INTERNATIONAL JOURNAL OF ENVIRONMENT
Volume-3, Issue-3, Jun-Aug 2014 ISSN 2091-2854
Received:7 July *Revised:27 July* *Accepted:20 August*

IMPROVISED MEDIA FOR *IN VITRO* POLLEN GERMINATION OF SOME SPECIES OF APOCYNACEAE

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Abstract

Pollen germination forms one of the most important stage post pollination prior to fertilization. This is essential for proper seed setting and seed development. *In vitro* pollen germination test is the most reliable way of assessing the pollen viability. In the present study pollen grains of seven genera under Apocynaceae family namely, *Allamanda*, *Alstonia*, *Catharanthus*, *Nerium*, *Plumeria*, *Thevetia* and *Tabernaemontana* were tested in some basic cultural media, such as Brewbaker's media, 6% Glucose solution, 4% Calcium Nitrate solution and 3% Boron solution. *Alstonia* pollen grains exhibited highest percentage of germination rate in all the cultural media. Glucose and Brewbaker's media is found to be highly suitable for efficient pollen germination in all the genera. Boron solution is effective for germination of pollen grains of tree species. *In vitro* pollen germination can be easily carried out in laboratories. These results can be utilised in plant breeding programmes to improve cultivar and varieties.

Key Words: *In vitro*, Pollen Germination, Apocynaceae, Sitting-Drop Culture, Basic Culture

Introduction

Pollen grain constitutes the male reproductive unit of higher group of vascular plants (Gymnosperms and Angiosperms). They can be easily collected, desiccated and stored in a viable condition for a considerable period of time that can be put into use at any time of the year. Pollen germination and pollen tube growth forms two of the most important pre-requisite stage post pollination prior to fertilization. It is essential for successful fertilization and proper seed setting for seed development (Kester et al., 1991; Martinez-Gomez et al., 2002; Imani et al., 2011). *In vitro* germination techniques have provided us with the physiological and biochemical mechanisms that control pollen germination and pollen tube growth. Storage of pollen grains causes loss of viability and therefore their ability to germinate and establishes successful seed-set. Cross-checking the viability of stored pollen grains becomes essential in case of *in vitro* fertilization in laboratory.

Tetrazolium test is the most commonly used way of testing the pollen viability (Stanley and Linskens, 1974). It is based on the dehydrogenase enzymes (present in pollen grains cytoplasm) capability to reduce the colourless tetrazolium salt to red-coloured insoluble formazen. If tetrazolium incubated pollen grains take up red colour, they can be scored as viable. However, this result is often confusing, i.e., tetrazolium test result may not correlate with the *in vitro* germination test or *in vivo* seed-set data (Barrow, 1983; Heslop-Harrison *et al.*, 1984). Therefore, in such a scenario *in vitro* pollen germination test becomes the most acceptable way of assessing the pollen viability (Heslop-Harrison, 1987; Steer and Steer, 1989; Taylor, 1997). This is rather a rapid and simple test of evaluating the stored pollen viability which shows positive correlation with the seed-set data as well (Akihama *et* al., 1978; Janssen and Hermsen, 1980). There are generally four types of techniques for testing the *in vitro* pollen germination capability, namely: (i) Sitting-Drop Culture, (ii) Surface Culture, (iii) Suspension Culture and (iv) Hanging Drop Culture.

"Surface Culture" is considered as the most convenient method for testing the *in situ* pollen germination. However, "Sitting-Drop Culture" is considered as the simplest and most convenient amongst all these methods. Since, in a short span of time, a large number of cultures can be simultaneously raised.

Pollen grains require specific environmental condition along with certain external factors that favours fasten germination (Boavida & McCormick, 2007; Chebli & Geitmann, 2007).

Temperature and pH forms two of the most essential external factors that are pre-requisite for pollen germination, besides humidity and air pressure (Snope & Ellison, 1963; Linskens, 1964). Calcium, boric acid, magnesium, potassium and sucrose can be considered as the most basic constituents for pollen germination (Mehan & Malik, 1975; Brewbacker & Kwack, 1963; Khan & Perveen, 2006). Pollen normally germinate in receptive stigma of flowering plants in response to moisture and appropriate basic nutrients. *In vitro* condition can be created to mimic the *in vivo* stigmatic situation. *In vitro* pollen germination can be done to know the basic nutrients requirements of pollen for successful germination.

Therefore, to know the basic requirements for successful *in vitro* pollen germination the present experiment has been done to assess the pollen viability of seven economical species of Apocynaceae.

Material and Methods

To evaluate the *in vitro* germination capability of seven genera under the Apocynacean members (species of *Allamanda*, *Alstonia*, *Catharanthus*, *Nerium*, *Plumeria*, *Thevetia* and *Tabernaemontana*), mature pollen grains were collected soon after anther dehiscence to get optimized result. The grains were collected from freshly opened flower buds preferably of same age to obtain uniformity in result, just before anthesis.

Surface sterilization of the mature anthers was done in 10% sodium hypochlorite solution. Pre-hydration of pollen grains was done since pollen grains are essentially in arrested developmental stage therefore high humidity essentially improves germination. *In vitro* germination was assessed using the hanging drop method. Thereafter the grains were transferred to microslides containing culture media in solution (Brewbaker's media, 6% Glucose solution, 4% Calcium Nitrate solution and 3% Boron solution). Pollen density was kept at optimal level to avoid population effect and from getting the nutrients as a limiting factor.

Since, scoring of pollen counts under different cultural treatments could be carried out simultaneously, the pollen tube growth was arrested after desired period of time by adding Formaldehyde: Glacial Acetic Acid: 70% Ethanol= 5:5:90 v/v. Average pollen germination is measured after specified time. 5-10 different fields were selected arbitrarily for observing the overall germination rates under different treatments at 1, 2 and 3 hours respectively. The rate of

pollen germination at 1, 2 and 3 hours are measured and plotted in the form of bar-diagram (Figure 1).

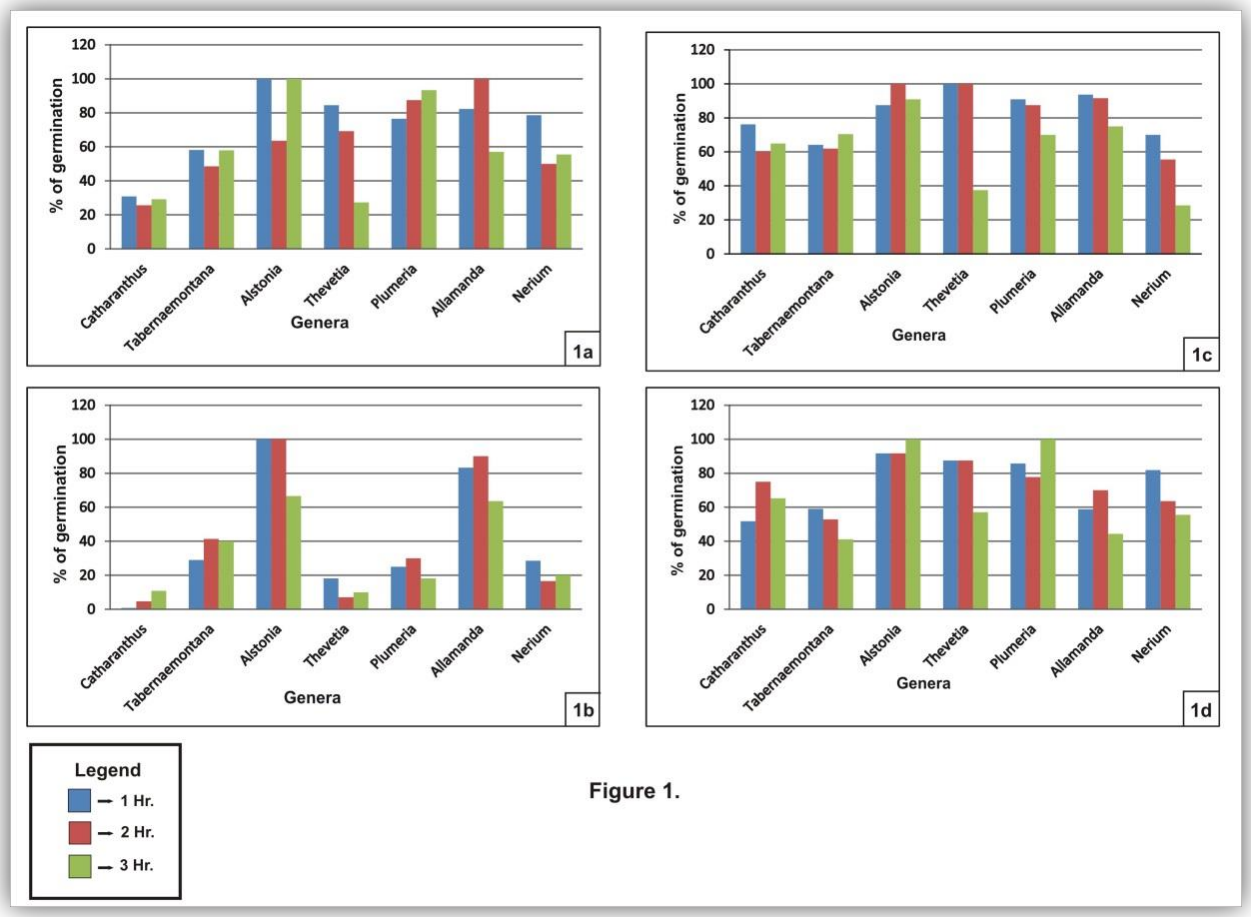

Figure 1.

Figure 1. Graphical representation of germination rate of Apocynaceae species(s) pollen grains after specific time intervals under

(a) 4**%** Calcium nitrate solution
(b) 3% Boron solution
(c) 6 % Glucose solution
(d) Brewbaker's Media

Results and Discussion

Calcium Nitrate concentration is much lesser in pollen compared to vegetative parts and seeds (Ge *et al.*, 2006). Calcium ion (Ca^{2+}) is responsible for proper functioning of K^+ and K^+/Na^+ channels (Akhtar *et al.*, 2013). Calcium gives rigidity to pollen tube wall post germination by binding Pectic Carboxylase group. Presence or absence of Calcium Receptor

Protein (CRP), Calmodulin is found to play role in initiating and inhibiting germination, indicating the direct role of calcium in pollen tube germination (Luan *et al.*, 2002; Yang and Poovaiah 2003). In the present set of test, 4% $Ca(NO_3)_2$ is found to be highly efficient in the initiation of germination of all the Apocynacean members. Grains of *Thevetia* shows cent percentage of germination at 1 and 2 hours but it gradually declines to only 37.5% at 3 hour. Least rate of germination is observed in the grains of *Tabernaemontana* and *Nerium* (Figure 1a). Boron solution (H_3BO_3) is found to have stimulatory effect in the germination of *Nymphaea* sp. pollen (Schmucker, 1933). Boron have direct influencer role in the metabolic process, hence nowadays it is very commonly used in different cultural media to catalyse pollen germination rate. However, the present test in Apocynaceaen pollen grains indicates lesser preference for boron at the onset of germination. On a contrary, tree species namely *Alstonia* shows preference for 3% boron solution in the culture media. Grains of *Alstonia* show 100% germination rate at 1 and 2 hours (Figure 1b).

Sugars form the end-products of photosynthesis which acts as building blocks for the respirational (oxidative catabolism) activity. Stigmatic exudates of *Yucca aloifolia* L. is considerably rich in sugars including, glucose, fructose and sucrose (Pellmyr *et al.*, 1996). Glucose and fructose are found in stigmatic fluid of *Oenothera* sp. Hook. (Dickinson and Lawson, 1975). *In vitro* pollen germination experiments have revealed that germination of grain is substantively high if artificial medium is considerably rich in glucose and sucrose. 6% glucose solution has been used in the present experimental set-up. All the grains show considerably high percentage of germination in presence of 6% glucose solution, indicating its vital nature for successful pollen germination. Grains of *Alstonia* show the highest specificity for glucose (Figure 1c).

Brewbaker and Kwack tested 86 species of flowering plants pollen for their germination and tube growth in presence of a medium suitable for these purposes (1963). Brewbaker's medium is a complex medium consisting of sucrose (100% w/v), Boric acid (100 mg/l), $Ca(NO_3)_2$ (300 mg/l), $MgSO_4$ (200 mg/l) and KNO_3 (100 mg/l). It is widely used in the preparation of pollen culture medium. It contains all the basic requirements for successful pollen germination. Apocynacean pollen grains gives positive response to the *in vitro* test in presence of Brewbaker's medium. The pollen of *Alstonia* exhibits the highest level of specificity for Brewbaker's media (Figure 1d).

Conclusion

Pollen, the haploid male gametophytic cell can be utilised in generating haploid plants and therefore in maintaining monohybrid lines. *In vitro* pollen germination test can be utilised to know the exact count of viable pollen. It is relatively easier and cheaper method for assessing the same.

The present study was done to assess the *in vitro* pollen germination test of Apocynacean pollen grains. From the foregoing study it is firmly established that compound media namely, Brewbaker's media is a better substitute over the simple media. Glucose and Calcium Nitrate solution are essential and ubiquitously required for the successful pollen germination of all the genera studied.

Knowing *in vitro* pollen germination of Apocynacean members can easily be adapted for use in the field by breeders and gardeners who are interested in improving the pollination potential and thereby increasing the vigour of existing lineages of species belonging to Apocynaceae.

Acknowledgement

This research paper is made possible through the help and support from everyone, including: parents, teachers, family, friends, and in essence, all sentient beings. The author expresses sincere appreciation to the unanimous reviewer for his/her critical remarks that helped in improving the quality of paper.

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