# POLLEN GERMINATION CAPACITY OF TWO CULTIVATED SPECIES (JASMINUM SAMBUC (L.) AIT. AND NYCANTHES ARBOR-TRISTIS L.) OF FAMILY OLEACEAE

# ANJUM PERVEEN<sup>1</sup> AND GHULAM RASOOL SARWAR<sup>2</sup>

<sup>1</sup>Department of Botany, University of Karachi, Karachi 75270, Pakistan, <sup>2</sup>Centre for Plant Conservation, University of Karachi, Karachi 75270, Pakistan

### Abstract

Pollen germination and viability of two species viz., *Jasminum sambuc* (L.) Ait., and *Nycanthes arbor-tristis* L. of the family Oleaceae were examined . Viability was determined on the basis of germination ability of pollen on sucrose medium with boric acid solution up to 48 weeks. Stored pollen were germinated under different conditions  $(+4^{\circ}C)$ ,  $(-20^{\circ}C, -30^{\circ}C)$ ,  $(-60^{\circ}C)$ , vacuum chamber and organic solvents. Pollen stored at low temperature  $-60^{\circ}C$  showed better germination as compared to pollen stored at  $+4^{\circ}C$  and fresh. Among two species *Jasminum sambac* (L.) Ait. showed much better germination percentage at  $-60^{\circ}C$  ( 40.60% ) whereas, *Nycanthes arbor-tristis* L., showed 37.0% germination after 48 weeks of storage. Pollen stored over silica jel under vacuum of both the species showed good germination as compared to organic solvents.

### Introduction

Pollen storage is a good conservation tool and time saving technique in hybridization and crops improvement programs (Towill, 2004). Germination capability of pollen is related to cultivars, nutrition conditions and environmental factors (Polat & Pirlack, 1999; Dafni & Firmago, 2000; Kelen & Demitas, 2003). Pollen viability is strongly influenced by temperature, moisture, genotypic differences, plant vigor and physiological stage and flower age. Different nutrition conditions and germination method for many plant species and varieties were used by researchers ( Kelen & Demitas, 2003). Pollen of different plants requires varying range of growth media like water, sugar solution, inorganic salts and vitamins for successful germination. Temperature is a basic factor in the control of the environmental conditions and influences pollen germination and longevity in stored pollen (Kelen et al., 1996). Towill (2004) suggested that low moisture content, within limits and lowering the storage temperature increase the pollen viability in stored pollen. Several researchers have studied the pollen viability and pollen germination of different taxa in different storage conditions such as liquid nitrogen (-196°C), refrigerator (+4°C), freezer (different minus temperatures), freeze dried and organic solvents (Nair & Singh, 1972; Hanson & Campbell, 1972; Shivanna & Rangaswamy, 1992; Kopp et al., 2000; Pansonen et al., 2001; Kenta et al., 2002; Perveen & Khan, 2009; Khan & Perveen, 2009; Khan & Perveen, 2010; Perveen & Ali, 2010; Perveen & Ali, 2011).

Present investigation is undertaken to examine the pollen viability and germination of two important ornamental species belonging to Oleaceae viz., *Jasminum sambuc* and *Nyctanthes arbor-tristris* in storage condition. Both these species are cultivated in number of tropical and subtropical countries including Pakistan for their beautiful flowers.

# **Material and Methods**

During the flowering period of *Jasminum sambuc* and *Nycanthes arbor-tritis* flowers were collected in large quantity from cultivated fields. They were placed in paper

bags and transferred to the laboratory. Anthers were separated and placed in petri dishes for the release of pollen. Pollen gathered and their germination and tube growth rate were tested immediately according to the method of Alexander (1969) and then stored for 48 weeks at +4°C, -20°C, -30°C, -60°C vacuum chamber and organic solvents. Pollen culture media were prepared according to standard method of Brewbaker & Kwack (1963). The germination was scored after 3-6 hours of incubation at room temperature in humid chambers using different solutions. 100 pollen per slide and 10 slides per plant and total 100 plants from different cultivated fields were studied. Pollen produced pollen tube and grew at least twice the diameter of pollen were counted as germinated. The viability of stored pollen was assessed in terms of percent germination.

## **Results and Discussion**

In the present investigation an attempt has been made to compare the pollen germination capacity of two cultivated species belonging to family Oleaceae viz. Jasminum sambuc (L.) Ait. and Nycanthes arbor-tristris L. Pollen stored up to 48 weeks in different conditions i.e., refrigerator, freezer, freeze drier, vacuum and organic solvents. Among two species Jasminum sambuc showed good germination percentage after 4 weeks of storage at all conditions (Table 1). Pollen stored at low temperature freeze drier showed the better germination percentage in first 4-28 weeks, but after that germination percentage was decreased slowly. Lora et al., (2006) examined the storage of Cherimoya (Annona cherimola) pollen at low temperature and demonstrated that the germination was progressively reduced with storage time and reached minimum after three months of storage. Our findings are also in agreement with that of Lora et al. (2006). Pollen stored at -20°C and -30°C showed good germination but as the time proceed and the germination percentage gradually decreased and after 48 weeks the germination was 21% and 27.10% in Jasminum sambuc. (Table 1 and Fig. 2).

Period in week	Different temperature									
	% Of germination at 4°C	% Of solutions	% Of germination at -20°C	% Of solutions	% Of germination at -30°C	% Of solutions	% Of germination at -60°C	% Of solutions		
4	39.20	30	51.00	30	50.00	30	59.00	30		
8	37.10	30	50.10	30	48.10	30	59.00	30		
12	35.30	30	46.90	30	46.00	30	58.10	30		
16	32.10	30	43.00	30	44.30	30	56.00	30		
20	29.40	30	41.20	30	44.00	30	55.40	30		
24	27.10	30	38.10	30	42.00	30	53.60	30		
28	25.10	30	35.00	30	40.60	30	54.90	30		
32	23.50	30	32.40	30	36.60	30	47.00	30		
36	21.00	30	29.70	30	35.00	30	45.10	30		
40	20.60	30	25.40	30	31.30	30	43.60	30		
44	17.10	30	23.60	30	28.60	30	42.00	30		
48	13.10	30	21.60	30	27.10	30	40.60	30		

 Table.
 1. Germination capacity of stored pollen grains of Jasminum sumbac (Oleaceae) at different temperature in sucrose and boric acid solutions.

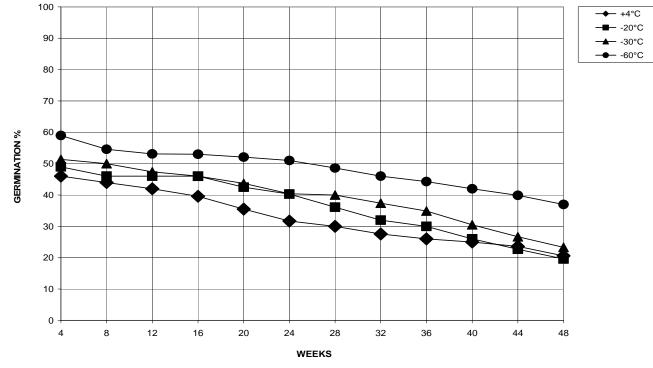


Fig. 1. Germination rate of stored Nycanthes arbor-tristis pollen over 48 weeks.

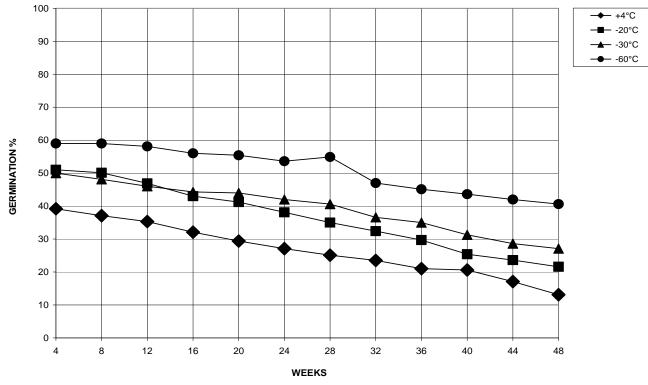
Maximum pollen germination was observed 59% at -60°C while minimum was observed 46% at 4°C in 4 weeks stored pollen of *Nycanthus arbor-tristis*. Percentage of pollen germination decreased with increasing storage time. The germination percentage at 4°C and fresh pollen was almost same in first week i.e., 46% and 45.3% respectively. Pollen stored at +4°C showed 39.20% germination in early weeks but then germination decreased rapidly and after 48 weeks, germination percentage fell to almost half of the maximum value (Table 2 and Fig. 1). Differences observed in *invitro* pollen germination was due to the results of complex interaction between morphology and physiology of pollen and components used in the culture medium (Gwata *et al.*, 2003). Kakani *et al.*, (2005)

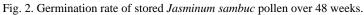
showed that the variations in *In vitro* germination and pollen tube growth due to the variations in the varieties of the plant species.

Souza-Lang & Pinto Junior (1997) revealed that the highest pollen germination of *Araucaria angustifolia* in medium without sugar but our result showed that highest germination percentage in the medium containing sucrose. However, pollen did not germinate in a high concentration of sucrose, which confirms the Premachardra *et al.*, (1992) hypothesis i.e., when concentration of sucrose increases in the medium, the concentration of carbon is also increased which inhibit the pollen germination.

Period in week	Different temperature									
	% Of germination at 4°C	% Of solutions	% Of germination at -20°C	% Of solutions	% Of germination at -30°C	% Of solutions	% Of germination at -60°C	% Of solutions		
4	46.00	30	49.00	30	51.40	30	59.00	30		
8	44.00	30	46.00	30	50.00	30	54.60	30		
12	42.00	30	48.00	30	47.40	30	53.10	30		
16	39.60	30	46.00	30	46.00	30	53.00	30		
20	35.50	30	42.50	30	43.70	30	52.10	30		
24	31.70	30	40.30	30	40.40	30	51.00	30		
28	30.00	30	36.10	30	40.00	30	48.60	30		
32	27.56	30	32.00	30	37.40	30	46.00	30		
36	26.00	30	30.00	30	34.90	30	44.30	30		
40	25.00	30	26.00	30	30.50	30	42.00	30		
44	23.60	30	22.70	30	26.7 0	30	39.90	30		
48	20.60	30	19.60	30	23.30	30	37.00	30		

 Table 2. Germination capacity of stored pollen grains of Nycanthes arbor-tristis (Oleaceae) at different temperature in sucrose and boric acid solutions.





Pollen germination was also observed by soaking the pollen in the solvent benzene, acetone and chloroform. Among these benzene showed better germination percentage than other solvents. Ikada & Numata (1998) also reported benzene was the most effective solvent for pollen storage of *Chrysanthemum*.

Present findings are also in agreement with that of Udomedee *et al.*, (2003). Who has also reported that the pollen stored at low temperature showed better germination results in *Cercuma*. The pollen of *Jasminum sambuc* and *Nycanthes arbor-tristis* stored at  $-60^{\circ}$ C showed better results and had a viability up to 40.60% after storing 48 weeks. In the remaining condition

germination percentage was very low after 48 weeks of storage (Tables 1 & 2).

#### Acknowledgement

We are thankful to PSF (Pakistan Science Foundation) for providing financial support.

#### References

Alexander, M.P. 1969. *Different staining of aborted and non-aborted pollen*. Stain Technology, 44: 117-122.

Brewbacker, J.L. and B.H. Kwack. 1963. The essential role of calcium ion in pollen tube growth. *Amer. J. Bot.*, 50: 859-865.

- Dafni, A. and D. Firmage. 2000. Pollen viability and longevity: practical, ecological and evolutionary implicatipons. *Plant Syst. Evol.*, 222: 113-132.
- Gwata, E.T., D.S. Wofford, P.L. Pfahler and K.J. Boote. 2003. Pollen morphology and *In vitro* germination characteristics of nodulating and non-nodulating soybean (*Glycine max* L.) genotypes. *Theor. Appl. Genet.* 106: 837-839.
- Hanson, C.H. and T.A. Campbell. 1972. Vacuum dried pollen of alfalfa (*Medicago sativa* L.) viable after eleven years. *Crop. Sci.*, 12: 874.
- Ikeda, H. and S. Numata. 1998. Pollen storage of *Chrysanthemum. Acta Hort*. (ISHS) 454: 329-334.
- Kakani, V.G., K.R. Reddy, T.P. Wallace, P.V. Prasad, V.R. Reddy and D. Zhao. 2005. Differences invitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Ann. Bot.*, 96: 59-67.
- Kelen, M. and I. Dematas. 2003. Pollen viability, germination capability and pollen production level of some grapes varieties (*Vitis vinifera* L.). Acta Physiologies Plantarum, 25: 229-233.
- Kelen, M., M. Sautyemez, O. Behen and A. Yalinkiliq. 1996. A study on fertilization biology of some grape varieties. *Acta. Hort.*, 441: 325-329.
- Kenta, T., K.K. Shimizu, M. Nakagawa, K. Okada, A.A. Hamid and T. Nakashizuka. 2002. Multiple factors contribute to out crossing in a tropical emergent *Dipterocarpus tempehes*, including a new pollen-tube guidance mechanism for self-incompatibility. *Amer. J. Bot.*, 89: 60-66.
- Khan S.A. and A. Perveen. 2010. In vitro pollen germination capacity of *Citrullus lanatus* L., (cucurbitaceae). *Pak. J. Bot.*, 42(2): 681-684, 2010.
- Khan, S.A. and A. Perveen. 2009. Pollen germination capacity of three mango cultivars (*Mangifera indica* L., anacardiaceae) from Pakistan. *Pak. J. Bot.*, 41(3): 1009-1012.
- Kopp, R.F., C.A. Maynard, P.R. de Niella, L.B. Smart and L.P. Abrahamson. 2000. Collection and storage of pollen from *Salix* (Salicaceae). *Am. J. Bot.*, 89: 248-252.
- Lora, J., M.A. Perez De Oteyz, P. Fuentetaja and J.I. Hormaza. 2006. Low temperature storage and *In vitro* germination of

cherimoya (Annona cherimola Mill.) pollen. Sci. Hort., 108: 91-94.

- Nair, P.K and B.V. Singh. 1972. Pollen germination studies in some legumes. J. Palyn., 8: 63-68.
- Pansonen, H-L., P. Pulkkinen and M. Kapyla. 2001. Do pollen donors with fastest-growing pollen tubes sire the best offspring in an anemophilous tree. *Betula pendula* (Betulaceae). *Amer. J. Bot.*, 88: 854-860.
- Perveen, A. and S. Ali. 2011. Pollen germination capacity and maintenance of pollen in *Praecitrullus fistulosus* (stocks) Pangola (cucurbitaceae). *Pak. J. Bot.*, 43(1): 47-50.
- Perveen, A. and S.A. Khan. 2009. Maintenance of pollen germination capacity of *Glycine max* (L.) Merr., (Papilionaceae), *Pak. J. Bot.*, 41(5): 2083-2086.
- Perveen, A., and S. Ali. 2010. Maintenance of pollen germination capacity of *Vitis vinifera* L. (vitaceae). *Pak. J. Bot.*, 42(5): 3001-3004.
- Polat, I. and L. Pirlak. 1999. An investigation on pollen viability, germination and tube growth in some stone fruits. *Turk. J. Agric.*, 23: 383-388.
- Premachandra, G.S., H. Saveoka, K. Fuji La and S. Ogata. 1992. Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax loand and growth as affected by increasing water deficits in *Sorghum. J. Exp. Bot.*, 43: 1569-1376.
- Shivanna, K.R. and N.S. Rangaswamy. 1992. Pollen biology. A laboratory manual. New Springer, Verlag. Berlin, Heidelberg, New York. Stain Trchnology, 44: 117-122.
- Souza–Lang, V.A. and J.E. Pinto Junior. 1997. Influencia do meio de cultura na germinacao do polen de tres species de *Eucaliptus. Bol. Pes. Fl.*, 34: 45-54.
- Towill, L.E. 2004. *Pollen storage as a conservation tool.* pp.180-188. In: Ex Situ Plant Conservation: Supporting species survival in the wild. (Eds.): E. Guerrent, K. Havens and M. Maunder. Island Press, Covela, CA.
- Udomdee, W., S. Fukai, L. Petpradap and J.A. Teixeira da Silva. 2003<sup>-</sup> *Curcuma*: Studies of tissue culture, pollen germination and viability, histology and flow cytometry, *Prop. Orn. Pl.*, 3(1): 34-41.

(Received for publication 3 October 2010)