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GERMINATION CAPACITY AND VIABILITY OF STORED POLLEN IN TWO ORNAMENTAL SPECIES OF THE GENUS *CAESALPINIA* L. (CAESALPINI OIDEAE-FABACEAE) AND THEIR MAINTENANCE

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Abstract

Pollen germination capacity and viability of two species *viz.*, *Caesalpinia gellisii* (Hook.) Dietr. and *Caesalpinia pulcherrima* (L.) Swartz. of the family Fabaceae were examined up to 48 weeks. Viability was determined on the basis of germination ability of pollen on sucrose medium with boric acid solution. Fresh pollen were stored at different storage conditions including refrigerator (4°C), freezer (-20°C, -30°C), freeze drier (-60°C), vacuum chamber and organic solvents. Pollen stored at low temperature -60°C showed better germination as compared to pollen stored at 4°C and fresh. *C. pulcherrima* showed much better germination percentage at -60°C (61.00%) whereas, *C. gellisii*, showed 30.80% germination after 48 weeks of storage. Pollen stored over silica gel in vacuum of both the species showed good germination as compared to organic solvents. Freezer (-20ºC & -30ºC) and freeze dryer (-60ºC) maintained pollen germination capacity and viability for a longer period as compared to fresh, refrigerated, pollen treated in vacuum over silica gel and organic solvents. Conclusively both low temperature and RH humidity are the most influential factors in controlling germination and viability of pollen grains.

Key words: Pollen, Viability, Temperature, Humidity, Germination, Sucrose, Boric acid.

Introduction

Maintaining the germination capacity of stored pollen can be most useful and particularly time saving in hybridization programmes and in situations where artificial pollination is necessary, such as allogamous or male sterile plants. Wild plants facing extinction and cultivated taxa could benefit from wild genomes contributions are also cases where stored viable pollen can be used.

Pollen storage is considered as the most efficient method to overcome barriers to hybridization between plants flowering at different time or growing in different regions. Long term storage of pollen has been achieved among different storage methods and cold storage proved to be the most economical and widely used method for preserving pollen. Alba *et al*., (2011) suggested the use of cryopreservation of pollen using liquid nitrogen at -196ºC. Several studies have been published on the determination of optimal conditions to maintain the pollen viability for a longer period. Pollen of different plants requires varying range of growth media like water, sugar solution, inorganic salts and vitamins for successful germination. The main factor affecting the pollen viability and germination capacities of stored pollen are temperature and relative humidity. Polito & Pinney, (1990) obtained good result when olive pollen were stored at 28-33 RH at 20ºC. *In-vitro* pollen germination and short term pollen storage in *Caladium* was studied by Deng *et al*., (2004). Conservation of the germination capacity of pollen grains in three varieties of *Zea mays* L. has been examined by Youmbi *et al.*, (2007). Zhang *et al.*, (2011) examined the effect of temperature on *In vitro* pollen germination and storage of Peony pollen. Soares *et al*., (2013) studied the *In-vitro* pollen germination capacity and viability in ornamental Passion flower (*Passiflora* spp.). Mankad (2012) examined *In vitro* pollen germination and storage of *Crinum asiatium* L. It is well known that the pollen have direct effect in fertilizing process in plant breeding (Androulakis & Loupassaki, 1990; Ateyyeh *et al*., 2000). The effect of temperature has been studied both *In vitro* and *In vivo* germination of self incompatible pollen in Olive by Mehri *et al*., (2003), in Apple by Montalti & Filiti, (1984). Various worker studies pollen germination in various conditions and medias (Hanson & Campbell, 1972; Nair & Sing, 1972; Shivanna & Rangaswamy, 1992; Polat & Pirlak, 1999; Kopp *et al*., 2000; Pansonen *et al*., 2001; Kenta *et al*., 2002; Towill, 2004; Khan & Perveen, 2009; Perveen & Ali, 2011). Present investigation is undertaken to examine the viability and germination of two important ornamental species belonging to subfamily Caesalpinioideae-Fabaceae *viz*., *C. gellisii* and *C. pulcherrima* in storage condition. Both these species are cultivated in number of tropical and subtropical countries including Pakistan for their beautiful flowers.

Material and Methods

Both species of the genus *Caesalpinia* produce flowers throughout the year, flowers were collected in large quantity from University campus and Botanic Garden, University of Karachi. They were placed in paper bags and transferred to the laboratory. Pollen viability was tested immediately according to the method of Alexander (1969) and then stored at different stored conditions including refrigerator (4°C), freezer (-20°C, -30°C), freeze drier (-60°C), vacuum chamber over silica gel and organic solvents (Acetone, Benzene &

Chloroform). Pollen culture medium was prepared according to the 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 (5%-50%). Approximately 100 pollen per slide and 10 slides per species were prepared to find out the germination percentage. Pollen produced pollen tube and grew at least twice the diameter of pollen were counted as germinated, while burst pollen as un-germinated. The viability of stored pollen was assessed in terms of percent germination.

This is the first report on the pollen germination capacity of two *Caesalpinia* species from Pakistan.

Results and Discussion

In the present investigation an attempt has been made to compare the pollen germination capacity of two cultivated species namely *Caesalpinia gellisii* (Hook.) Dietr. and *Caesalpinia pulcherrima* (L.) Swartz belonging to family subfamily Caesalpinioideae-Fabaceae. Pollen were stored up to 48 weeks at different conditions i.e. refrigerator (4°C), freezer (-20°C & -30°C, freeze drier (-60°C), in vacuum over silica and in organic solvents. Among two species *Caesalpinia pulcherrima* showed better germination percentage and viability after 48 weeks at all storage conditions as compared to *C. gellisii*, the later species showed better germination and viability in early weeks particularly pollen maintain viability and showed 30.8% of germination after 48 weeks at freeze dried condition (Fig. 1), while pollen stored at 4°C showed poor germination and lost viability after 28 weeks of storage. Udomedee *et al*., (2003), whom reported that the pollen stored at low temperature showed better germination in *Curcuma*, our findings also support his view. Pollen of *C. pulcherrima* showed better germination and viability rates in all stored conditions after 48 weeks of storage 19.6% at 4°C, 35.0% at -20°C, 39.6% at -30°C and 59.9% at -60°C as compared to 0%, 3.0%, 1.0% and 30.8% of *C. gellisii* at mentioned conditions (Figs. 1 & 2). Lora *et al*., (2006) examined the storage of Cherimoya (*Annona cherimola*) pollen at low temperature and demonstrated that germination was progressively reduced with storage time and reached minimum after three months of storage, our findings and results of *C. gellisii* pollen also supported the findings of Lora *et al*., (2006) but the results of *C. pulcherrima* pollen showed contradiction to the statement and maintained better viability & germination percentage even after 48 weeks. Maximum pollen germination 73.0% was observed in *C. pulcherrima* at -60°C after 4 weeks of storage as compared to 56.7% of *C. gellisii*. Difference in *In vitro* pollen germination percentage may be due to the result of complex interaction between morphology and physiology of pollen and components used in culture medium (Gwata *et al*., 2003). Kakani *et al*., (2005) showed that the variation in *in-vitro* germination and pollen tube growth was due to the variation in varieties of the plant species. Souza-Lang & Pinto Junior (1997) reported highest pollen germination of *Araucaria angustifolia* in a medium without sugar but our results showed better germination in a medium having 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 was increased in the medium, the concentration of was also increased which inhibited pollen germination.

Pollen germination was also studied by soaking fresh pollen in organic solvents including Acetone, Benzene and chloroform. Among solvents benzene showed better germination results than others. Ikada & Numata (1998) reported benzene as the most effective solvent for pollen storage of *Chrysanthemum*.

Conclusively both low temperature and relative humidity maintained the viability and germination percentage of pollen in stored conditions for a longer period.

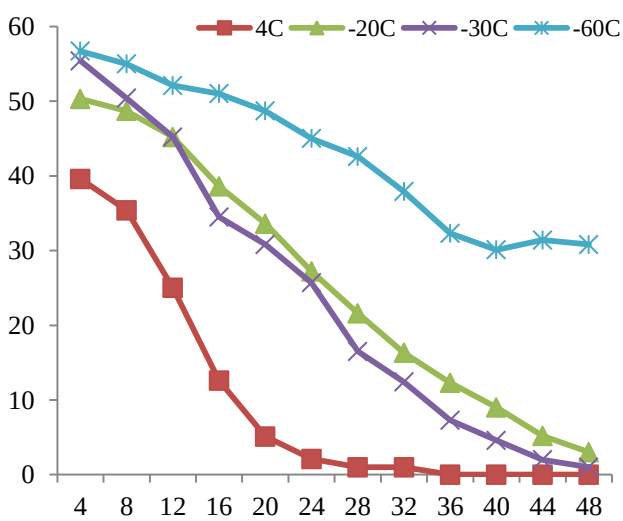

Fig. 1. Germination percentage of stored *Caesalpinia gilliesii* pollen up to 48 weeks.

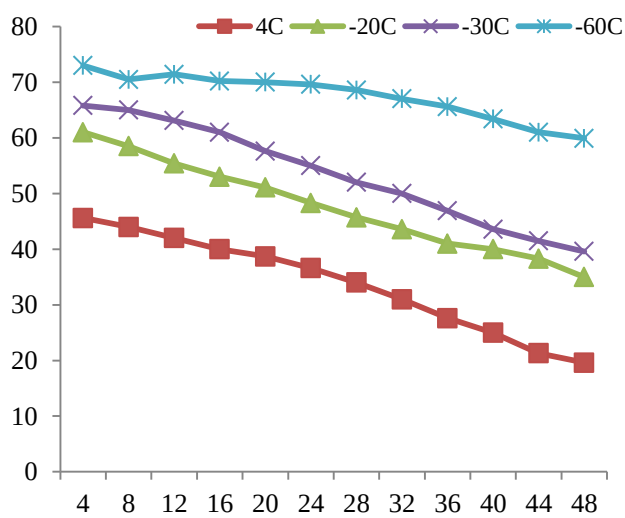

Fig. 2. Germination percentage of stored *Caesalpinia pulcherrima* pollen up to 48 weeks.

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