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In vitro Germination of Cotton Pollen Grains Using two Different Approaches

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Abstract: Gene overlap between the sporophytic and gametophytic phases and successful *in vitro* germination of cotton pollen grains have motivated plant breeders that if more reliable and consistent protocols of *in vitro* pollen germination become available, they could impose certain stresses like heat, salinity and others and selection could be made accordingly in controlled laboratory conditions instead of time consuming and expensive field conditions. Our studies were aimed to utilize two methods of *in vitro* germination in an artificial medium. One was a droplet method and another was cellophane paper method. The difference in percent of pollen germination and pollen tube length were obvious where droplet method gave higher % of pollen tubes also longer tube length as compared to cellophane paper method. The pollen busting in droplet was also very high and very uncommon on cellophane paper. These results thus suggest that when both the methods are made consistent and reliable, the percent of pollen germination and tube length may be used as criterion of selection for different stress conditions.

Key words: Cotton pollen grains, *in vitro* germination, Germination approaches

Introduction

Emphasis is being continued to explore the possible use of pollen selection as tool in plant breeding programmes. However, the basic question in this respect arises as to what extent the genes expressed at sporophytic phases are correlated with the genes expressed in the gametophytic phases. These types of questions were supported with the studio', mainly on the basis of correlation at isozymes and RNA hybridization levels. Tanksley *et al*. (1981) explored the evidence of gene overlap between gametophytic and sporophytic cycles in Tomato (*Lycopersican esculentum* L.) by comparing activity of nine enzymes. They found that about 82% of the isozymes have expressed in the pollen grains and 58% in both the phases, whereas 3% were specific to only pollen grains (gametophyte Phase). Further studies still supported the gene overlaps, such as 72% in *ha mays* (Sari Gorla *et al*., 1986), 81% in *prunus* (Weeden, 1986). Since, overlap between the genetic expression of both the phases has now been well established, the application of gametophytic selection in plant breeding programmes has yet remained uncommon, specially in cotton where *in vitro* germination of pollen grain. is extremely difficult and inconsistent (Banerji, 1929). However, Bronkers (1961) reported an excellent germination % and later embryologists have been even more successful in cotton pollen germination *in vitro* (Barrow, 1981). *In vitro* germination of cotton pollen grains still poses lot of difficulties, that is inconsistency in germination percent and tube length which are assumed to be used as the criterion of pollen selection. Literature also is still lacking to actually demonstrate the real pollen tubes and Its differentiation from the pollen bursts. This study was therefore aimed to demonstrate two procedures of germinating cotton pollen grains *in vitro*. One is droplet of medium placed on microscope glass slides and another is cellophane paper moistened with the medium. If we are fairly consistent in *in vitro* pollen germination, studious on the external effects like heat, salinity, herbicide, insecticides and tolerance to other chemical compounds may be based on pollen selection in the laboratory instead at plant level in un-controlled field conditions.

Materials end Methods

The studies were carried out at Central Cotton Research Institute, Sakrand during 1999 for determining the usefulness of two methods for *in vitro* cotton pollen germination.

In vitro Germination of Cotton Pollen Grains on medium placed on microscope Glass Slides.

A medium containing three different concentrations:

1. $[CaNO_3]_2$ = 140 mg, $MgSO_4.4H_2O$ = 140 mg, H_2BO_3 = 50 mg, sucrose 20%.
2. $[CaNO_3]_2$ = 300 mg, $MgSO_4.4H_2O$ = 140 mg, H_2BO_3 = 50 mg, sucrose 30%.
3. $[CaNO_3]_2$ = 30O mg, $MgSO_4.4H_2O$ = 140 mg, H_2BO_3 = 50 mg, sucrose 40%.

Each concentration was dissolved separately in 100 ml of distilled water.

A drop or two of medium was placed on clean microscope glass slide in the form of circle with camel hair brush. Five plates batch of each concentration were prepared for pollen germination. At about 10.00 a.m., when the flowers dehisced and the anthers burst, the pollen grains from tetrapolid cotton (4x = 52) were shed onto the droplets of medium, scattered with brush so as to assure that pollens were completely saturated with the medium. The distilled water moistened filter papers were placed in the base of Petri plates. Additional drop or two of distill water was also left in the Petri plates, in order to provide germinating pollens a required humidity of about 70% as indidcated by earlier workers. The slides were then carefully (not to shuffle the medium) placed on moistened filter papers in Petri plates, covered half a way, in order to allow the air flow freely. After this preparation, the Petri plates were either kept at $30 \pm 2^{\circ}C$ in incubator or placed in the room if room temperature was around 30°C. The slides were observed under microscope on 10x objective from 10 minutes to 3.0 hours during incubation period. To permanalize the slides, a coverslip was gently placed on the droplets, excessive medium was removed by the piece of filter papers. In order to drain the bursts and dehydrate pollen tubes, 100% alcohol was slowly passed through the edges of coverslip. Care was taken not to drain out the tubes but only the mass of bursts. The edges of coverslip were then sealed with Canada balsam and the slides were allowed to dry-of.

***In vitro* Pollen Grain Germination on Cellophane paper:** Similar concentrations of media as mentioned in procedure one were prepared. Clean filter papers of Petri plate size were cut and placed into Petri dishes and saturated with each concentration of medium. Two to three additional

drops of medium were also left in the Petri plates to assure that filter papers should not dry-up otherwise there was risk that pollen grains itself can dry-of and ultimately will not germinate. About 2 cm^2 pieces of cellophane papers were cut and placed on medium moistened filter papers, half an hour before the pollens could be shed-on gelatin papers. It was assumed that waiting for half an hour before the pollens were shed on cellophane the papers will be saturated with medium through its semi-permeability. These pieces of cellophane papers were arranged in each Petri plate. The dehisced pollen grains were shed onto the cellophane papers. The Petri plates were then covered with their tops and placed in incubator at about $30 \pm 2°C$. A plastic beaker with 500 ml of water was also kept in incubator so as to provide the germinating pollen grains the requried humidity. After about three hours of incubation, the pollen germination was recorded by taking carefully the pieces of cellophane papers with forceps and placed on glass slides. These slides were then observed under microscope on 10x objective for recording the pollen germination. After germination was completed, a drop or two of 0.5% acetocarmine was added on the cellophane papers to stain the pollen tubes.

Results and Discussion

In vitro Pollen Germination in Droplet of Medium Placed on Microscope Glass Slides: The effect of two different concentrations of $[CaNO_3]_2$ and three concentrations of sucrose with equal quantity of $MgSO_4.4H_2O$ and H_3BO_3 on pollen germination was astonishing. Surprisingly, at 20 to 30% of sucrose and 140 mg of $[CaNO_3]_2$, about 100% of pollen have bursted explosively in a minute or two and the mass of cytoplasm was dispersed all over the medium (Fig. 1a). These pollen bursts ware not the real pollen tubes. Luckily in a medium containing 40% sucrose, 300 mg/l $[CaNO_3]_2$, 140 mg $MgSO_4.4H_2O$ and 50 mg H_3BO_3, the actual pollen tubes with symmetrical prominent walls and granular mass of cytoplasm with about 50-98% germination were recorded. Miravalle (1966) used Bronkers (1961) method and obtained 35-73% germination. The tube length in our study ranged from 15.0-20.5 mm (Fig. 1c). The pollen tubes remained attached with the pollen grains as long as they were properly handled. A drop or two of 0.5% acetocarmine was added so as to stabilize the tubes and take colour photographs. While observing our pollen tubes, I became skeptical about the tubes Barrow (1981) has obtained. Those are probably pollen bursts and not the tubes as shown in Fig. 1b. Our keen observations further revealed that sucrose is very important in bringing the pollen gains in osmotic equilibrium or nutritional source. However, $CaNO_3$ and $MgSO_4.4H_2O$ serve as nutritional sources whereas H_3BO_3 stabilizes the germinated tubes (Giles and Prakash, 1987). Though, 40% sucrose and 300 mg $CaNO_3$ seem an appropriate quantity for pollen germination, however we used magnesium instead of manganese as used by Taylor (1972). We also observed that our pollen tubes were quite different from those of Taylors. Miravalle (1966), nevertheless succeeded in obtaining real pollen tubes by using Bronkers (1961) methods. We have also observed in our study that after 23 hours of incubation, pollen tubes do not grow any longer.

***In vitro* Pollen Germination on Cellophane paper:** The percent of pollen germination on cellophane paper was less than the droplet method, may be because of little semi-permeability of medium though cellophane paper. Thus, may not have allowed sufficient quantity of medium to the pollen to grow into tubes. However, fewer pollen tubes if germinated, they were not as straight as in droplet method (Fig. 1d). The possible explanation for curved pollen tubes

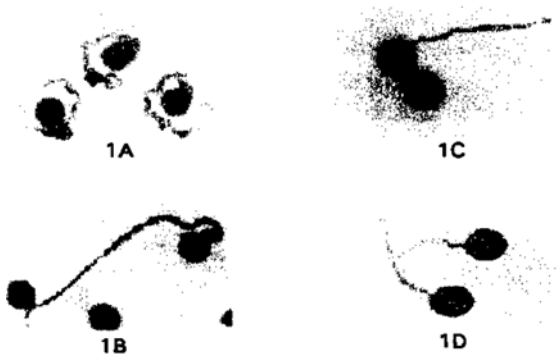

Fig. 1a: Pollen bursts in circular shape 1b: Pollen burst in tube like structure 1c: Pollen tube grown by droplet method 1d: Pollen tubes grown on cellophane paper

of cellophane method may be due to interference caused by the surface of cellophane method on germinated pollen tubes as compared to droplet method. There was no osmotic equilibrium problem with cellophane paper method in any of the three concentrations. It is because this method did not completely saturate the pollen grains. However, 30 to 40% sucrose and 300 mg $[CaNO_3]_2$ seemed appropriate quantities for cotton pollen grain germination as suggested by Taylor (1972), used by Barrow (1981) whose pollen tubes are very different to ours. However, Miravalle (1966) succeeded in germinating real *in vitro* cotton pollen tubes by using Bronkers (1961) method. It was also observed that after 3 hours of incubation the pollen tubes did not grow any longer. No double pollen tubes were recorded in celcophane paper method whereas double and triple tubes were very common in droplet method.

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