The Bioscan 11(4): 2683-2686, 2016 (Supplement on Agronomy)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

POLLINATION STUDIES IN WALNUT (*JUGLANS REGIA* L.)

K. M. BHAT[1]*, SHAZIYA HASSAN[1], M. A. BHAT[2], M. A. MIR[1] AND S. N. KIRMANI*[1]
[1]Division of Fruit Sciences,
[2]Department of Plant Biotechnology,
Sher-e-Kashmir University of Agricultural Sciences and Technology- Kashmir, Shalimar - 190 025 (J&k) INDIA
e-mail: shoaibkirmani@gmail.com

KEYWORDS
Germination
Pollination
Pistillate flower
abscission
Viability
Walnut

Received on :
03.03.2016

Accepted on :
14.10.2016

***Corresponding author**

ABSTRACT
The present investigation entitled "Pollination Studies in Walnut (*Juglans regia* L.)" was carried out on the walnut selections available at Horticultural Farm, Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar Srinagar. Pollen viability of all investigated genotypes was greater than 87 %. Highest viability of 91.40 % was observed in selection SKAU-W-0020 and the lowest (87.40 %) in SKAU-W-0022. Maximum pollen germination was recorded in SKAU-W-0020 (51.51 %) on media containing agar agar powder (1%) + Sucrose (15%) + boric acid (100 ppm) + $CaCl_2$ (1 mM). There was a significant effect of genotype and pollination method on pistillate flower abscission and was maximum (42.40 %) in SKAU-W-0015 through open pollination and minimum (15.77 %) in SKAU-W-0025 through cross pollination. Fruit retention also varied significantly with maximum (90.61 %) in SKAU-W-0025 X SKAU-W-0022 combination and minimum (81.49 %) in SKAU-W-0024 under open pollination. Taking into consideration the pollen viability and germination values, investigation revealed that these selecyed selections of walnut can be used as pollinizers for walnut genotypes that bloom in the same period and the level of pistillate flower abortion was significantly reduced under controlled pollination when compared with open pollination.

INTRODUCTION

The 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, walnut grows in North-western Himalayan belt, expanding to Darjeeling and Sikkim. Jammu and Kashmir state is the major producer of walnut covering an area of 93,642 ha and production of about 2, 09,051 metric tonnes (Anonymous, 2012). Pollen functional quality is important for crop improvement and fertility studies. One of the important factors for fertilization success is pollen viability and germination. Pollen viability is an ability of a pollen grain to germinate and develop as a pollen tube (Prajapati and Jain, 2011). *In vitro* germination studies of pollen has been used as powerful tool for genetical, physiological, biochemical and cytochemical processes for a wide range of plant species belonging to different families (Sarika Gupta and Mary Varkey Boswal, 2012). The rate of pollen germination of fruit and nut species and cultivars varies depending on the medium or chemical concentration. For this reason the suitable germination medium should be obtained for each species. Germination percentage is affected by many factors, including the concentration of calcium, hydrogen and borate in the germination media (Holdaway-Clarke *et al*., 2003). The addition of boron and calcium to the germination media increases germination percentage and length of pollen tube growth in many fruit species. Sucrose primarily serves to control the osmotic potential of the germination media but may also provide a base for polysaccharide, synthesis and metabolic energy (Kwack, 1965). Some studies have been carried out on walnut pollen production, viability and amount of pollen for different cultivars (Saglam and Gulcan, 1995; Sutyhemez and Eti, 2005) but such pollen tests have not been carried out in walnuts grown in Kashmir. Pistillate flower abortion (PFA) is understood as the loss of flowers early in the season due to excess of pollen. This loss is stimulated by an excess of pollen on stigmas due to high rate of ethylene biosynthesis which activates preformed abscission zone, resulting in flower abscission which can seriously reduce yield (Gonzalez *et al.,* 2008) and pollen donor has its effect on other paramerters of nut and kernel. (Golzari *et al*., 2010). Many observations made in countries like France, USA, Spain, Chile demonstrated that PFA exists nearly in all walnut cultivars (Polito, 1998).

However, such studies regarding pollen performance and effect of pistillate flower abortion on walnut production have not been undertaken under Kashmir condition. The paper deals with the objective to determine viability, germination, pollination ability of different walnut varieties and to determine efficient mode of pollination on pistillate flower abscission and fruit retention.

MATERIALS AND METHODS

Plant material

The present study was carried out to determine the viability, germination, and pollen production capacity of walnut varieties and also to determine efficien mode of pollination in walnut (*Juglans regia* L) during the year 2012 at the Horticulture farm, Division of Fruit Science, Sher-e-Kashmir University of

Agricultural Sciences and Technology of Kashmir, Shalimar Campus , Srinagar. The present investigation was conducted on two promising pollen parents of walnut (SKAU-W-0022 and SKAU-W-0020) crossed with following female parents of walnut (SKAU-W-0024, SKAU-W-0025, SKAU-W-0008 and SKAU-0015).

Pollen germination and viability

Pollen germination and pollen viability tests were performed according to Eti (1990). The freshly dehisced pollen grains were dusted on sterilized petri plates containing media agar agar powder (1%) solidifying against different concentrations of sucrose (10, 15, 20 , 25%) alone and in combination with 100 ppm boric acid and 1mM calcium chloride. The petri plates were incubated at 27 ± 1ºC for 24 hours. The pollen grains were considered as germinated when length of pollen tube was equal or exceeded pollen diameter. Pollen viability was estimated using two staining techniques, TTC (2,3,5-triphenyl tetrazolium chloride) and Fluorescence diacetate. TTC and FDA solutions were prepared according to (Norton, 1966) and Heslop and Harrison and Heslop and Harrison (1970) respectively. Pollen grains were scattered onto TTC and FDA solutions, and stained pollen grains were counted after 2 hours and fifteen minutes respectively. Under TTC test, the pollen grains that stained orange or bright red colour were counted as viable. In the second staining method, pollen grains that fluorescence were counted as alive. The experiments were designed as completely randomized blocks with three replications. Randomly selected visual areas, including about 100 pollen grains were counted in each replicate. All observations of germination and viability were made at x100 magnification using a light microscope.

Modes of pollination

In open pollination four different branches on four sides of a tree in each genotype were selected and number of healthy female flowers were counted and marked for open pollination. In cross pollination pollen from two (male) genotypes viz., SKAU-W-0022 and SKAU-W-0020 was collected by shaking the catkins in glass flasks. The pollen was then diluted with talcum powder in order to obtain 5 per cent concentration of pollen (w/w) to carry out manual pollination test. Each replicate of female genotypes, were labeled and bagged with muslin cloth bags. At the bi-fid stage which is the stage of stigma receptivity with an angle of 45° between two stigmatic lobes, the bags were removed and the pollen of the SKAU-W-0022 was applied on pistillate flowers of SKAU-W-0025 and SKAU-W-0024 and pollen of the SKAU-W-0020 was applied on pistillate flowers of SKAU-W-0008 and SKAU-W-0015. Pollination was performed by depositing a prepared load of pollen on the stigmas of each flower using the eraser end of pencil , considered as the contact surface of a known area as earlier used by (Gonzalez *et al*., 2008) while performing controlled pollination in walnut. The degree of covering was superficial without agglomeration of pollen. After pollen application the stigmas were covered with cotton to avoid contamination. Fig. 1. Shows the steps involved for artificial pollen application and state of development of pistillate flowers. 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 characteristics were recorded. The final fruit retention was determined on the basis of number of fruits retained at the time of harvest on the selected branches.

RESULTS AND DISCUSSION

Pollen germination and viability

Different media using different sucrose were used to test the germination of walnut pollen. The *in vitro* germination was highest in the pollen parents (51.51% in SKAU-W-0020 and 50.41 per cent in SKAU-W-0022) in medium containing Agar Agar 1% + Sucrose 15 % + boric acid 100ppm + $CaCl_2$ 1Mm and lowest germination of 34.83 per cent was recorded in medium containing Agar Agar 1Mm + Sucrose 25% (Table

Table 1: Effect of different sucrose concentrations alone and in combination with Boron and Calcium Chloride on pollen germination percentage in pollen parents

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

Table 2: Determination of pollen viability of pollen parent genotypes in walnut using T.T.C. and FDA stain tests.

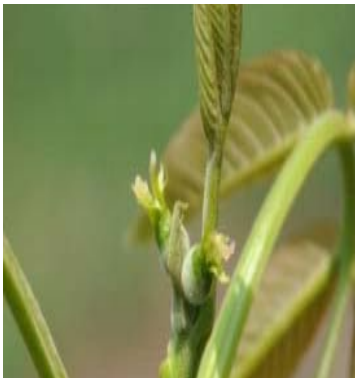
Male genotype	TTC Viable	Semi-viable	Dead	FDA Viable	Dead
SKAU-W-0022	87.40 ±2.07	6.80±0.84	5.20±0.83	88.40 ±1.15	11.60 ±0.62
SKAU-W-0020	90.20±0.83	5.20±0.84	3.20±0.83	91.40 ±1.15	8.30 ±0.41
t_{cal}	2.80*	3.02*	3.77**	4.16**	7.68**
p-value	0.035	0.016	0.005	0.003	0.002

Table 3: Effect of open and hand pollination on pistillate flower abscission (%) after 3 weeks of pollination and fruit retention (%) at harvest.

Female genotype	Pollen parent		Pistillate flower abscission (%)		Fruit retention at harvest (%)	
SKAU-W-0025	:	Open pollination	26.53	(5.24)	86.47	(9.35)
	X	SKAU-W-0022	15.77	(4.09)	90.61	(9.57)
SKAU-W-0024	:	Open pollination	30.74	(5.63)	81.49	(9.08)
	X	SKAU-W-0022	19.25	(4.49)	87.53	(9.40)
SKAU-W-0008	:	Open pollination	40.13	(6.41)	82.14	(9.11)
	X	SKAU-W-0020	27.75	(5.36)	88.23	(9.44)
SKAU-W-0015	:	Open pollination	42.40	(6.58)	86.49	(9.35)
	X	SKAU-W-0020	26.44	(5.23)	89.78	(9.52)
$CD_{0.05}$			0.27		0.14	

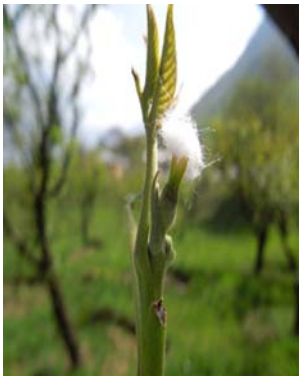
*Figures within parenthesis are the square root transformed values

Bagging of flowers



Stage of stigma receptivity

Dusting of pollen with help of Pencil rubber end



Stigma covered with cotton to avoid contamination

1). Results showed that pollen germination rates were improved by addition of boric acid and calcium to sucrose. Boric acid is generally used as boron source. Boron is believed to promote pollen germination by affecting H^+- ATPase activity, which initiates pollen germination and tube growth (Feijo *et al*., 1995). Calcium is involved with pectin synthesis and the control of the osmotic conditions. Similar results were also achieved by (Mert, 2009; Kamrani, 2012; Mondal and Ghanta, 2012) in different crops. Highest pollen viability (91.40% and 90.20 %) was observed in SKAU-W-0020 selection under both FDA and TTC stain tests respectively. Semi-viable percentage of pollen was highest (6.80 %) in SKAU-W-0022 and the lowest (5.20%) in SKAU-W-0020 through TTC test while as dead pollens were maximum (11.60 %) in SKAU-W-0022 through FDA test and minimum (3.20 %) in SKAU-W-0020 through TTC test (Table 2). The TTC test is based upon dehydrogenase enzyme activity. Upon seed/tissue hydration, the activity of this enzyme increases, resulting in release of H^+ ions which reduce the colourless Tetrazolium salt into a red compound Formazen which stains living cells with red colour while dead cells remain colourless. In FDA test the Fluorescein diacetate, a non-polar and non-fluorescent molecule is hydrolysed by enzyme pollen esterase into Fluorescein, a polar and fluorescent molecule which after accumulation inside the pollen grain appears fluorescent in blue light. The results of the present study are in accordance with previous studies (Cosmulescu *et al.,* 2009; Sutyemez, 2011).

Modes of pollination

The pistillate flower abscission under different modes of pollination is given in Table 3. Present investigation revealed that the highest (42.40%) pistillate flower abscission (PFA) was recorded under open pollination in SKAU-W-0015 which was reduced to 26.44 % under cross pollination with SKAU-W-0020. The lowest pistillate flower abscission (PFA) was recorded under open pollination of SKAU-W-0025 (26.53%) which was further reduced to (15.77%) under cross pollination in SKAU-W-0025 X SKAU-W-0022 (Table 3). The reason for this is the excess pollen load on stigmas as in open pollination the pollen load can be higher but in case of hand cross pollination we use a particular concentration and quantity of pollen. Excessive pollen tubes growing down the style of the female flower produce higher amounts of ethylene, which is associated with organ senescence. Beede and Polito (2003) also reported that elevated ethylene levels are cause of flower abortion. The degree of losses due to pistillate flower abscission was different in different walnut cultivars. Climatic conditions may also influence the flower drop level due to PFA in some cultivars (Rovira and Aleta, 2006). Hassani *et al*. (2006) reported that PFA was 11 to 92% among Iranian Genotypes. Gun *et al*. (2010) reported that the level of PFA in Turkish walnut cultivars ranged from 65.4% to 100%. The results of the present investigation were supported by results of previous work (Gonzalez *et al.,* 2008; Lemus, 2010).

The highest fruit retention (90.61%) at harvest was recorded under cross pollination in SKAU-W-0025 crossed with SKAU-W-0022 followed by 89.78 per cent under cross pollination in selection SKAU-W-0015 crossed with SKAU-W-0020 and lowest (81.49%) under open pollination in selection SKAU-W-0024 (Table 4). Fruit retention is higher as little fruit drop is observed in walnut as compared to other fruit and nut species

after fruit set. McGranahan and Leslie (1990) reported that there is sporadic fruit drop in walnut which may continue throughout the growing season. The results of our study are supported by Kumar *et al*. (2005) who reported high values of fruit retention in walnuts (52.93 %- natural pollination; 68.02% - self pollination and 65.37% under cross pollination). Lower values of fruit set and fruit retention may be attributed to younger age of plants, genetic makeup of the varieties/selections and also to the climatic and environmental factors (Pandey and Tomar, 2012).

From the above observations it is concluded that both staining methods gives good response in determination of pollen viability. The *in vitro* germination assey was highest in the pollen parents (51.51% in SKAU-W-0020 and 50.41 per cent in SKAU-W-0022) in medium containing agar agar 1% + Sucrose 15 % + boric acid 100ppm + $CaCl_2$ 1Mm. Highest pollen viability (91.40% and 90.20 %) was observed in SKAU-W-0020 selection under both FDA and TTC stain tests respectively. On the basis of pollen viability and germination walnut genotypes tested in this study have the characteristics of good pollinizers and can be used as pollinizers for those walnut genotypes that bloom in the same period. Pistillate flower abscission under hand pollination reduced significantly when compared to the open pollination. Results showed that the degree of Pistillate Flower Abscission varied with the variety. SKAU-W-0022 proved to be effective pollinizer for SKAU-W-0024 & SKAU-W-0025; and SKAU-W-0020 for SKAU-W-0008 & SKAU-W-0015. The studies showed that the level of pistillate flower abortion in walnut genotypes was significantly reduced under controlled pollination when compared with open pollination.

REFERENCES

Anonymous 2012. Production and area statement for year 2010. *Department of Horticulture, Government of Jammu and Kashmir.*

Beede, R. H. and Polito, V. S. 2003. Effect of Retain on reducing pistillate flower abortion in 'Serr' walnut. *Walnut Research Reports.* p. 113.

Cosmulescu, S., Baciu, A. and Gruia, M. 2009. Walnut tree pollen quality in cultivars with various flowering times. *Bulletin UASVM Horticulture.* **66(1):** 650

Eti, S. 1990. Cicek tozu miktarýný belirlemede kullanýlan pratik bir yöntem. *C.U.Z.F. Dergisi.* **4(5):** 49-58.

Feijo, J. A., Malho, R. and Obermeyer, G. 1995. Ion dynamics and its possible role during *in vitro* pollen germination and tube growth. *Protoplasma.* **187:** 155-167.

Golzari, M., Rahemi, M., Vahdati, K. and Hassani, D. 2010. Effect of pollen source on Persian walnut characteristics. *Acta Hort.* **861:** 99-102.

Gonzalez, R. C., Gamalur, L. S. and Reginato, G. 2008. Pistillate flower abscission symptoms of Serr walnut (*Juglans regia* L.) *Chil. J. Agric. Res.* **68**: 183-191.

Gun, A., Erdogen, V., Akcay, M. E., Fidanci, A. and Tosun, I. 2010. Pistillate flower abscission in Turkish walnut cultivars and its reduction by AVG. 2nd International Symposium on Sustainable Development (ISSD' 10). *Proceedings Science and Technology, Sarajevo.* **3 :** 29-34.

Hassani, D., Eskandari, S. and Jarrahi, K. 2006. Pistillate flower abscission of walnut genotypes. *Acta Hort.* **705:** 257-260.

Heslop-Harrison, J. and Heslop-Harrison, V. 1970. Evaluation on pollen viability of enzymaticaliy induced fluorescence intracullular hydrolysis of fluorescein diacetate, *Stain Technol.* **45:** 115-120.

Holdaway-Clarke, T., Weddle, N., Kim, S ., Robi, A., Parris, C., Kunkel, J. and Helper, P. 2003. Effect of extracellular calcium, pH and borate on growth oscillations in lilium formosanum pollen tubes. *J. Expt. Bot*. **54:** 65-72.

Kamrani, R. 2012. Study on pollen germination and pollen tube growth of five Iranian apricot cultivars on *in vitro* condition. *International Conference on Applied Life Sciences.* pp. 299-302.

Kumar, A., Kumar, K. and Sharma, S. D. 2005. Extent of fruit set and retention under different modes of pollination in Persian walnut (*Juglans regia* L.). *Acta Hort.* **696:** 327-330.

Kwack, B. 1965. The effect of calcium on pollen germination. *Proc. Amer. Soc. Hort. Sci*. **86:** 818-823

Lemus, G. 2010. PFA control in 'Serr' in Chile. *Acta Hort.* **861**: 263-266.

McGranahan, G. H. and Leslie, C. 1990. *Walnuts* (*Juglans*). *In*: Ballington J. R and J.N. Moore (Eds.), *Genetic Resources of Temperate Fruit and Nut Crops*, Vol. II. ISHS Secretariat, The Netherlands, pp. 907-951.

Mert, C. 2009. Temperature responses of pollen germination in walnut (*Juglans regia* L.). *J. Biol. Environ. Sci.* **3(8):** 37-43.

Mondal, S. and Ghanta, R. 2012. Effect of sucrose and boric acid on in vitro pollen germination of Solanum macranthium dunal. *Ind. J. Fund. App. life Sci.* **2(2):** 202-206.

Norton, J.D. 1966. Testing of plum pollen viability with tetrazolium salts. *Am. Soc. Hort. Sci.* **89:** 132-134.

Pandey, C. and Tomar, C. S. 2012. Growth, flowering, fruit set and yield in some cultivars/selections of walnut (*Juglans regia*). *Ind. J. Agric. Sci.* **82(5):** 12-14.

Polito, V. 1998. Floral biology: flower structure, development and pollination. *In*: Ramos.D(Ed.), *Walnut production manual*. University of California, Publ. 3373, Oakland, California, USA, pp. 127-132.

Prajapati, P. P. and Jain, B. K. 2011. Effects of leaf extract on in vitro pollen germination and pollen tube growth in Luffanaegypticamill. and Momordicacharantia. *The Bioscan*. **6(3):** 447-449.

Rovira, M. and Aleta, N. 2006. Flower drop by precocious abortion in walnuts. *Nucis Newsletter.* **13:** 27-30.

Saglam, H. and Gulcan, R. 1995. Bazi me yvetur lerin decicek to zusakla mayontemleri Turkiye II Usual Bahce Bitkileri Kongresi, Ciltl, pp. 229-232.

Sarika, G. and Mary Varkey, B. 2012. Spirodelapolyrrhiza extract induced changes in pollen growth of barley plant. *The Bioscan*. **7(4):** 715-717.

Sutyemez, M. 2011. Pollen quality, quantity and fruit set of some self-compatible and self-incompatible cherry cultivars with artificial pollination. *Afr. J. Biotechnol.* **10(17):** 3380-3386.

Sutyhemez, M. and Eti, S. 2005. Pollen quality and pollen production rate of the selected walnut types from K.Maras region. *Acta Hort.* **705:** 382-398.