.89)	(42.96)	(47.79)	(47.25)	(44.34)	(46.46)	(46.46)	(45.18)	(47.11)	(42.87)	(43.67)	(44.71)	
5	47.59	59.70	45.76	51.95	51.25	43.86	56.94	42.85	47.44	47.77	45.72	58.32	44.30	49.70	49.51	
5.	(43.62)	(50.59)	(42.57)	(46.12)	(45.73)	(41.47)	(48.99)	(40.89)	(43.54)	(43.72)	(42.55)	(49.79)	(41.73)	(44.83)	(44.72)	
6	46.32	53.69	47.94	52.77	50.18	42.92	51.95	55.72	49.92	50.13	44.62	52.82	51.83	51.35	50.15	
0.	(42.89)	(47.12)	(43.82)	(46.59)	(45.10)	(40.93)	(46.12)	(48.29)	(44.96)	(45.07)	(41.91)	(46.62)	(46.05)	(45.77)	(45.09)	
7	45.35	60.89	50.86	43.85	50.24	48.92	51.91	50.85	48.82	50.12	47.13	56.40	50.85	46.34	50.18	
7.	(42.33)	(51.29)	(45.49)	(41.47)	(45.15)	(44.38)	(46.10)	(45.49)	(44.33)	(45.07)	(43.36)	(48.69)	(45.49)	(42.90)	(45.11)	
8	48.43	58.83	46.49	48.74	50.62	45.89	53.94	51.84	52.57	51.06	47.16	56.39	49.16	50.65	50.84	
0.	(44.10)	(50.09)	(42.99)	(44.28)	(45.36)	(42.65)	(47.26)	(46.05)	(46.47)	(45.61)	(43.37)	(48.68)	(44.52)	(45.38)	(45.49)	
0	49.54	58.76	45.63	47.86	50.45	45.65	53.93	49.86	50.92	50.09	47.60	56.34	47.75	49.39	50.27	
).	(44.74)	(50.05)	(42.50)	(43.78)	(45.26)	(42.51)	(47.25)	(44.92)	(45.53)	(45.05)	(43.62)	(48.65)	(43.71)	(44.65)	(45.16)	
10	50.72	54.64	47.68	50.59	50.91	43.31	41.94	47.82	51.64	46.18	47.02	48.29	47.75	51.11	48.54	
10.	(45.41)	(47.66)	(43.67)	(45.34)	(45.52)	(41.16)	(40.36)	(43.75)	(45.94)	(42.80)	(43.29)	(44.01)	(43.71)	(45.64)	(44.16)	
Mean	48.47	54.76	47.73	48.29	(49.99)	47.51	50.53	48.25	49.73	(50.27)	47.99	52.65	47.99	49.01	(50.12)	
Wiedii	(44.13)	(47.75)	(43.70)	(44.02)	(44.90)	(43.57)	(45.30)	(43.99)	(44.85)	(43.43)	(43.85)	(46.52)	(43.85)	(44.43)	(44.66)	
CD0.05	D				0.05					0.02					0.03	
	Р				0.07					0.04					0.04	
	D×P				0.14					0.08					0.08	

Table 3: Pollen germination (Sucrose 15% + 10 ppm boric acid) of wild pomegranate genotypes

Plant	t 2013-14						20	014-2015	;		Pooled					
no.	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	
1	66.15	54.52	56.39	54.63	57.92	66.15	54.85	58.72	54.96	58.67	66.15	54.69	57.55	54.80	58.30	
1.	(54.42)	(47.59)	(48.67)	(47.66)	(49.59)	(54.42)	(47.79)	(50.02)	(47.85)	(50.02)	(54.42)	(47.69)	(49.35)	(47.75)	(49.80)	
2	52.64	53.52	53.56	56.92	54.16	52.64	53.19	53.56	56.92	54.08	52.64	53.35	53.56	56.92	54.12	
Ζ.	(46.51)	(47.02)	(47.04)	(48.98)	(47.39)	(46.51)	(46.83)	(47.04)	(48.98)	(47.34)	(46.51)	(46.92)	(47.04)	(48.98)	(47.36)	
2	53.68	66.66	64.60	62.55	61.87	63.01	66.33	54.60	62.55	61.62	58.35	66.50	59.60	62.55	61.75	
5.	(47.11)	(54.74)	(53.49)	(52.27)	(51.90)	(52.54)	(54.53)	(47.64)	(52.27)	(51.75)	(49.83)	(54.63)	(50.56)	(52.27)	(51.82)	
4	51.78	64.78	56.70	58.73	57.99	61.78	66.78	66.70	58.73	63.49	56.78	65.78	61.70	58.73	60.74	
4.	(46.02)	(53.60)	(48.85)	(50.03)	(49.62)	(51.81)	(54.81)	(54.76)	(50.03)	(52.85)	(48.92)	(54.20)	(51.80)	(50.03)	(51.24)	
5	51.73	67.80	53.43	67.19	60.04	51.07	62.80	53.43	57.19	56.12	51.40	65.30	53.43	62.19	58.08	
5.	(45.99)	(55.43)	(46.97)	(55.05)	(50.86)	(45.61)	(52.42)	(46.97)	(49.13)	(48.53)	(45.80)	(53.93)	(46.97)	(52.09)	(49.70)	
6	59.52	66.61	55.60	58.14	59.97	51.52	66.61	69.60	58.14	61.47	55.52	66.61	62.60	58.14	60.72	
0.	(50.49)	(54.70)	(48.22)	(49.69)	(50.77)	(45.87)	(54.70)	(56.54)	(49.69)	(51.70)	(48.18)	(54.70)	(52.38)	(49.69)	(51.24)	
7	52.40	66.81	57.08	57.79	58.52	62.40	56.81	57.08	57.79	58.52	57.40	61.81	57.08	57.79	58.52	
7.	(46.38)	(54.82)	(49.07)	(49.48)	(49.94)	(52.18)	(48.91)	(49.07)	(49.48)	(49.91)	(49.28)	(51.87)	(49.07)	(49.48)	(49.93)	
8	55.40	66.58	54.44	58.72	58.79	55.40	66.58	62.44	58.72	60.79	55.40	66.58	58.44	58.72	59.79	
0.	(48.10)	(54.69)	(47.55)	(50.02)	(50.09)	(48.10)	(54.69)	(52.21)	(50.02)	(51.25)	(48.10)	(54.69)	(49.88)	(50.02)	(50.67)	
9	56.74	63.66	65.76	57.05	60.80	56.41	63.66	56.43	57.05	58.39	56.57	63.66	61.09	57.05	59.60	
).	(48.88)	(52.96)	(54.19)	(49.06)	(51.27)	(48.68)	(52.96)	(48.69)	(49.06)	(49.85)	(48.78)	(52.96)	(51.44)	(49.06)	(50.56)	
10	55.34	57.74	55.57	55.71	56.09	55.34	57.74	59.57	58.71	57.84	55.34	57.74	57.57	57.21	56.96	
10.	(48.07)	(49.45)	(48.20)	(48.28)	(48.50)	(48.07)	(49.45)	(50.52)	(50.02)	(49.51)	(48.07)	(49.45)	(49.36)	(49.15)	(49.01)	
Mean	55.54	62.87	57.31	58.74	58.61	57.57	61.54	59.21	58.08	59.10	56.55	62.20	58.26	58.41	58.86	
wicali	(48.20)	(52.50)	(49.22)	(50.05)	(49.99)	(49.38)	(51.71)	(50.35)	(49.65)	(50.27)	(48.79)	(52.10)	(49.78)	(49.85)	(50.13)	
CD _{0.05}	D				0.31					0.32					0.30	
	Р				0.48					0.50					0.48	
	D×P				0.97					1.01					0.96	

Table 4. Dallan av	mination	(Sumara 200)) of wild	nomonato	aanotrinor
rable 4: Pollen ge	ermination	(Sucrose 20%) or wha	pomegranate	genotypes

Plant	t 2013-14						20	14-2015			Pooled					
no.	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	Sirmour	Shimla	Solan	Mandi	Mean	
1	6.40	6.44	6.69	8.06	6.90	6.03	6.54	11.73	7.37	7.92	6.22	6.49	9.21	7.72	7.41	
1.	(2.53)	(2.54)	(2.59)	(2.83)	(2.62)	(2.45)	(2.56)	(3.42)	(2.71)	(2.79)	(2.49)	(2.55)	(3.01)	(2.77)	(2.70)	
2.	12.31	6.92	6.56	6.58	8.09	11.68	8.62	6.56	9.41	9.07	11.99	7.77	6.56	7.99	8.58	
	(3.51)	(2.63)	(2.56)	(2.56)	(2.82)	(3.42)	(2.94)	(2.56)	(3.07)	(3.00)	(3.46)	(2.78)	(2.56)	(2.82)	(2.91)	
2	13.32	6.81	6.73	6.67	8.38	11.65	6.78	9.73	5.83	8.50	12.49	6.79	8.23	6.25	8.44	
5.	(3.65)	(2.61)	(2.59)	(2.58)	(2.86)	(3.41)	(2.60)	(3.12)	(2.42)	(2.89)	(3.53)	(2.61)	(2.86)	(2.50)	(2.87)	
1	8.64	11.24	6.53	6.76	8.29	8.67	10.27	6.63	6.86	8.11	8.65	10.75	6.58	6.81	8.20	
4.	(2.94)	(3.35)	(2.55)	(2.60)	(2.86)	(2.94)	(3.20)	(2.57)	(2.62)	(2.84)	(2.94)	(3.28)	(2.56)	(2.61)	(2.85)	
5	13.52	9.78	6.40	11.86	10.39	12.18	9.91	6.47	10.86	9.85	12.85	9.84	6.43	11.36	10.12	
5.	(3.68)	(3.13)	(2.53)	(3.44)	(3.19)	(3.49)	(3.15)	(2.54)	(3.29)	(3.12)	(3.58)	(3.14)	(2.54)	(3.37)	(3.16)	
6	13.59	9.81	6.45	11.62	10.37	13.29	10.31	6.45	14.82	11.22	13.44	10.06	6.45	13.22	10.79	
0.	(3.69)	(3.13)	(2.54)	(3.41)	(3.19)	(3.64)	(3.21)	(2.54)	(3.84)	(3.31)	(3.67)	(3.17)	(2.54)	(3.63)	(3.25)	
7	13.27	9.70	7.77	8.64	9.84	12.63	8.63	7.77	8.77	9.45	12.95	9.16	7.77	8.71	9.65	
7.	(3.64)	(3.11)	(2.79)	(2.94)	(3.12)	(3.55)	(2.94)	(2.79)	(2.96)	(3.06)	(3.60)	(3.03)	(2.79)	(2.95)	(3.09)	
8	12.34	8.80	7.52	8.91	9.39	12.34	7.58	7.52	7.81	8.81	12.34	8.19	7.52	8.36	9.10	
0.	(3.51)	(2.97)	(2.74)	(2.98)	(3.05)	(3.51)	(2.75)	(2.74)	(2.79)	(2.95)	(3.51)	(2.86)	(2.74)	(2.89)	(3.00)	
9	12.37	8.65	6.77	7.80	8.90	12.21	9.64	6.44	11.80	10.02	12.29	9.15	6.60	9.80	9.46	
).	(3.52)	(2.94)	(2.60)	(2.79)	(2.96)	(3.49)	(3.11)	(2.54)	(3.44)	(3.14)	(3.51)	(3.02)	(2.57)	(3.11)	(3.05)	
10	12.41	9.95	8.14	7.81	9.58	13.59	9.15	10.80	9.81	10.84	13.00	9.55	9.47	8.81	10.21	
10.	(3.52)	(3.15)	(2.85)	(2.78)	(3.08)	(3.69)	(3.03)	(3.29)	(3.13)	(3.28)	(3.60)	(3.09)	(3.07)	(2.96)	(3.18)	
Mean	11.82	8.81	6.95	8.47	9.01	11.43	8.74	8.01	9.33	9.37	11.62	8.78	7.48	8.90	9.19	
wiean	(3.42)	(2.96)	(2.63)	(2.89)	(2.97)	(3.36)	(2.95)	(2.81)	(3.03)	(3.03)	(3.39)	(2.95)	(2.72)	(2.96)	(3.00)	
CD0.05	D				0.03					0.04					0.03	
	Р				0.05					0.06					0.04	
	D×P				0.10					0.12					0.08	

Conclusion

The wild pomegranate genotypes possesses a good germination percentage like that of cultivated one. Among the total germplasm, the genotypes from Shimla have higher germination percentage. Among the three different sucrose medium used, the, media containing 15 per cent concentration of sucrose was found best. Addition of boric acid 10 ppm to the germination medium even further increased the pollen germination.

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