MORPHOLOGY AND VIABILITY OF POLLEN GRAINS FROM *GOSSYPIUM* VARIETIES

A.A. Sangole[1] and J.A. Tidke[2]
anjalisangolc05@gmail.com
[1]Department of Botany, Shri. R.L.T. College Science, Akola, Maharashtra, India
[2]Laboratory of Reproductive Biology of Angiosperms, Department of Botany, Sant Gadge Baba, Amravati University, Amravati, Maharashtra India

ABSTRACT

The characterization and viability of pollen grains are useful tools in breeding programs. The objective of this study was to describe the morphological patterns and viability of pollen grain from selected varieties of Gossypium spp. Pollen morphological observation were made using light and scanning electron microscope, where as the viability was performed by in vitro germination and histochemical analysis(2,3,5 triphenyltetrazolium chloride). In-vitro pollen germination was carried out in different media such as sucrose, boric acid, calcium nitrate and magnesium sulphate with 10%, 20%, 30%, 40%, and 50% concentrations. Selected varieties shows the same shape , type and no alteration in ornamentation pattern. In vitro pollen germination shows that maximum in sucrose solution of 30% and 40% concentration in all varieties. The histochemical analysis overestimated pollen viability when compared with the in vitro results. The results is investigated that breeding of Gossypium varieties by increasing to understanding of their morphology and pollen viability.

Keywords: *in vitro germination, histochemistry, scanning electron microscopy, Gossypium varieties , pollen tube.*

Introduction

The genus *Gossypium*, to which cotton belongs, contains a number of species. Some authorities include many different varieties and forms under a few species, though others draw the species lines closer. For proper classification of the genus *Gossypium*, the difference and similarities between the several species comprising the genus must be recognized. The study of form and structure of cotton plants, therefore, is of fundamental importance (Balls, 1919; Brown, 1938; Hayward, 1938 and Hector, 1936). In cotton there are approximately 10,000 pollen grains in a flower. The mature pollen is three nucleate (Cannon, 1903; Balls, 1905; Denham, 1924 and Beal, 1928). The pollen grain of upland (4n) and Indian (2n) varieties are the smallest. The morphological characters of pollen grains or spores are embodies in the exine and are important criteria in consideration of the taxonomy and inter-relationships of plants at various taxonomic levels. Moreover, knowledge of the exine morphology of various sporomorphs is of primary importance. Pollen grains and spores are reproductive propagules of diagnostic value of virtue of the characters embodies in the exine (Erdtman, 1952). The pollen grain can also be useful tool in evolutionary consideration has been demonstrated by Wodehouse (1935).

Pollen histochemical analysis are carried out for the following reasons i) possible relation between the pollen content and the mode of pollination ii) study of pollinator foraging behavior, nutritional demands, pollination mode, pollen content and iii) composition in relation to phylogeny (Dafni, 1992). Lipids and starch are important constituents of the pollen grains to establish the relations with flower foragers.

Viability means ability to live; but pollen viability is the ability of pollen to complete post-pollination events and to effect fertilization. In the old literature the terms pollen viability and pollen sterility were used interchangeably (Shivanna and Rangaswamy, 1992). As viability refers to the ability of pollen to deliver functional gametes to the embryo sac, the most authentic test to viability would be to assess the fertilization capacity of the pollen as measured by fruit and seed set following controlled pollination (Heslop-Harrison and Shivanna 1984 and Shivanna and Rangaswamy, 1992).

Number of pollen grains viable to germinate at the time of germination after their deposition on stigma is an important event in the process

of fertilization leading to formation of fruits and seeds. Germination of pollen is the first morphogenetic event in fulfilling its function of transport and discharge of sperm cells into embryo sac. pollen grains of a large number of species readily germinate *in-vitro* on a simple medium, *in-vitro* germination has been extensively used in studies on structural and physiological details of germination and tube growth. Two celled pollen grains in general are more amenable to *in-vitro* germination as compared to 3-celled pollen. Number of methods have been used for *in-vitro* germination and have been comprehensively described by Shivanna and Rangaswamy (1992). Details of the processes involved in pollen germination and tube growth are of paramount importance. In *in-vitro* germination of pollen is the most frequently used method for checking pollen viability (Visser, 1955).

Studies of pollen viability and morphology are of high importance in relation to genetic breeding programmes, aimed at attaining potentially promising selections. Pollen viability is a male fertility measure widely used in the monitoring of stored pollen, aimed at ensuring fertility and achieving cross-fertilization between genotypes flowering during different periods (Oliveira *et al.* 2001). Determination of pollen viability can occur through the use of direct methods such as the inducement of *in vitro* germination (Acar & Kakani 2010; Alcaraz *et al.* 2011; Sorkheh *et al.* 2011) and *in vivo* germination (Fakhim *et al.* 2011) or other, indirect methods based on cytological parameters, such as pollen staining (Beyhan & Serdar 2008; Abdelgadir *et al.* 2012). However, *in vitro* germination of pollen is the most utilised method for viability testing and for genetic breeding programmes (Satish & Ravikumar 2010).

Material and Methods

For the pollen morphological studies pollen material was collected from matured flowers after anther dehiscence immediately by using the forceps. The collected material was kept in suitable paper packets or glass vials. Pollen grains from matured flowers were also collected by removing the standard and petals and the pollen mass was unload in to the suitable vials.

To study the pollen morphology of selected cotton varieties, pollen samples were collected from matured but undehisced anthers. The pollen samples were collected in 70% alcohol and for the further morphological studies acetolysis method was followed (Erdtman, 1952 and Nair, 1960).

Pollen samples were immersed in to IKI solution and examined under the microscope for the change in colour. Dark bluish-black color indicates the presence of starch. For the estimation of lipids pollen sample were kept in freshly prepared stock solution of Sudan IV and treated pollen sample was observed under microscope within 2-3 minute to note the change in colour. A red color indicates the presence of lipid (Baker and Baker, 1983).

To carry the pollen viability test, the pollen grains were collected in sterilized petridishes at the time of anthesis. 10% tetrazolium salt solution was prepared and added to 60% sucrose solution in the ratio of 1:5 at the time of preparation of slides for observations

For *in-vitro* pollen germination dehisced anthers from matured flowers of selected cotton varieties were collected. *In-vitro* pollen germination was carried out in different media such as sucrose, boric acid, calcium nitrate and magnesium sulphate with 10%, 20%, 30%, 40%, and 50% concentrations. The micro slides of pollen grains prepared in different concentrations were observed

Observation

Acetolysed and unacetolysed pollen grain studied showed any alterations in morphological characters. Except the varieties DHY 186, PA-348, LRA-5166, Ankur-216 and Banni- 145 all varieties showed no alterations in morphological characters.

From the histochemical tests it was noted that the pollen grains of all cotton varieties contains starch and lipids (Table No.03).

Pollen viability percentage of cotton varieties was found to be 98.4% and 95.7% in NHH-44, 99.0% and 94.2% in Ankur-651 , 98.4% and 95.9% in AKH-081, 98.1% and 97.8% in DHY-186, 98.4% and 93.8% in PA-348, 98.9% and 93.2% in Renuka-143, 98.0% and 95.9% in H-10, 96.0% and 97.2% in PKV-hy-2, 99.3% and 96.3% in H-8, 95.1% and 98.2% in NHH-52, 98.7% and 95.8% in BT-162, 98.3%and

97.7% in LRA-5166, 98.6% and 98.2% in Kaveri Kurnel ,98.4% and 95.8% in Ankur-216 ,97.8% and 97.5% in Banni- 145 and 98.8% and 96.6% in Ajeet-11 during the year 2003 and 2005. Pollen viability percentage was found to be maximum in H-8 and Ankur –651; however, it was minimum in Renuka-143 and PA-348.

In vitro pollen germination was found to be maximum in sucrose solution of 30% and 40% concentration in all varieties. It was found to be 16.1% and 19.3% in NHH-44, 13.8% and 20.7% in Ankur-651, 17.5% and 24.2% in AKH-081, 11.9% and 11.4% in DHY-186, 10.7% and 16.9% in PA-348, 24.4% and 19.2% in Renuka-143, 5.0% and 9.8% in H-10 , 23.1% and 14.5% in PKV-hy-2 , 19.5% and 27.8% in H-8, 12.6% and 20.5% in NHH-52 , 23.2% and 23.6% in BT-162 ,20.2% and 27% in LRA-5166 ,14.2% and 25.2% in Kaveri Kurnel , 20% and 22% in Ankur-216, 11.1% and 17.0% in Banni- 145 ,and 9% and 15.7% in Ajeet-11

The percentage of pollen germination in different concentrations of Potassium Nitrate, Boric Acid, Calcium Nitrate and Magnesium Sulphate was found to be very less or nil.

Result and discussion

Pollen grains are very conservative organ, facilitating the identification of plants at various taxonomic levels. Acetolysed and unacetolysed pollen grain studied during the year 2003 and 2005 hardly showed any alterations in morphological characters. Except the verities DHY 186,PA-348, Renuka-143, BT-162, LRA-5166 Kaveri-Kurnel, Ankur-216 and Banni- 145 all varieties showed no alterations in morphological characters.

From the histochemical tests it was noted that the pollen grains of all cotton varieties contains starch and lipids. Pollen histochemistry is possibly related to pollination mode, pollinator foraging behavior and phylogeny. The nutritive value of pollen also influences the behaviour of flower visitors

Pollen viability is considered as an important parameter of pollen quality. Environmental factors, particularly temperature and humidity greatly affect the pollen viability. From the observations, the percentage of viability was found to be maximum in H-8 (99.3%) and Ankur –651 (99.0%). It was minimum in Renuka-143; (93.2%) and PA-348; (93.8%). The data obtained during this investigation will be helpful for breeding programme of selected cotton varieties.

In vitro pollen germination was found to be maximum in sucrose solution of 30% and 40% concentration in all varieties. Cotton pollen has proved to be recalcitrant to traditional *in vitro* germination and pollen tube growth protocols.

The percentage of pollen germination in different concentrations of Potassium Nitrate, Boric Acid, Calcium Nitrate and Magnesium Sulphate was found to be very less or nil. An assessment of the role of these stimulants for *in vitro* pollen germination showed meager response.

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