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EVALUATION OF SOME POLLINIZERS AND THEIR SUITABILITY ON PRODUCTIVITY AND QUALITY OF WALNUT (*Juglans regia*) CULTIVARS

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ABSTRACT

The present study was carried out at Horticultural Farm, Division of Fruit Science, SKUAST, Shalimar, Srinagar (Kashmir) during 2012-13 to evaluate various walnut pollinizers in improving the productivity and nut quality. Pollen germination and its viability, pollen production rate, anther and pollen morphology of 'SKAU-W-0020' and 'SKUA-W-0022' and influence of pollination method i.e. open and cross on nut and kernel characteristics was studied. Highest pollen viability (91.4%) was observed in selection 'SKAU-W-0020'. Maximum pollen germination (51.5%) was observed in 'SKAU-W-0020' on medium containing agar agar powder (1%) + sucrose (15%) + boric acid (100 ppm) + $CaCl_2$ (1 mM). Pollen production varied significantly within genotypes with maximum (1,43,75000) in 'SKAU-W-0020' and minimum (1,20,60000) in 'SKAU-W-0022'. Maximum anther length (2457 µm) and width (1228 µm) was in 'SKAU-W-0020'; however, maximum pollen length (35 µm) and width (39.6 µm) was in 'SKAU-W-0022'. Nut weight, diameter, length and kernel length, weight and kernel percentage in 'SKAU-W-0025' and 'SKAU-W-0015' increased through cross pollination, however in 'SKAU-W-0024' and 'SKAU-W-0008' reverse trend was observed.

Key words: Anther, germination, pollen, viability, walnut

INTRODUCTION

Persian walnut (*Juglans regia* L.), also known as English walnut, belongs to family *Juglandaceae*. Walnut is one of the most important nuts grown in India. In India, Jammu and Kashmir is the major producer of walnut covering an area of 6,397 ha and production of about 1,81,443 metric tonnes (Anonymous, 2014). Jammu and Kashmir contributes around 85% of total walnut production of the country and assumes a unique distinction and has monopoly in the production of export quality walnut. It flourishes in temperate belts at an altitude of 900-3500 m masl. Walnut is a monoecious, wind pollinated and self-compatible nut crop. Abundant and healthy flower development is necessary to ensure a sufficient fruit set. Moreover, desired pollination of these plants by suitable pollinizers and occurrence of fertilization in flower are crucial. A good pollinizer should produce well developed pollen with both high viability and germination (Luza and Polito, 1985; Sutyemez, 2007). Previous studies have revealed that pollen germination of few fruit and nut species and their cultivars vary depending upon the chemical composition of medium. For this reason the suitable germination medium should be obtained for each fruit species. Some studies have been carried out on pollen production, viability and amount of pollen for different walnut types and cultivars (Luza and Polito, 1985; Saglam and Gulcan, 1995; Sutyemez, 2007).

The components of pollen production are important in determining the pollination ability of any cultivar or type. For a cultivar to be used as a pollinizer, in addition to high pollen viability and pollen germination rates, it is important that its anthers produce high amounts of pollens. Although all pollens germinated on stigma do not reach the carpels, however, for successful fertilization, pollinizer cultivars producing high amount of pollens are desired (Stosser and Anvari, 1981). Mert (2010) described the morphology and ultra structure of anthers and pollen grains for 8 walnut cultivars and reported that the pollen grains of walnut plants varied significantly with respect to length and had sub-oblate shape whereas the shape of anther was prolate and perprolate. Keeping in view the economic importance of walnut and complex pollination situations, an investigation was carried out to determine pollen viability and its germination, pollen production capacity and effects on nut and kernel characteristics of different walnut varieties to know their pollinizer efficiency.

MATERIALS AND METHODS

The present study was carried out at the Horticulture farm, Division of Fruit Science, SKUAST, Shalimar, Kashmir (India) in the year 2012-2013. Two promising pollen parents of walnut (SKAU-W-0022 and SKAU-W-0020) crossed with female parents of walnut (SKAU-W-0024, SKAU-W-0025, SKAU-W-0008 and SKAU-0015) were used. Fresh pollen was collected from male parents at flowering time before pollen dehiscence.

Pollen germination and pollen viability tests were performed according to Eti (1990). Solidified agar (1%) medium containing different concentrations of sucrose (*viz*., 10, 15, 20 and 25%) alone and in combination with boric acid (100 ppm) and calcium chloride (1 mM) were used as germination medium. After the pollen dusting, the petri-plates were incubated at 27±1^{o}C for 24 h and then observed under compound microscope (magnification of 10 x 40X). Total pollen grains observed under microscopic field were counted. The germinated pollen grains (pollen grain was considered as germinated when length of pollen tube was equal or exceeded its diameter) were also counted. The viability of pollen grains was determined by 2,3,5-triphenyl tetrazolium chloride (TTC @ 1%) and fluorescein diacetate (FDA @ 2 mg FDA mL^{-1} acetone) test (Eti, 1990). Pollen grains were dusted on TTC solution on slides and kept at room temperature for 2 h under day light. The pollen grains were then examined under light microscope (magnification of 10 x 40X). The viability of pollen was scored according to staining level i.e. pollen with bold red colour as viable, with light red colour as semi-viable and yellowish-green colour or colourless as non-viable (Eti, 1990). In FDA test, the pollen was dusted and kept at room temperature for 1 h and pollen grains observed under fluorescence microscope (Olympus, model: CX-41; magnification of 10 x 40X). The pollen grains which fluoresced green were considered as viable.

Pollen production rate in genotypes 'SKAU-W-0020' and 'SKAU-W-0022' was assessed by selecting 20 randomly catkins per plant. Total number of staminate flowers per catkin (FC) and anthers per flower (AF) were counted. The number of pollen grains per anther (PA) = PF/AF was calculated using haemocytometric method (Eti, 1990). The number of pollen grains per flower (PF) was calculated as PF = PA x AF and the number of pollen grains per catkin (PC) was calculated as PC = FC x PF. The length and width of each anther and pollen were taken with the help of previously calibrated stage and ocular micrometer at a magnification of 400X. The shapes of anther and pollen were determined on the basis of length and width ratio (Tousum and Koyuncu, 2007).

For determining the effect of pollination mode and pollen sources on nut and kernel characters, four different branches in open pollination on four sides of a tree in each genotype were selected and number of healthy female flowers were counted and marked for open pollination. Twenty five pistillate flowers were selected from each replicate of female genotypes, labeled and bagged with muslin cloth In cross pollination the pollens from two (male) genotypes viz., 'SKAU-

W-0022' and 'SKAU-W-0020' were collected. At bifid stage [the stage of stigma receptivity with an angle of 45^{o} between two stigmatic lobes], the bags were removed and the pollen of 'SKAU-W-0022' was applied on pistillate flowers of 'SKAU-W-0025' and 'SKAU-W-0024' and pollen of 'SKAU-W-0020' was applied on pistillate flowers of 'SKAU-W-0008' and 'SKAU-W-0015' using rubber end of pencil. After pollen application the stigmas were covered with cotton to avoid contamination. Then the pollinated flowers were again bagged. The bags covering the pollinated flowers were removed 3 weeks after pollination. Percent of pistillate flowers showing PFA (pistillate flower abortion) characteristics (either still attached to the shoot ready to drop, or already abscised) were recorded after three weeks of pollination. The final fruit retention was determined on the basis of number of fruits retained at the time of harvest on the selected branches. To study the influence of pollen parent, the nut and kernel characters like nut length, nut diameter, nut weight, shell thickness, kernel length, kernel weight and kernel percentage were recorded. Nut length, nut diameter and kernel length was determined using Vernier calliper while nut and kernel weight was determined using top balance. The thickness of the selected nuts from both cross and open pollination was measured from suture to suture with the help of Vernier calliper. Average of 25 nuts from both self and cross pollination were used to determine above parameters. The data generated during an experiment was analyzed in a completely randomized and block randomized design. To satisfy module, assumptions of experiment were subjected to critical difference.

RESULTS AND DISCUSSION

Pollen germination

Different media were used to test the germination of walnut pollen. *In vitro* germination was highest in pollen parents [51.51% in 'SKAU-W-0020' (Plate I) and 50.41% in 'SKAU-W-0022'] in medium containing 1% agar + 15% sucrose + 100 ppm boric acid + 1 Mm $CaCl_2$ (Table 1) and lowest germination of 34.83% was recorded in medium containing 1% agar and 25% sucrose (Table 1). Pollen germination was improved by the addition of boric acid and calcium to sucrose. Boron is believed to promote pollen germination by affecting H^{+}-ATPase activity, which initiates the pollen germination and tube growth (Feijo *et al*., 1995) and calcium is involved in pectin synthesis and the control of the osmotic conditions (Gibernau *et al*., 2003). Similar results have also been achieved by Sutyemez (2007); Mert (2009); Kamrani (2012) and Mondal and Ghanta (2012).

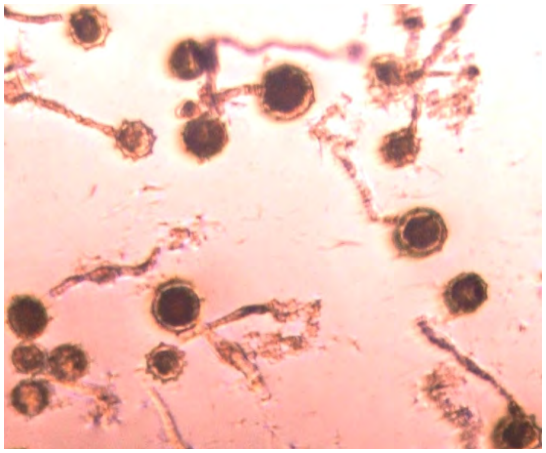

Plate I: Pollen germination of SKUA-W-0020 using 1% agar + 15 % sucrose + 100 ppm boric acid + 1mM $CaCl_2$

Pollen viability

The pollen viability was high (91.4%) in 'SKAU-W-0020' as detected through fluorescein diacetate test (FDA), followed by 90.2% when detected through TTC in the same genotype (Table 2 and Plates II-III). Semi-viable percentage of pollen was high (6.8%) in 'SKAU-W-0022' and low in 'SKAU-W-0020' (5.2%) through TTC test while dead pollens were maximum (11.6%) in 'SKAU-W-0022' through FDA test and minimum (3.2%) in 'SKAU-W-0020' through TTC test (Table 2). At ambient temperature, walnut pollen was short-lived and due to dichogamy it is important for

Table 1: Effect of agar media fortified with different levels of sucrose, boric acid, calcium chloride on pollen germination percentage of walnut

Treatments	Pollen germination (%)	
	SKAU-W-0022	SKAU-W-0020
10% sucrose	39.46	37.88
15% Sucrose	43.48	40.89
20% Sucrose	37.43	35.29
25% Sucrose	38.93	34.83
10% Sucrose + 100 ppm boric acid + 1 mM $CaCl_2$	48.09	49.68
15% Sucrose + 100 ppm boric acid + 1 mM $CaCl_2$	50.41	51.51
20% Sucrose + 100 ppm boric acid + 1 mM $CaCl_2$	46.65	45.69
25% Sucrose + 100 ppm boric acid + 1 mM $CaCl_2$	42.67	43.17
$CD_{0.05}$	3.88	2.73

fruit breeders to determine the viability of pollen before pollination so that pollen can be stored for later use. The TTC test is based upon dehydrogenase enzyme activity. Upon seed/tissue hydration, the activity of this enzyme increases, resulting in the release of H^+ ions which reduce the colourless tetrazolium salt into a red compound formazein which stains living cells red while dead cells remain colourless. Meanwhile with FDA test the fluorescein diacetate, a non-polar and non-fluorescent molecule, is hydrolyzed by enzyme pollen esterase into fluorescein, a polar and fluorescent molecule, which on accumulation inside pollen grain appears fluorescent in blue light. Our findings are in line with Cosmulescu *et al.* (2009) and Sutyemez (2011a).

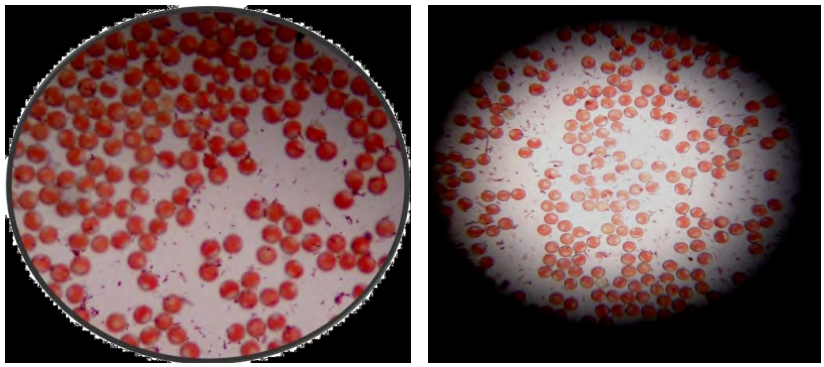

Plate II: Pollen viability [using TTC stain] in SKUA-W-0020 (left) and SKUA-W-0022 (right)

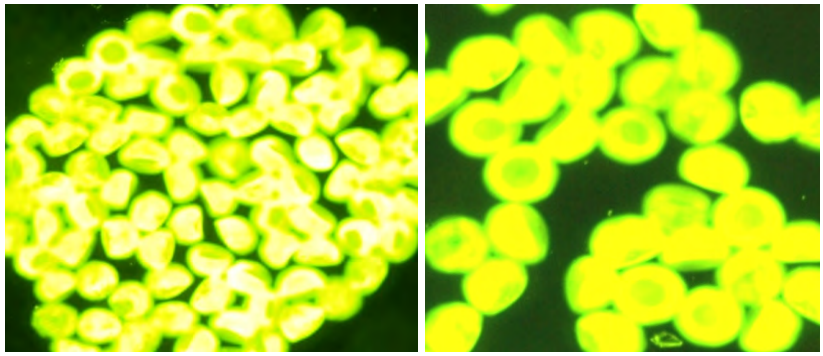

Plate IV: Pollen viability {using FDA stain} in SKUA-W-0020 (left) and SKUA-W-0022 (right)

Pollen production rate

The pollen production study helps in potentially practical uses in fertilization biology.

Table 2: Pollen viability percentage of two male genotypes of walnut detected by TTC and FDA methods

Male genotypes	TTC			FDA	
	Viable	Semi-viable	Dead	Viable	Dead
SKAU-W-0022	87.40 ±2.07	6.80 ±0.84	5.20 ±0.83	88.40 ±1.15	11.60 ±0.62
SKAU-W-0020	90.2 ±0.83	5.20 ±0.84	3.20 ±0.83	91.40 ±1.15	8.30±0.41
t_{cal}	2.80*	3.02*	3.77**	4.16**	7.68**
p-value	0.035	0.016	0.005	0.003	0.002

Table 3: Pollen production rate of two male walnut genotypes

Male genotype	FC	AF	PF	PA	PC
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
SKAU-W-0022	114 ±1.58	20 ±1.57	90,000±38.2	4500 ±32.6	10260000 ±412.31
SKAU-W-0020	125 ±1.58	23 ±1.53	1,15,000 ±37.6	5000 ±22.6	14375000 ±412.31
t_{cal}	11.00***	3.00*	1042.57***	28.17***	15780.3*
p-value	0.000	0.017	0.000	0.000	0.03

FC: Number of staminate flowers per catkin; AF: Number of anthers per flower; PF: Number of pollen grains per flower; PA: Number of pollen grains per anther =PF/AF; PC: Number of pollen grains per catkin =FCXPF

The components of pollen production are important in determining the pollination ability of any cultivar or type. For a cultivar to be used as a pollinizer, in addition to high pollen viability and pollen germination rates, it is important that its anthers produce high amounts of pollens. As all pollens germinated on stigma do not reach the carpels, thus for successful fertilization pollinizer, cultivars producing high amount of pollens are desired (Sutyemez, 2011a). In the present study pollen production rate of two walnut genotypes *viz.*, SKAU-W-0022 and SKAU-W-0020 varied significantly. Cultivar 'SKAU-W-0020' showed high number of staminate flowers per catkin (FC = 125), number of anthers per flower (AF = 23), number of pollen grains per flower (PF = 115000), number of pollen grains per anther (PA = 5000) and number of pollen grains per catkin (PC = 14375000) while cv. 'SKAU-W-022' showed low production rate (Table 3). The results of present investigation were in accordance with the results of previous study (Derin and Eti, 2001; Sutyemez, 2007; Mert, 2010).

Anther and pollen morphology

Highest anther length (2457 µm) and width (1228 µm) was observed in walnut cultivar **'**SKAU-W-0020' and lowe anther length (2262 µm) and width (1198 µm) was in cv. 'SKAU-W-0022'. The anther length to width ratio was maximum (2.13) in cv. SKAU-W-0020 and minimum (1.92) in cv. 'SKAU-W-0022'. Prolate shape of anther was observed in cv. 'SKAU-W-0022' while the shape of anther in selection 'SKAU-W-0020' was perprolate. The highest pollen length (35 µm) and width (39.6 µm) was observed in cv. 'SKAU-W-0022' and low pollen length (31 µm) and width (36.2 µm) in SKAU-W-0020. The pollen length : width ratio was maximum (0.88) in cv. 'SKAU-W-0022' and minimum (0.85) in cv. 'SKAU-W-0020'. Sub-oblate shape of pollen was observed both in cv. 'SKAU-W-0022' as well as in selection 'SKAU-W-0020' (Table 4). The findings are supported by Belhadj *et al. (*2007); Evrenosoglu and Misirli (2009), Mert (2010) and Sutyemez (2011b).

Effect of pollen parent and pollination method on nut and kernel characters

The influence of pollen parent on nut and kernel characteristics of walnut under open and cross pollination was also studied. Results of present investigation revealed cross pollination in 'SKAU-W-0025' and 'SKAU-W-0015' slightly increase nut weight, diameter, length and kernel length, weight and kernel percentage. However, in 'SKAU-W-0024' and 'SKAU-W-0008' the nut weight,

Table 4: Anther and pollen morphology of two male walnut genotypes

Male genotype	Anther characters				Pollen characters			
	Length (µm)	Width (µm)	Length: width ratio	Shape	Length (µm)	Width (µm)	Length: width ratio	Shape
	Mean ±SD	Mean ±SD	Mean		Mean ±SD	Mean ±SD	Mean	
SKAU-W-0022	2262±11.2	1198±9.14	1.92	Prolate	35±2.3	39.6 ±2.40	0.88	Suboblate
SKAU-W-0020	2457±9.72	1228±8.22	2.13	Perprolate	31±3.16	36.2 ±1.92	0.85	Suboblate
t_{cal}	29.36***	5.46***			2.83*	2.46*		
p-value	0.000	0.000			0.02	0.04		

Table 5: Influence of pollination and pollen parent on nut characters (length, diameter, weight and shell thickness) of female genotype

Female genotype	Pollen parent	Nut length (mm)	Nut diameter (mm)	Nut weight (g)	Shell thickness (mm)
SKAU-W-0025	: Open pollination	32.54[a]	30.54[b]	10.45[a]	1.51[b]
	X SKAU-W-0022	32.75[a]	30.84[b]	10.98[a]	1.49[b]
SKAU-W-0024	: Open pollination	33.86[a]	30.78[b]	10.65[a]	1.84[a]
	X SKAU-W-0022	33.78[a]	30.69[b]	10.32[a]	1.79[a]
SKAU-W-0008	: Open pollination	30.98[b]	33.45[a]	9.99[b]	1.51[b]
	X SKAU-W-0020	30.92[b]	33.25[a]	9.78[b]	1.58[b]
SKAU-W-0015	: Open pollination	32.66[a]	32.15[a]	9.35[b]	1.74[b]
	X SKAU-W-0020	32.95[a]	32.47[a]	9.54[b]	1.69[b]
$CD_{0.05}$		1.35	1.42	0.85	0.12

diameter, length and kernel length, weight, and kernel percentage were slightly decreased by cross pollination (Table 5 and 6). The pollen from different sources affects readily discernible characteristics of seeds and fruits in the period immediately following fertilization and such immediate or direct effects are termed as "xenia" and have been described in many species. Ak (2010) in his experiment on *Pistacia* species concluded that fruit weight, width and thickness were affected by the pollen source and shelling percentage also changed, however fruit length was not affected. Golzari *et al.* (2010) also reported that the nut and kernel diameter as well as shell thickness were affected by pollen sources. As the nut and kernel characters within genotypes under open and cross pollination were non-significant it can be concluded that there was no xenia effect. The results of the present investigation confirmed that the nut and kernel characteristics in selections under study through open and cross pollination were at par.

Table 6: Influence of pollination and pollen parent on Kernel length, weight, percentage and colour of female genotypes

Female genotype	Pollen parent	Kernel length (mm)	Kernel weight (g)	Kernel percentage
SKAU-W-0025	: Open pollination	26.52[b]	5.28[a]	50.52[c]
	X SKAU-W-0022	26.87[b]	5.57[a]	50.72[c]
SKAU-W-0024	: Open pollination	28.92[a]	5.21[ab]	48.92[d]
	X SKAU-W-0022	28.65[a]	5.03[b]	48.74[d]
SKAU-W-0008	: Open pollination	25.32[c]	5.35[a]	53.55[a]
	X SKAU-W-0020	25.15[c]	5.22[a]	53.37[a]
SKAU-W-0015	: Open pollination	26.38[b]	4.85[b]	51.87[b]
	X SKAU-W-0020	26.65[b]	4.96[b]	51.99[b]
$CD_{0.05}$		0.96	0.39	1.18

*Figures within parenthesis are the square root transformed values
Values superscripted with same alphabet are not significantly different

Conclusion: Testing of pollen performance could be helpful for the fruitful cultivation of genetic progeny for the breeding purpose, and especially for selecting, which cultivars should be used by growers. Cross pollination resulted in better kernel and nut quality as compared to open pollination. On the basis of pollen viability, germination and pollen production rate walnut genotypes tested in this study have the characteristics of good pollinizers. SKAU-W-0020 proved to be better pollen parent in terms of its pollen viability, its germination and pollen production rate as compared to SKAU-W-0022. Nut weight, diameter, length and kernel length, weight and kernel percentage in SKAU-W-0025 and SKAU-W-0015 increased through cross pollination, however in SKAU-W-0024 and SKAU-W-0008 reverse trend was observed. Thus, this study may provide useful information for facilitating the evaluation of walnut cultivars based on their pollen performance.

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