In vitro pollen germination and pollen viability study in brinjal

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Abstract

For hybrid seed production, it is prerequisite to know the floral biology of the crop and pollen biology is an integral part of flower biology of any crop to understand artificial hybridization more efficiently. After pollination, germination of the viable pollen is important for proper fertilization of ovary and to develop optimum fruit and seed set. So, the study aimed to evaluate the *in vitro* pollen germination and viability of brinjal in ambient as well as cold storage conditions. The pollen germination was tested *in vitro*, by incubating the pollen grains for 3 hours at 25°C in different concentrations of sucrose, boron and Agar media [(T₁ = 2 % Sucrose + 4 ppm Boron, T₂ = 2 % Sucrose + 8 ppm Boron, T₃ = 5 % Sucrose + 4 ppm Boron, T₄ = 5 % Sucrose + 8 ppm Boron, T₅ = 10 % Sucrose + 4 ppm Boron, T₆ = 10 % Sucrose + 8 ppm Boron, T₇ = 0.5 % Agar + 10 % Sucrose + 100 ppm Boron, T₈ = 1 % Agar + 10 % Sucrose + 300 ppm Boron, T₉ = 0.5 % Agar + 12 % Sucrose + 100 ppm Boron, T₁₀ = 1 % Agar + 12 % Sucrose + 300 ppm Boron, T₁₁ = Distilled water (Control)]. The viability of the pollen grains was evaluated following acetocarmine staining technique. The best germination of fresh pollen was recorded in treatment of 1 % Agar + 12 % Sucrose + 300 ppm Boron. A close perusal of the results of *in vitro* pollen germination showed that the lowest germination percentage was recorded in media with different concentration of sucrose and boron, not containing agar, and there is no germination of pollen takes place in distilled water. Significantly the highest *in vitro* pollen germination and pollen viability percentage was recorded in fresh pollen (95.00%) and as the duration of pollen storage increased in both the storage conditions, the *in vitro* pollen germination decreased. Of the two storage conditions of pollen, the higher *in vitro* germination percentage of pollen found in pollen stored in refrigerator compared to ambient storage condition. This study help in identification for culture media used to determine *in vitro* pollen germination and time period for pollen viability by *in vitro* pollen germination and pollen viability methods.

Introduction

Evaluation of pollen viability is crucial in the process of hybridization. The viability of pollen grains in brinjal was found to be affected by many endogenous factors like nutritional status of the plant (Howlett, 1936), agricultural pesticides and other chemicals (Mac Daniels and Hildebrand, 1939; Dubey and Mall, 1972), plant species, nutritional status of the plants, time of year and harvest time, photoperiod, temperature, harvest method, incubation period, and presence of micro and macronutrients in the culture medium (Soares *et al.*, 2008) and adjustments of the composition of culture media for each species (Chagas *et al.*, 2010; Sinimbu *et al.*, 2011) etc. Pal and Singh (1943) observed maximum pollen fertility on the 2nd day of flower opening in brinjal. Popova (1958) found that pollen grains remained viable up to 7-10 days in brinjal. The angiosperms pollen requires a carbon source, boron and often other nutrients also to induce their germination (Galleta, 1983). Sugar is used mainly to control the osmotic balance between the solution and pollen germination, and provides energy to assist the pollen tube developmental process (Miranda and Clement, 1990). Keeping these facts in view, the present study was carried out to trace the *in vitro* pollen germination and viability of pollen grains as well as the standardize the *in vitro* pollen germination media for brinjal.

Material And Methods

The experiment was carried out at Laboratory of Department of Genetics and Plant Breeding, College of Agriculture, Junagadh Agricultural University, Junagadh, during *Kharif* 2019 with Male parent (JBR 03-16) of hybrid brinjal GJBH 4 using Completely Randomized Design with three replications. The pollen (anther) collected for *in vitro* germination study was stored under ambient storage condition as well as cold storage condition (refrigerator) and *in vitro* germination was carried out immediately at the time of anthesis followed by twelve hours interval. Flowers were collected at the time of anthesis and anthers were carefully removed using forceps; the collected anthers are dehisced under sunlight for about 5-10 min, depending upon the intensity of light. Dehisced pollens were subjected to *in vitro* pollen germination, in different eleven media containing different concentration of sucrose, boron and Agar media [(T₁ = 2 % Sucrose + 4 ppm Boron, T₂ = 2 % Sucrose + 8 ppm Boron, T₃ = 5 % Sucrose + 4 ppm Boron, T₄ = 5 % Sucrose + 8 ppm Boron, T₅ = 10 % Sucrose + 4 ppm Boron, T₆ = 10 % Sucrose + 8 ppm Boron, T₇ = 0.5 % Agar + 10 % Sucrose + 100 ppm Boron, T₈ = 1 % Agar + 10 % Sucrose + 300 ppm Boron, T₉ = 0.5 % Agar + 12 % Sucrose + 100 ppm Boron, T₁₀ = 1 % Agar + 12 % Sucrose + 300 ppm Boron, T₁₁ = Distilled water (Control)]. The dehisced pollen dusted on petri plate which contain a solidify media and for liquid media cavity slides (sitting drop technique) is used. After that incubating the pollen for 3 hours at 25°C, the pollen germination percentage was calculated under stereo-microscope (10 X magnification of Leica Laborlux K microscope with dedicated camera of model Leica EC3) and image capture by MagVision software. Beyond three hour of storage pollen used to start burst (Guler *et al.*, 1995). Outer growth of the pollen tube beyond the diameter of the pollen was assumed as germinated.

The viability of pollen grain was estimated following acetocarmine staining technique (Moore and Webb, 1972). Pollen grains were obtained by squeezing the anthers with a sharp needle. They were then stained with one per cent acetocarmine. The well stained pollen grains evaluated after one hours and well stained pollen grains were considered to be fertile and the unstained ones as sterile.

Pollen germination and viability in per cent of male parent of hybrid GJBH 4 was counted immediately at the time of anthesis followed by eight hours interval as per the below formula.

$$\text{Pollen germination (\%)} = \frac{\text{Number of germinated pollen}}{\text{Total number of pollen}} \times 100$$

Results And Discussion

In vitro pollen germination

The results presented in Plate 1 clearly showed that among eleven different treatments (10 media with varied concentrations of sucrose, boron and agar, and one control/distilled water), the high germination of fresh pollen was recorded in media containing agar, sucrose and boron (treatment T₇ to T₁₀), of which the best germination of fresh pollen was recorded in treatment T₁₀ (1 % Agar + 12 % Sucrose + 300 ppm

Boron). A close perusal of the results of *in vitro* pollen germination (Plate 1) showed that the lowest germination percentage was recorded in media with different concentration of sucrose and boron, but not containing agar, and there is no germination of pollen takes place in distilled water. The result clearly indicates the importance of sucrose, boron and agar for *in vitro* pollen germination of brinjal. The results are in accordance with the reports of Guler *et al.* (1995) and Rathod *et al.* (2018). Guler *et al.* (1995) reported that most convenient germination medium was 1 per cent agar, 12 per cent sucrose, 300 ppm H_3BO_3 and 300 ppm $Ca(NO_3)_2$ for *in vitro* pollen germination of eggplant. Rathod *et al.* (2018) compared three *in vitro* pollen germination media in *Momordica* spp and their interspecific hybrids reported that sucrose @ 15 % + Boric acid @ 0.25% + Calcium nitrite @ 300mg solution showed the maximum per cent of pollen germination and pollen tube growth.

After standardized the protocol, *in vitro* germination of pollen in brinjal, the best treatment combination, 1 % Agar + 12 % Sucrose + 300 ppm Borons was utilized in the present study for *in vitro* pollen germination study. For that, the pollen (anther) collected for *in vitro* germination study was stored under ambient storage condition as well as cold storage condition (refrigerator) and *in vitro* germination was carried out immediately at the time of anthesis (fresh pollen) followed by twelve hours interval of stored pollen. Data on pollen germination were recorded from an average of three repetitions of each treatment. Mean data for pollen germination percentage were analyzed in CRD (Cochran and Cox, 1957) to optimize the standard germinating medium. The mean data for pollen germination percentage of fresh pollen (at the time of anthesis) as well as of 12 hours interval of pollen stored in different conditions (refrigerator and ambient) presented in Table 1 (Plate 2 and 3) clearly showed that all the eleven treatments manifested significant differences for *in vitro* pollen germination percentage. Significantly the highest *in vitro* pollen germination percentage was recorded in fresh pollen (95.00%) and as the duration of pollen storage increased in both the storage conditions, the *in vitro* pollen germination decreased. Of the two storage conditions of pollen, the higher *in vitro* germination percentage of pollen found in pollen stored in refrigerator compared to ambient storage condition. These results may be attributed to sufficient loss of pollen viability due to diurnal fluctuations in ambient temperature and relative humidity. These results are in accordance with the findings of Islam and Khan (1998) in *Momordica dioica* (spine gourd) and Khan and Anjum (2006) in brinjal.

Pollen viability in brinjal

The mean data for pollen viability percentage of fresh pollen (at the time of anthesis) as well as of 12 hours interval of pollen stored in different conditions (refrigerator and ambient) presented in Table 2 (Plate 4 and 5) clearly showed that all the eleven treatments manifested significant differences for pollen viability percentage. Significantly the highest pollen viability percentage was recorded in fresh pollen (97.00%) and as the duration of pollen storage increased in both the storage conditions, the pollen viability decreased. Of the two storage conditions of pollen, the higher viability percentage of pollen found in pollen stored in refrigerator compared to ambient storage condition.

Conclusion

The best germination of fresh pollen was recorded in treatment of 1 % Agar + 12 % Sucrose + 300 ppm Boron. A close perusal of the results of *in vitro* pollen germination showed that the lowest germination percentage was recorded in media with different concentration of sucrose and boron, not containing agar, and there is no germination of pollen takes place in distilled water. Significantly the highest *in vitro* pollen germination and viability percentage was recorded in fresh pollen and as the duration of pollen storage increased in both the storage conditions, the *in vitro* pollen germination and pollen viability decreased. Of the two storage conditions of pollen, the higher *in vitro* germination percentage and viability of pollen found in pollen stored in refrigerator compared to ambient storage condition.

Declarations

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations. The authors collected plants material from the university where the author got a degree and plant materials (genotypes) used in research which is developed by same university so plant material was free for authors. There is no permission or license obtained for using of plant material.

Consent for publication

Not Applicable

Availability of data and materials

All relevant data are within the manuscript

Competing interests

The authors whose names are listed they have NO involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript. In short, the authors declare no conflicts of interest.

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Authors' contributions

All authors have been personally and actively involved in study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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References

1. Chagas EA, Pio R, Chagas PC, Pasqual M and Bettiol Neto JE (2010) Medium composition and environmental conditions for the germination of pollen grains of pear root socks. *Ciencia Rural* **40**(2), 261- 266.
2. Cochran WG and Cox GM (1957) Experimental Designs. 2nd Edition. Wiley, New York.
3. Dubey PS and Mall LP (1972) Herbicidal pollution. Pollen damage by the herbicide vapours. *Experientia* **28**, 600.
4. Galleta GJ (1983) Pollen and seed management. In: Methods in Fruits Breeding. Moore, J. N. and Janick, J. (Eds.). Purdue University Press, India, pp 23-47.
5. Guler HY, Abak K and Eti S (1995) Method, medium and incubation time suitable for in vitro germination of eggplant (*Solanum melongena*) pollen. *Acta Horticulturae* **412**, 99-105.
6. Howlett FS (1936) The effect of carbohydrate and nitrogen deficiency upon microsporogenesis and the development of the male gametophyte in the tomato (*Lycopersicon esculentum* Mill.). *Annals. Bot* **50**, 767-804.
7. Islam S and Khan S (1998) Pollen viability of *Momordica dioica* Roxb. as affected by storage period and temperature. *Bangladesh J. Bot* **27**(2), 153-155.
8. Khan SA and Anjum P (2006) Germination capacity of stored pollen of *Solanum melongena* L. (*Solanaceae*) and their maintenance. *Pak. J. Bot* **38**(4), 917-920.
9. Mac Daniels LH and Hildebrand EMA (1939) Study of pollen germination upon the stigmas of apple flowers treated with fungicides. *Proceedings of the American Society for Horticultural Science* **36**, 137.
10. Miranda IPdeA and Clement CR (1990) Germination and storage of pejobaye (*Bactris gasipaes*) palmae pollen. *Rev. Biol. Trop* **38**(1), 29-33.
11. Moore PD and Webb JA (1972) An Illustrated Guide To The Pollen Analysis. Hodder and Stoughton, London, 133p.
12. Pal BP and Singh HB (1943) Floral characters and fruit formation in the egg plant. *I. J. Genet* **3**(1), 45-58.
13. Popova D (1958) Some observations on the flowering, pollination and fertilization of the eggplant. *Inst. Rasten. Plant. Ind* **5**, 211.
14. Rathod V, Behera TK, Munshi AD, Durgesh K, Jat, GS, Boopala KG and Sharma N (2018) Pollen viability and *in vitro* pollen germination studies in *Momordica* species and their intra and interspecific hybrids. *Int. J. Chemical Studies* **6**(6), 32-40.
15. Sinimbu NFA, Martins ABG and Barbosa JC (2011) *In vitro* viability of "bacury" pollen grains. *Revista Brasileira de Fruticultura* **33**(2), 593-600.

16. Soares, TL, Silva SO, Costa MAPC, Santos-Serejo JA, Souza AS, Lino LSM, Souza EH and Jesus ON (2008) *In vitro* germination and viability of pollen grains of banana diploids. *Crop Breed, Appl. Biotech* **8**, 111-118.

Tables

Table 1 *In vitro* pollen germination percentage

Treatments	Pollen germination (%)
P ₁ = Fresh pollen	95.00
P ₂ = 12 hour old pollen stored in refrigerator	89.00
P ₃ = 24 hour old pollen stored in refrigerator	78.00
P ₄ = 36 hour old pollen stored in refrigerator	67.00
P ₅ = 48 hour old pollen stored in refrigerator	56.66
P ₆ = 60 hour old pollen stored in refrigerator	49.66
P ₇ = 12 hour old pollen stored in ambient condition	85.33
P ₈ = 24 hour old pollen stored in ambient condition	70.33
P ₉ = 36 hour old pollen stored in ambient condition	58.33
P ₁₀ = 48 hour old pollen stored in ambient condition	45.66
P ₁₁ = 60 hour old pollen stored in ambient condition	35.00
Mean	66.36
C.D. at 5 %	3.63
S. Em.±	1.23
C.V.	3.21

Table 2 Pollen viability in brinjal

Treatments	Pollen Viability (%)
V_1 = Fresh pollen	97.00
V_2 = 12 hour old pollen stored in refrigerator	92.33
V_3 = 24 hour old pollen stored in refrigerator	81.00
V_4 = 36 hour old pollen stored in refrigerator	69.33
V_5 = 48 hour old pollen stored in refrigerator	58.00
V_6 = 60 hour old pollen stored in refrigerator	51.33
V_7 = 12 hour old pollen stored in ambient condition	88.00
V_8 = 24 hour old pollen stored in ambient condition	73.00
V_9 = 36 hour old pollen stored in ambient condition	60.33
V_{10} = 48 hour old pollen stored in ambient condition	46.67
V_{11} = 60 hour old pollen stored in ambient condition	36.33
Mean	68.48
C.D. at 5 %	4.51
S. Em.±	1.54
C.V.	3.89