DOI : 10.5958/0974-0112.2018.00041.5

Indian J. Hort. 75(2), June 2018: 237-244

Development of pollen germination medium to test pollen viability of eggplant and its wild species

P. Jayaprakash*, Sheeba D, Vikas, V.K., Sivasamy. M, and T. Sabesan**
ICAR-Indian Agricultural Research Institute, Regional Station, Wellington 643 231, Tamil Nadu

ABSTRACT

In this study, we report *in vitro* pollen germination medium (PGM) for the egg plant and four of its wild species belonging to tertiary gene pool *viz*., *Solanum torvum*, *S. khasianum*, *S. trilobatum* and *S. surrettence*. The medium developed by Brewbaker and Kwack has been modified and improved with addition of poly ethylene glycol, ε-amino caproic acid etc which supported over 78 % pollen germination and pollen tube growth of these species. The complete pollen germination medium for eggplant consists of 20 % maltose + 250 mg l^{-1} Boric acid + 300 mg l^{-1} Calcium nitrate + 15% PEG 4000 + 750 mg l^{-1} EACA + 0.5-1% agar at 25 ºC température for 3h of incubation. During the study, pollen sterility of 8-12% was observed at flowering period invariably in all *Solanum* species.

Key words: Brinjal, *S. melongena*, wild species, *in vitro* pollen germination.

INTRODUCTION

Eggplant (*Solanum melongena*.L) is an important vegetable crop of significant economic importance and its fruits are consumed worldwide. It is believed to have originated in warmer region of India and China .The demand for this vegetable has increased owing to its use in diverse culinary preparations. The F_1 hybrids have been preferred well than the varieties because they exhibit heterosis for various agronomic traits including resistance to pests and disease. European catalogue of registered varieties have >75% of eggplant varieties of F_1 hybrids and the well developed vegetable industry of Japan , Netherland, the USA and Canada have > 90% varieties of hybrid origin .In eggplant, commercial hybrids are produced by hand emasculation and pollination which results in higher seed cost. One of the strategy to reduce seed cost is to use male sterile lines as female parent which require only pollination thereby avoiding emasculation. A functional cytoplasmic male sterility (CMS) has been reported in eggplant. Here, fresh or stored pollen can be used for hybrid seed production. Pollen grain which carries male genetic material plays important role in compatible pollination and fruit set. The germination rate of pollen grains and rate of pollen tube growth determines the pollen vigor.

In vitro pollen germination is the most widely used technique for testing the viability of pollen grains in breeding programs. This valuable tool also addresses basic questions in sexual reproduction such as pollen preservation, pollen selection, pollen transformation, etc. The media used for *in vitro* pollen germination of different species range from simple sucrose/boric acid media to complex media containing polyethylene glycol), EACA and various amino acids etc Brewbaker and Kwack (1) have developed a pollen germination medium which was found suitable for more than 86 plant species.

Earlier attempts to germinate eggplant pollen in nutrient medium were not successful. The highest *in vitro* pollen germination of 10.8 % and 66 % *in vivo* pollen germination was reported by Franca *et al*. (2). Standardization of *in vitro* pollen germination was earlier attempted by Khan and Perveen (8) and Guler *et al.* (5). However, the pollen germination achieved by them was less than 50 per cent and it was also mentioned that even the germinated pollen burst. These reports clearly indicated that there is no reliable *in vitro* pollen germination protocol to test the viability of eggplant available. The requirements for pollen germination under *in vitro* condition would reveal information about the different constituents, temperature etc. which in turn may reflect the nutrient status of the stigmatic surface of respective species. With this rationale, this study was conducted to standardize in *vitro* pollen germination medium for *Solanum melongena and* its wild species (*S. torvum*, *S. khasianum, S. trilobatum* and *S. surretance).* These wild species are important source for shoot borer resistance. Having this rationale, both staining and *in vitro* pollen germination for eggplant and its wild species have been standardized.

*Corresponding author's E-mail: jpsarit@gmail.com
**Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu

MATERIALS AND METHODS

The study was conducted at Indian Agricultural Research Institute (IARI) Regional Station, Wellington, Tamil Nadu, India during 2012-2016. Seeds of Annamalai variety eggplant was collected from Annamalai University, were grown at temperature range of 15 to 25ºC and pollen was collected from freshly opened flowers.

The eggplant pollen viability is tested by Fluorochromatic Reaction (FCR) .The fluorescein diacetate (FDA) (2mg/ml) dissolved in acetone solution was used. Using above pollen extracting method, pollen grains were mounted on FDA solution. The bright green or yellowish green fluorescence pollen grains are viable and non viable grains fail to fluoresce brightly. Pollen viability was tested at weekly interval during flowering period. Pollen grains were collected from freshly opened flower in the morning at 9.00AM. Since the eggplant pollens are sticky, it is treated with few drops of hexane solution and allowed for drying on slide. This dried pollen is handled carefully using needle and gently tapped on the medium for their germination studies. To standardize pollen germination medium, different kinds of media were prepared with different compositions including diverse level of maltose, polyethylene glycol (PEG) 4000, boric acid, calcium nitrate, EACA etc. These experiments were performed in complete randomized design with five replications.

In vitro pollen germination medium(PGM)was prepared following Brewbaker and Kwack (1). In this study, maltose was used as osmoticum instead of sucrose. In a preliminary investigation, media having BK salts at different concentrations of maltose (18, 19, 20, 21 and 22%) and/or polyethylene glycol 4000 (13, 14, 15, 16 and 17%) were screened and the medium with appropriate constituents for perfect osmoticum. Medium was made in Falcon tube consists 20% maltose, 15% of PEG 4000, 50 to 150 mgl^{-1} of Boric acid and 350 to 450 mgl^{-1} of Calcium nitrate. After confirming the exact osmoticum (20%) and PEG 4000 (15%) concentration, the combined effect of boric acid (50, 75, 100, 125 and 150 mgl^{-1}) and calcium nitrate (200, 300, 400, 500 and 600 mgl^{-1}) at different concentration were studied. The medium selected from the above was modified with EACA (200, 225, 250, 275 and 300 mgl^{-1}) and tryptone (30,40,50,60,70 mgl^{-1}) to fine tune pollen tube growth. The medium was placed on the petriplate using PGM droplet technique (Jayaprakash *et al*., 7)

Effect of pH (4, 4.5, 5, 5.5, 6, 6.5, 7 and 7.5) and temperature (21,22, 23, 24,25.26,27,28,29 and 30ºC) on eggplant pollen germination was also tested. The pH of the PGM was adjusted using 1N of HCL and 0.1 N of NaOH and measured using digital pH meter. The temperature was varied in BOD incubator to see its effect upon pollen germination. Stigma position variability was observed within each inflorescence. Four types of stigma arrangement explained by Pradeepa (12) were tested for germination percentage. Flower with stigma below, flower with stigma on the same level, flower with stigma above and flower with stigma much below anther tip were studied. Pollen was extracted from each flower type and pollen germination was calculated.

Fresh pollen grains from eggplant were shed on selected final medium to study its morphology. Eggplant pollens were shed on the medium and kept for incubation. After three and half hours, slide were viewed for germination percentage and fixed using acetic alcohol fixative (glacial acetic acid: ethanol, 1:3 v/v) and viewed under SEM(FEI-Quanta 250). Fixed pollens were mounted on one side of the double sided adhesive carbon conducting tape, and then mounted on the 8mm diameter aluminum stub and viewed (Shyla and Natarajan, 13). Sample surface were observed at different magnification and the images were recorded. SEM operated at an accelerating voltage of 5KV.

Slides were examined with Olympus India fluorescence microscope. Germinated pollen was counted using random field in order to avoid duplication. A total of 200-250 pollens were scored from 5-6 microscopic fields. The average pollen tube length was measured from samples of at least 50-60 pollen grains for each treatment. The pollen having pollen tube length more than its diameter is scored as germinated. The pollen tube length was measured using an ocular micrometer calibrated with stage micrometer 0.01mm.

RESULTS AND DISCUSSION

Currently, the demand for eggplant (*Solanum melongena* L.) in the world has increased and it has some properties that reduce the level of cholesterol. In past, people maintained varieties of their region and nowadays the hybrids of eggplant were preferred than open-pollinated cultivars because of its yield and diseases resistance. Furthermore, the production of hybrid seeds is facilitated by the size of the flower in this species (França *et al*., 2). The eggplant is important vegetable in India and the demand for this vegetable has increased owing to its diverse use. This vegetable comes in different shape, color and size which can be used in various of culinary preparations. Even in varieties, low fruit set was observed (62%) (Suganiya *et al*., 14). It is imperative that the quality of pollen determines the fruit set percentage in hybrid eggplant seed production.

Though the fruit set reflects the quality of pollen used, there are many quick and easy techniques to assess the viability of pollen reported . FDA flurochrome clearly differentiated the viable and non-viable pollen. The viable pollen fluoresces in dark green colour whereas the non-viable pollens were shriveled and did not stain (Fig. 1). It was invariably observed that sterility of 8-12% was seen during crop season over years. Among the various staining techniques, the FDA staining was found to be the reliable indicator of pollen viability The *in vitro* pollen germination was considered as the best technique to assess pollen viability of any species .

Sucrose (10-30%) in the initial medium gave inconsistent results with less than 10% pollen germination and bursting (*data not shown*) and the medium was subsequently substituted with maltose. Based on pollen bursting, initially a medium with 20 % maltose +100 mgl^{-1} boric acid + 14 % PEG 4000+ 300 mgl^{-1} calcium nitrate + 100 mgl^{-1} magnesium sulfate + 200 mgl^{-1} potassium nitrate was selected. Among different maltose concentrations (18, 19,….22 per cent) tried, pollen germination was highest at 20% maltose with 47.56% pollen germination with 670 µm after 12h (Fig. 2a). There was reduction in pollen germination upto 22% in maltose concentration just above or below 20 per cent. Hence, in further treatments 20% maltose was maintained.

Addition of PEG 4000 reduced the pollen busting and stabilized eggplant pollen germination. Among the PEG 4000 concentration(13,14,15, 16 and 17%) tested, pollen germination was maximum(66.9%) at 15% concentration with mean pollen tube length (PTL) of 794 µm followed by 14% PEG with pollen germination of 62.5% and 600 µm PTL (Fig. 2a).

To culture pollen, different media ranging from simple sucrose media to complex ones containing PEG, amino acids, vitamins etc have been reported (Jayaprakash *et al*, 7).

In eggplant, Guler *et al*. (4) used agarified medium containing 12% sucrose, 300 ppm H_3BO_3 and 300 ppm calcium nitrate and reported high amount of bursting. Khan and Perveen (8) tested the viability of stored pollen in Brewbaker and Kwack (1) medium and concluded that pollen stored at -20 to-30 ºC showed better germination than fresh or pollen stored at 4 ºC. In both the reports, there has been no clear cut mention on pollen germination rate, pollen tube length, pollen and pollen tube bursting etc. Later, França *et al* (2) cultured eggplant pollen in Brewbaker and Kwack medium and observed a maximum of 10.8% pollen germination at 7.5gl^{-1} sucrose concentration. They emphasized the necessity for more studies concerning the development of a more suitable culture medium to test the viability of *in vitro* eggplant pollen grains. Also stated that, Brewbaker and Kwack medium might be suited for

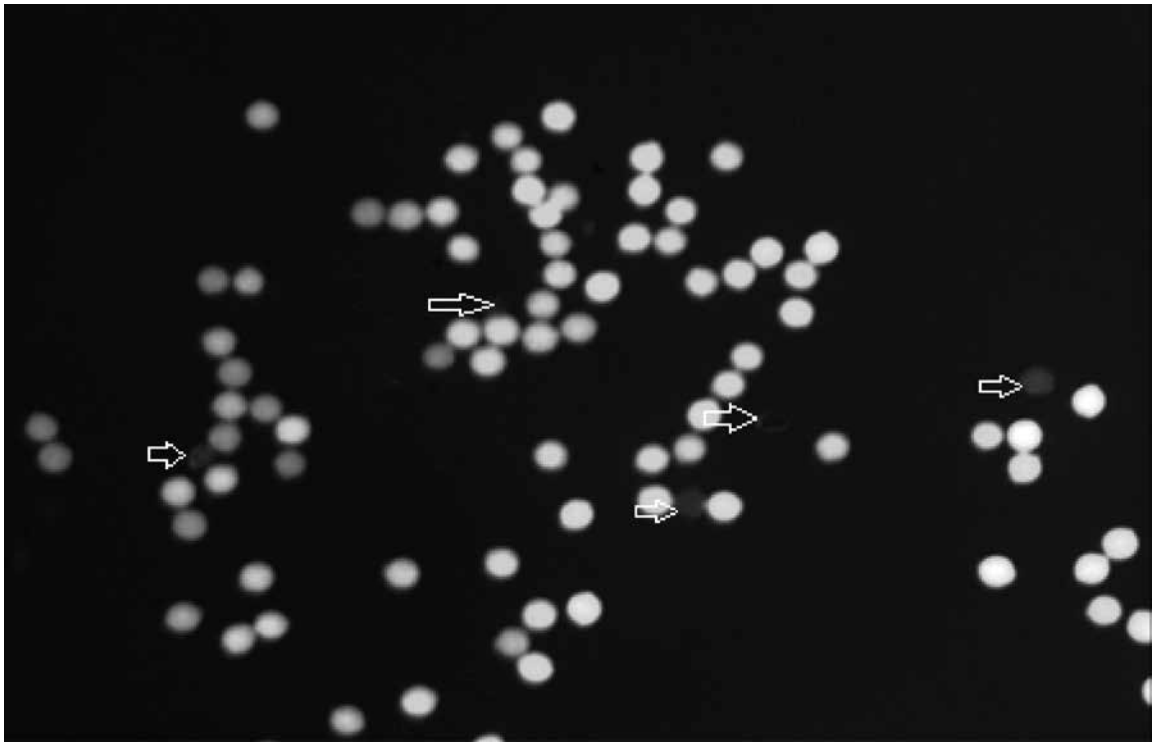

Fig. 1. Eggplant pollen : FDA staining showing viable pollen stained darkly (*Arrows indicate sterile pollen*).

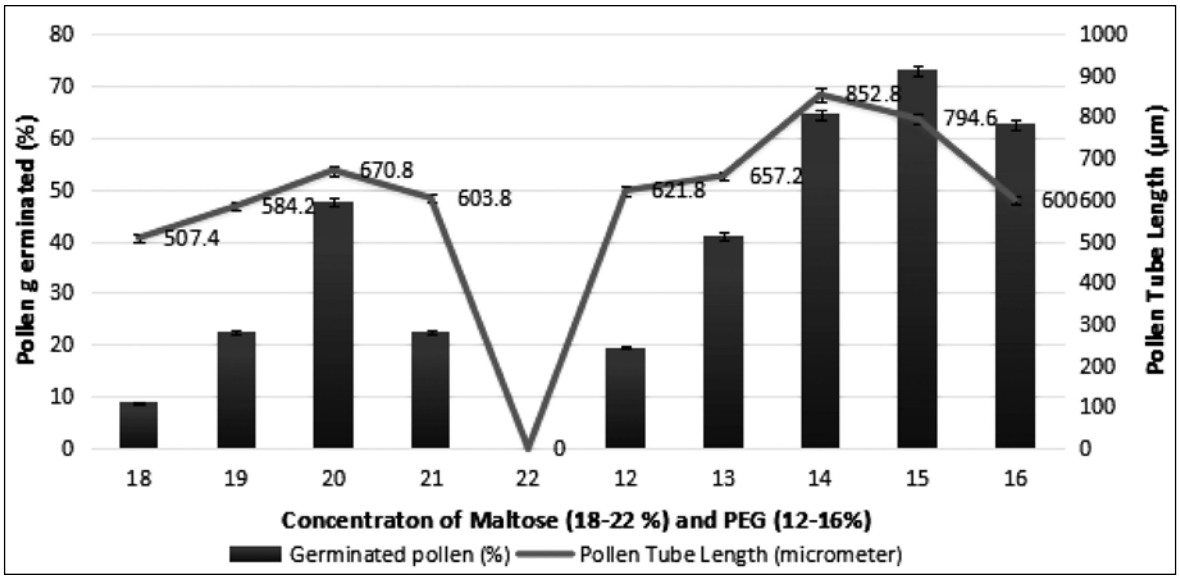

Fig. 2a. Effect of Maltose and Poly ethylene Glycol on eggplant pollen germination.

tomato but not for eggplant. In our study, replacing sucrose with maltose in BK medium did the work.

In this study, maltose at 20% supported 47.56% pollen germination initially and with other media constituents it enhanced pollen germination upto 78%. Brewbaker and Kwack (1) reported a pollen germination medium containing sucrose which was found to be suitable for more than 83 species. There were few instances where carbon source other than sucrose has been used as part of PGM. Maltose was earlier preferred as better carbon source in PGM than sucrose for *Eucalyptus marginata*. Maltose seems to be better osmoticum as it has also supported pollen germination of wheat and rye (Jayaprakash*et al*., 7). Addition of PEG to pollen germination medium reduced pollen tube bursting and stabilized the pollen germination in many species (Jayaprakash *et al*., 7). However the molecular weight of PEG differs with species. PEG 4000 at 15% concentration increased the eggplant pollen germination from 47.5% to 66.9%.

Sucrose maintains osmotic potential of medium in increased concentration (i.e. above 12%) at the same time increases the permeability and damage the membrane. Hence maltose along with PEG 4000 was used as osmotium which reduces penetration and plasmolysis of membrane (Leduc *et al*., 11). Large Molecular size PEG bind water and allow slow uptake by membrane which regulate its permeability. PEG even when penetrating the extracellular space and circulating through the apoplast, it has no recognized effect on the metabolism of the cell.

When boric acid levels was tested, the highest pollen germination (PG) of 71.73% with 810.47 µm PTL was observed at 100mgl^{-1} concentration followed by boric acid at 125 mgl^{-1} (60.99PG: 624.3 µm PTL). Keeping maltose at 20%, PEG 15% and boric acid 100mgl^{-1}, the level of calcium nitrate was varied (200, 225, 250, 275, 300mgl^{-1}). The effective concentration of 400 mgl^{-1} calcium nitrate encouraged 72.3% pollen germination with 827 µm PTL (Fig. 2b)

Role of boric acid and calcium nitrate *in vitro* pollen germination has been well established. Eggplant required 100 mg/l of boric acid and elevated level of calcium nitrate for pollen germination (400mg/l). In eggplant, application of boric acid at 150mg/l increased flower number, flower set and fruit set (Pradeepa, 12). Usually addition of minerals, starts pollen tube growth and each mineral concentration maintain the cytoplasm content and allowed gradually through the pollen tube (Helper, 6).

Inclusion of EACA at 250mg/l to the medium (Maltose 20% +15% PEG 4000 + 100mg/l boric acid + 400 mg/l calcium nitrate + BK salts+ 1% agar) enhanced the pollen germination from 66.91% to 75.61%. EACA is an immunosuppressor and was first used in PGM. It helped to establish complete PGM for pigeonpea. Similarly, EACA was one of component of wheat PGM (Jayaprakash *et al*., 7). EACA might have played an important role in pollen germination by increasing lipid availability by membrane solubilization.

Among the EACA levels tested (200, 225, 250, 275, 300 mgl^{-1}), 250 mgl^{-1} concentration supported 75.6% with mean pollen tube length 660.4 µm. Beyond this concentration a reduction in pollen germination and pollen tube length was observed.

To improve pollen germination further, different

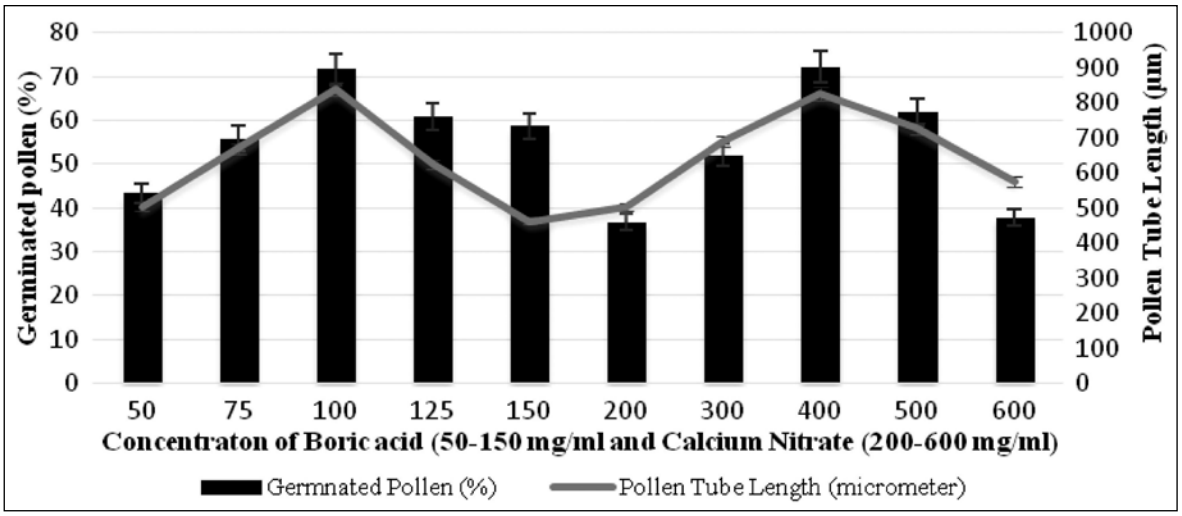

Fig. 2b. Effect of boric acid and calcium nitrate on *in vitro* pollen germination of eggplant.

level of tryptone (30,40,50…70mgl^{-1}) was added to the medium consisting of maltose at 20%, PEG 15% and boric acid 100 mgl^{-1},BK salts, 400 mgl^{-1}calcium nitrate and 250 mgl^{-1} EACA. Tryptone concentration of 50 mgl^{-1} showed maximum pollen germination (78.1%) with 602.1 µm pollen tube length (Fig. 2c). Pollen germination in final medium with smooth pollen tube was shown in Fig. 3a & b.

Addition of organic nitrogen source (peptone) in pollen germination medium showed increased pollen germination in wheat. In eggplant PGM, tryptone in lower concentration (50 mg l^{-1}) showed little increase in pollen germination. Along with EACA, tryptone enhanced the pollen germination. So, the selected medium (Maltose 20% +15% PEG 4000 + 100mg/l boric acid + 400 mg/l calcium nitrate + EACA 250mg/l + T50 mg/l + 1% agar) was assumed to give same compatibility between the pollen grains and specialized sporophytic surface of stigma. Similarly, PGM has been standardized for four wild *Solanaum* species viz.. *Solanum torvum*, *S. khasianum, S. trilobatum* and *S. surrettence.*

Among temperature tested (21, 22, 23, 24, 25, 26, 27, 28, 29, 30ºC), maximum pollen germination of 76.56% with 624.67 µm pollen tube length was observed at 25ºC. Temperatures either above or below 25ºC reduced both the pollen germination and rate of pollen tube growth. Similarly, maximum pollen germination of 75.47% with 586.98 µm mean pollen tube length was observed.

All medium are incubated at 25ºC and pH 6 throughout the pollen germination studies. Initially,

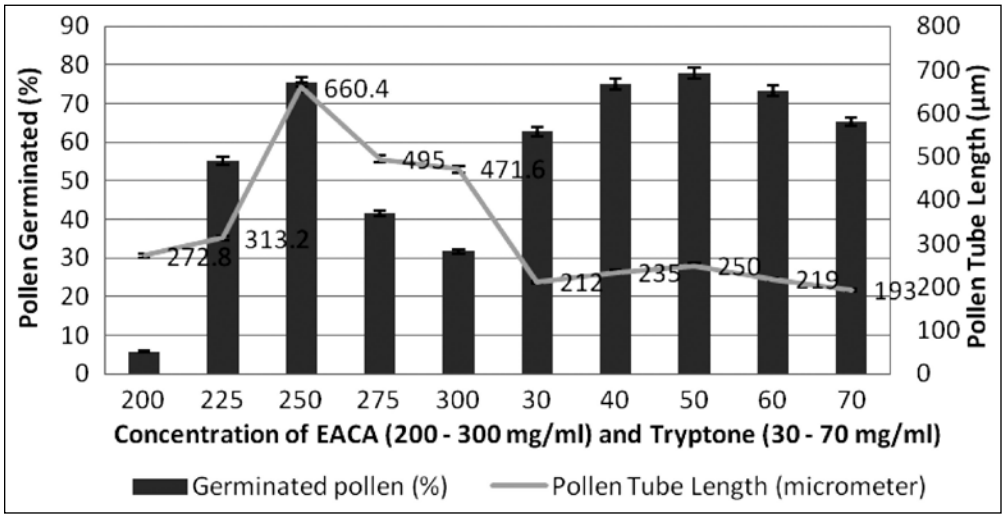

Fig. 2c. Effect of ε-amino carproic acid and Tryptone on pollen germination *in vitro*.

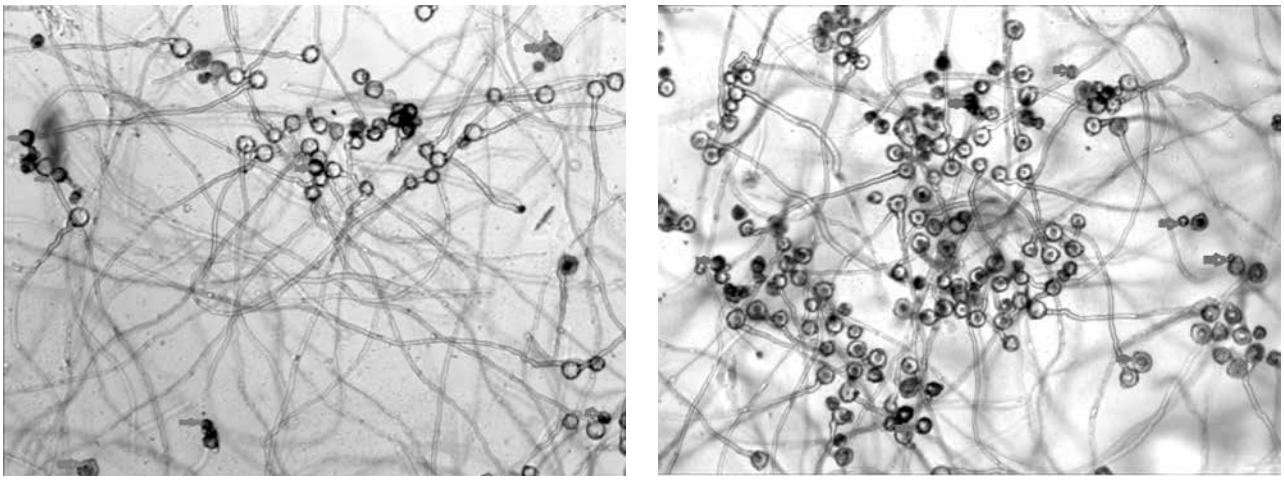

Fig. 3. Photomicrograph showing the pollen germination and smooth intact pollen tube growth of variety eggplant pollen in medium (Maltose 20% +15% PEG 4000 + 100mg/l boric acid + 400 mg/l calcium nitrate + EACA 250mg/l + T50 mg/l + 1% agar) (A&B) shows the smooth pollen tubes and ungerminated pollen (*Arrows indicate sterile pollen sterile pollen*).

basal medium showed pollen germination after 12 hrs, where as the timing was reduced to 8hrs for germination when boric acids and calcium nitrate were added. Addition of EACA the pollen fastens pollen germination after 3 and half hours itself. So this addition greatly helped in fast recording of germination percentage. Here the pollen showed maximum germination but tube length got reduced to 660.4 and 602.1 um. Since eggplant pollen tube can grow very long, it the medium constituent does not give correct nutrition, less pollen grain were germinated with long tube length. Hence, after calcium nitrate 72.3% with 827.6 um tube length of uneven pollen germination was seen. But after EACA and tryptone addition, evenly germinated pollens with maximum percentage and reduced pollen tube length recorded. This reveals this final medium gives nearly the same nutrition as *in vivo*. Henceforth, E250 and T50 medium were finally selected.

In the beginning, the initial basal medium showed pollen germination after 12 hrs, where as the timing was reduced to 8 hrs for germination by the addition of minerals like boric acids and calcium nitrate. It was surprising that by addition of EACA pollen starts germination after 3 and half hrs. So this addition greatly helped in fastening of germination percentage. Pollen showed maximum germination at the cost of pollen tube length from 660.4 to 602.1 µm. Since eggplant pollen tube can grow very long, the medium constituent does not give correct nutrition, less pollen grain were germinated with long tube length. Hence after calcium nitrate 72.3 % with 827.6 um tube length of uneven pollen germination was seen. But after EACA and tryptone addition, evenly germinated pollens with maximum percentage and reduced pollen tube length recorded. This reveals this final medium gives nearly the same nutrition as *in vivo*. Henceforth, EACA 250 and T50 medium were finally selected.

In some vegetables the observed pollen *in vitro* germination rates were reported to be less than 90 percent, for example, Gomes *et al*. (4) observed 49.8% germination in onion pollen grains, Franzon *et al*. (3) 79.7% in beans etc. In eggplant, the observed fruit set was 62%, (Suganya *et al.*, 14) while the *in vivo* pollen germination was 66% (Franca *et al.*, 2) which indicate that there is above 30 percent sterility/ non-viable pollen. In both staining and *in vitro* pollen germination studies sterile/ non-viable pollen count 10-12% has been observed. Considering the above pollen sterility, the medium reported here may be concluded as complete PGM for testing the viability of eggplant pollen.

Basically four forms of eggplant flowers based on stigma position in relation to anthers are usually seen. The pollen extracted from each flower was germinated in the PGM reported here.

Stigma position in relation to anthers in flower	Pollen germination (%)
1. Flower with stigma below anthers	78.1
2. Flower with stigma on the same level of anthers	50-60
3. Flower with stigma above the anthers	20-40 with pollen bursting
4. Flower with stigma much below anthers	0-10

So, flowers with stigma below anther tip were selected throughout the experiments since it has shown highest pollen germination than the rest. Scanning electron microscopy of pollen grains of selected eggplant variety showed average equatorial axis of 28.28 µm (27-29 µm) and polar axis of 38.24 µm (37-39 µm) (Fig. 4a & b). In final medium germinated pollen tube diameter showed 7.23 µm – 7.21 µm initially and as tube growth progressed, diameter decreased to 6.89 µm (Fig. 4c & d). Since eggplant pollen tube grows long the exact end of tube couldn't be located properly. Fig. 4e & f showed mat growth of eggplant pollen grains.

SEM studies helps to resolve many structural based questions. Koti *et al.* (9) studied SEM pattern of soybean pollen after UV- B radiation which showed no apertures on pollen grains. Earlier studies showed pollen morphology of three Solanaceae family from Saudi Arabia using SEM and explained eggplant pollen grains are generally radially symmetrical, isopolar, tricolporates, zonoaperatures, prolate and with non perforatetectum. Lashin (10) reported detailed studies of six Solanum varieties pollen morphology using SEM. Pollen tube structure on selected medium was studied using this technique showed medium nearly consists of all nutrition for intact tube growth and long growth.

The composition of improved Brewbaker and Kwack medium suited for pollen germination of each wild solanum is presented in Table 1. Sucrose (10%) was found to be the appropriate sugar which supported pollen germination of these species. Some solanum species required EACA in PGM where as other solanums germinated without it. All the observation on pollen germination and pollen tube growth were taken 3h after incubation except *S.khasianum* which germinated quickly (1h)

Again, in all the wild species during pollen germination we observed sterility upto 10.12%. The pollen germination results of eggplant were compared with wild *Solanum*(Table 1). As compared to domesticated species, the wild *Solanum* required minimum nutrition for pollen germination *in vitro.*

There exists lot of variability in the whole gene pool of *Solanum*. The PGMs reported here may be used in variety of ways (1) the variability in each species may be explored (2) help in undertaking pollen selection experiments (3) help in attempting *in vitro* pollination/ fertilization (4) intra specific variability based on pollen germination medium will be useful for the selection of appropriate accession for hybridization.

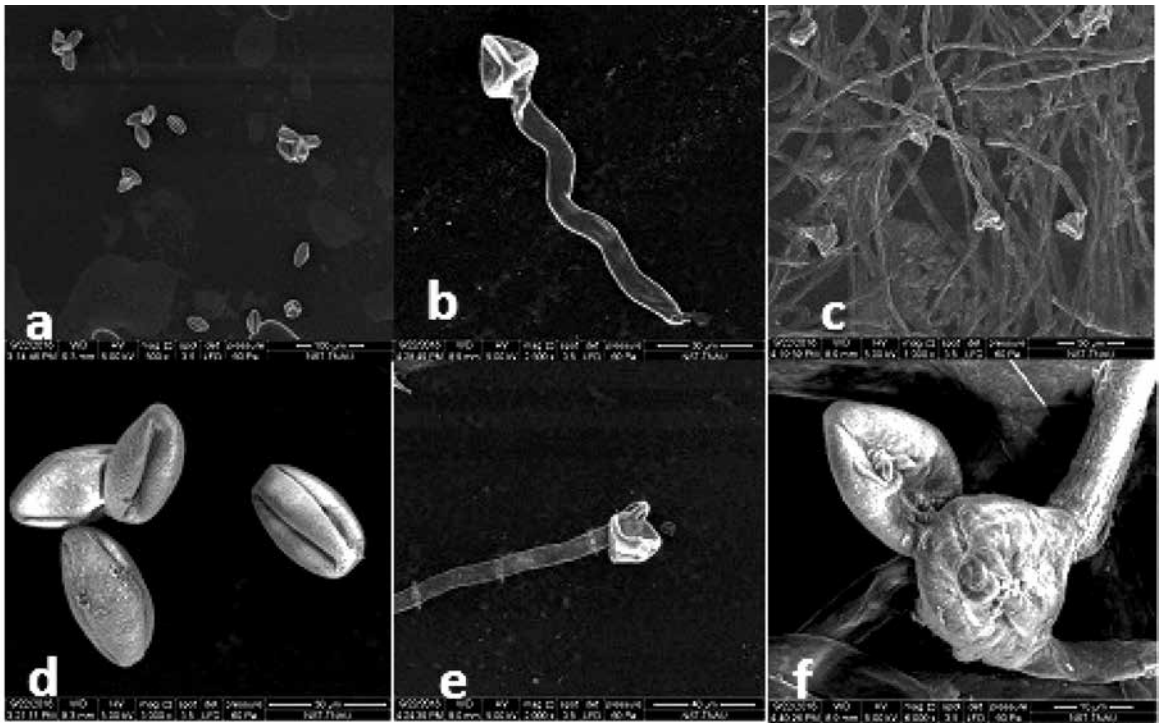

Fig. 4: Pollen morphology of eggplant (a) pollen grains showing prolate-spheroidal and trizonocolporate, x500 (b) Polar view showing large mesocolpium, x3000 (c & d) Germinated pollen in final medium, x2500 & x2000 (e & f) Mat growth of eggplant pollen with long tube length, x500 & x6000.

Table 1. Composition of pollen germination medium and other conditions for eggplant and its wild species.

Parameter	*S. melongena*	*S. torvum*	*S. khasianum*	*S. surettense*	*S. trilobatum*
Sucrose (%)	-	10	10	10	10
Maltose (%)	20	-	-	-	-
Boricacid (mg l^{-1})	250	200	300	200	200
Calcium nitrate (mg l^{-1})	300	300	300	100	100
PEG 4000 (%)	15	15	-	-	-
EACA (mg l^{-1})	750	200	-	-	-
Temperature (°C)	25	24	25	24	25
Period of incubation (hours)	3h	3h	1h	3h	3h
Maximum per cent germination achieved	78.1 %	76.53%	78.63%	77.58%	76.25%

*100 mgl^{-1} Potassium nitrate + 200 mgl^{-1} Magnesium sulphate + 1% Agar are comman for PGM.

CONCLUSION

The complete pollen germination medium for eggplant consists of 20 % maltose + 250 mg l^{-1} Boric acid + 300 mg l^{-1} Calcium nitrate + 15% PEG 4000 + 750 mg l^{-1} EACA + 0.5-1% agar which supports > 78% pollen germination at 25 ºC température after 3h of incubation

REFERENCES

1. Brewbaker, J.L. and Kwack, B.H. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *American J. Bot.* **50**: 859-65

2. Franca, L.V., Nascimento, W.M., Carmona, R. and Freitas, R.A. 2009. Viability of eggplant pollen. *Crop Breed. Appl. Biotech.* **9**: 320-27.

3. Franzon, R.C., Corrêa, E.R. and Raseira, M.C.B. 2005. *In vitro* pollen germination of feijoa (*Acca sellowiana* (Berg) Burret). *Crop Breed. Appl. Biot.* **5**: 229-33.

4. Gomes, P.R., Raseira, M.C.B., Baudet, L.L. and Peske, S.T. 2003. Armazenamento do grão de pólen de cebola (*Allium cepa* L.). *Revista Brasileira de Sementes*, **25**: 14-17.

5. Guler, H.Y., Abak, K. and Eti, S. 1995. Method, Medium and incubation time suitable for in vitro pollen germination of eggplant (*Solanum melongena*.L). *Acta Hort.* **412**: 99-105.

6. Hepler, P.K. 2005. Calcium: A central regulator of plant growth and development. *Plant Cell.* **17**: 2142-55.

7. Jayaprakash, P., Annapoorani, S., Vikas, V.K., Sivasamy, M., Kumar1, J., Saravannan, K., Punniakotti, E. and Sheeba, D. 2015. An improved *in vitro* germination medium for recalcitrant bread wheat pollen (*Triticum aestivum*L.).*Indian J. Genet.* **75**: 446-52.

8. Khan, S.A. and Perveen, A. 2006. Germination capacity of stored pollen of *Solanum melongena* L., (Solanaceae) and their maintenance. *Pak. J. Bot.* **38**: 917-20.

9. Koti Sailaja, Reddy, K. Raja, Reddy, V. R., Kakani, V. G. and Zhao, D. 2015. Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. *J. Exp. Bot.* **56**: 725-36.

10. Lashin, G.M.A. 2012. ultrastructure and pollen morphology significance of some species of *Solanum* (Solanaceae). *Egyptian J. Bot.* 141-56.

11. Leduc, N., Monnier, M. and Douglas, G.C. 1990. Germination of trinucleatted pollen: formulation of a new medium for Capsella bursa-pastoris. *Plant Reprod.* **3**: 228-35.

12. Pradeepa, G.L. 2002. Fruit set in relation to floral morphology of eggplant (*Solanum melongena* L.). *Tropical Agric. Res. and Exten.* **5**: 12-16.

13. Shyla, K.K. and Natarajan, N. 2014. Customizing Zinc oxide, silver, titanium dioxide nanoparticles for enhancing groundnut seed quality. *Indian J. Sci. Tech.* **7**: 1376-81.

14. Suganiya, S., Kumuthini, A. and Harris, D. 2015. Effect of Boron on flower and fruit set and yield of ratoon Eggplant crop. *International J. Sci. Res. and Innov. Tech.* **2**: 135-41.

Received : July, 2017; Revised : April, 2018;
Accepted : May, 2018