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Short Note

Studies on pollen fertility, viability and *in vitro* germination in different exotic cultivars and indigenous selections of Persian walnut

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Abstract

Freshly collected pollen showed 77.65-94.89 per cent fertility; however, viability was recorded between 64.75-91.30 per cent. Medium B (20% sucrose + 1.0 mM $CaCl_2$ + 0.65% agar + 10ppm boric acid) registered higher *in vitro* pollen germination with a mean value of 48.60 per cent. Higher concentration of sucrose supplemented with calcium chloride and boric acid is required for higher pollen germination.

Key words: Persian walnut, fertility, viability, *in vitro* pollen germination.

Persian walnut (*Juglans regia* L.) is known by the name of 'akhrot' over the greater part of India. It is monoecious, wind pollinated and deciduous tree. Flowering is dichogamous i.e. within an individual plant, the timing of functioning of male and female organ differs. Walnut produces non-sticky and light pollen in abundance, averaging about 900 pollen grains per anther and about 1,800,000 per catkin. The number of anthers per flower averaged 18 at the stalk end and 13 at the tip of catkin (Impiumi and Ramina, 1967). A mature walnut tree produces an average of 5000 catkins, each of which will have between 1,000,000 to 4,000,000 pollen grains (Westwood, 1988). Walnut pollen shows the highest germination capacity when it was first dehisce from the anther. Pollen stored under stimulated natural conditions lost its viability within 2-3 days after collection. In some cases, freshly collected pollen lost its viability within 1-3 hours after exposure to sunlight at 25-30°C. Due to this short life of the pollen grains and dichogamous nature of the species necessitates the breeders to determine the pollen viability prior to pollination. Thus, the present study was carried out to see the *in vitro* germination and fertility of pollen in different exotic and indigenous selections of Persian walnut.

Fresh pollen grains were collected from seven exotic cultivars and ten indigenous selections from the Departmental orchard of Fruit Breeding and Genetic Resources, UHF, Nauni-Solan (HP). The orchard was located at an elevation of 1225m asl and between 31°N latitude and 77°E longitude. The pollens were stained in 1 per cent acetocarmine to determine pollen fertility and in 1 per cent tetrazolium salt for pollen viability. The pollen grains were dusted on a clean microscopic slide and 1-2 drops of acetocarmine and tetrazolium salt was put on these pollen grains for determining pollen fertility and pollen viability, respectively and a cover glass was placed on it and was left for 5-10 minutes for proper staining. For the observation of viable and non-viable pollen grains twenty microscopic fields were taken and average percentage was determined. For determining *in vitro* pollen germinability four solid sterilized media were used:

Medium A: 20% sucrose + 0.1mM $CaCl_2$ + 0.65% agar + 10ppm boric acid

Medium B: 20% sucrose + 1.0mM $CaCl_2$ + 0.65% agar + 10ppm boric acid

Medium C: 25% sucrose + 0.1mM $CaCl_2$ + 0.65% agar + 10ppm boric acid

Medium D: 25% sucrose + 1.0mM $CaCl_2$ + 0.65% agar + 10ppm boric acid

The catkins prior to pollen shedding were covered with the butter paper bag and the anthers were allowed to dehisce in the paper bag. The shed pollen grains were cleaned and a thin layer of pollen was sprinkled on the surface after the medium had solidified. For observations ten plates were taken from each media and twenty microscopic fields were taken in each plate. The plates were observed after 24 hours under the microscope and the pollen grains with pollen

tubes (at least 3 times longer than pollen size) were considered to be germinated. The data recorded were analysed as per the method suggested by Gomez and Gomez (1984) using completely randomized design.

The study reveals that maximum pollen fertility with 1 per cent acetocarmine was recorded in 'Kandaghat Selection' (94.89%) which was statistically at par with most of the cultivar/selection, with a mean value of 86.19 per cent, however, minimum fertility of pollens was observed in 'Luxmi Akhrot' (77.65%). The maximum pollen viability was recorded in 'Netar Akhrot' (91.30%) followed by 'Plant No. 45' (90.97%). The minimum pollen viability was observed in 'Plant No. 32' (64.75%) (Table 1). Almost similar results were reported earlier by Parihar and Bajpai (1986) and Vergano *et al.* (1990) for pollen fertility and viability.

Table 1. Pollen fertility, viability and *in- vitro* germination in different exotic cultivars and indigenous selections of walnut

Cultivars/Selections	Pollen fertility (%)	Pollen viability (%)	*In vitro* pollen germination			
			Medium A	Medium B	Medium C	Medium D
ACO 38853	89.30	66.31	43.33	44.01	45.40	32.72
Blackmore	88.96	81.74	35.41	51.57	46.47	28.82
Gobind	89.35	74.66	30.52	53.46	49.96	27.97
Plant No. 10	88.09	75.48	43.75	48.94	44.00	31.86
Netar Akhrot	89.40	91.30	44.71	59.58	49.18	37.07
Roopa Akhrot	90.24	77.82	48.55	46.35	42.47	34.04
KX Giant	91.29	80.51	41.82	52.17	41.12	27.02
Rattan Akhrot	84.35	83.48	46.54	44.10	45.63	30.70
Kandaghat Selection	94.89	73.35	43.14	47.19	38.18	31.24
Inder Akhrot	83.89	83.60	42.47	57.98	39.58	32.10
Plant No. 32	81.77	64.75	31.14	39.51	40.59	24.24
Xenia	82.90	83.35	37.82	44.91	37.90	27.65
Plant No. 45	85.56	90.97	38.79	52.96	46.42	35.85
Plant No. 46	90.64	75.83	37.48	47.27	38.90	28.78
Plant No. 47	78.23	73.68	34.05	48.55	48.46	21.65
Solding Selection	78.65	82.34	32.79	40.24	44.57	32.81
Luxmi Akhrot	77.65	75.28	31.91	47.37	37.39	24.63
Mean	86.19	78.50	39.07	48.60	43.31	29.95
CD (P=0.05)	5.98	3.88	4.20	10.18	8.75	4.03

Maximum germinability of the pollen grains were observed in Medium B (20% sucrose + 1.0mM $CaCl_2$ + 0.65% agar + 10ppm boric acid) with a mean value of 48.60 per cent followed by Medium C (25% sucrose + 0.1mM $CaCl_2$ + 0.65% agar + 10ppm boric acid) i.e. 43.31 per cent, however, least pollen germination was registered in Medium D (25% sucrose + 1.0mM $CaCl_2$ + 0.65% agar + 10ppm boric acid) i.e. 29.95 per cent (Table 1). From the present findings, it is concluded that *in vitro* pollen germination differs significantly among different media. Under conditions of Medium A (20% sucrose + 0.1mM $CaCl_2$ + 0.65% agar + 10ppm boric acid) maximum (48.55%) *in vitro* pollen germination was recorded in 'Roopa Akhrot', which was statistically at par with 'Rattan Akhrot' (46.54%) and 'Netar Akhrot' (44.71%), whereas minimum germination (30.52%) was observed in Gobind cultivar. In case of Medium B, maximum (59.58%) germination of the pollen grains were recorded in 'Netar Akhrot' which was statistically at par with most of the cultivars/selections, however, minimum (39.51%) germinability was observed in 'Plant No. 32'. Maximum germination of the pollen grains in Medium C was registered in 'Gobind' with a value of 49.96 per cent, which was statistically at par with most of the cultivars/ selections, whereas, minimum (37.39%) was observed in 'Luxmi Akhrot'. With a value of 21.65 per cent, 'Plant No. 47" scored minimum pollen germinability under Medium D conditions, however, maximum (37.07%) germination was registered in 'Netar Akhrot' which was statistically at par with 'Plant No. 45'

(35.85%) and 'Roopa Akhrot' (34.04%). The present findings were in accordance with the findings of Luza and Polito (1985) which showed that maximum pollen germination was in 20 per cent sucrose +1.0 mM $CaCl_2$ + 0.65 per cent agar + 0.16 mM boric acid. Eliseev and Kharaziya (1983), Parihar and Bajpai (1986) and Samushia *et al.* (1998) also reported similar results with different concentrations of sucrose and glucose.

Present studies indicated that for proper germination, Persian walnut pollens require higher concentration of sucrose supplemented with calcium chloride and boric acid.

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