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In Vitro germination, viability and morphology of eggplant (*Solanum melongena* L.) Pollen

A. DAS, M. K. PANDIT, [1]B. N. PANJA, [2]PANDIYARAJ P. AND S. SAHA

Department of Vegetable Crops, [1]Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741252, West Bengal
[2]Division of Vegetable Crops, Indian Institute of Horticultural Research, Bengaluru– 560 089, Karnataka

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ABSTRACT

Pollen morphology, viability and in vitro germination of 21 eggplant genotypes were evaluated for two subsequent autumn-winter season during 2014-16. The optimization of sucrose and boron medium for pollen germination has been standardized from eleven germinating media. The medium containing 5 per cent sucrose and 4ppm boron recorded the highest germination of pollen in all the three types (Round, oval and slender). For the first season, the range of pollen germination percentage was 57.44% (BCB-14) to 22.52% (BCB-5) and in the second season 60.05% (BCB-14) to 22.07% (BCB-8). Pooling the mean data over the seasons, it has been found that BCB-10 had the highest pollen viability (76.59%) and BCB-16 the minimum (57.14%). Range of pollen diameter was from 34 (BCB-27) to 22.84 micrometer (BCB-9). Pollens are mostly round in shape. Micro pollen were also noted in all 21 genotypes. Percentage of micro pollen (pollens which are smaller than actual pollen size) was the lowest in BCB-14 (10.03%) and the highest in BCB-1 (32.56%). The higher the micro pollen percentage is the lower the hybrid seed production. Micro pollen diameter ranged from 8.37 (BCB-22) to 14.74ìm (BCB-4).

Keywords: Eggplant, in vitro germination, morphology, pollen and viability.

Eggplant, brinjal or aubergine (*Solanum melongena* L.) is the most popular and widely cultivated warm season vegetable crop in the central, southern and south-east Asia as well as India. Vavilov (1928) regarded brinjal as the native of India and the crop has immense variability in India covering an area of 0.71 million hectares with a production of 13.56 million tonnes (NHB, 2015). World-wide different hybridization programme is going on to get different desirable traits and hybrid seed production in brinjal. But for hybridization experiment or 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. Lack of fertile pollen formation generally occurs in less light (glass houses) and low temperature area (Guler, 1995). The angiosperms pollen requires a carbon source, boron, and often other nutrients also to induce their germination (Galleta, 1983). Thompson and Batjer (1950) reported that the additional use of boron in the medium markedly increased the germination percentage and pollen tube length. 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 (Stanley and Linskens, 1974; Galleta, 1983; Miranda and Clement, 1990). Germination capability of viable pollen is directly linked with the nutrition in medium and environment. Normal meiotic events lead to the formation of normal tetrads and normal pollen while abnormal meiosis results in abnormal segregation of chromosomes in the tetrads resulting in macro and micro pollen. Since long occurrence of micro or dummy pollen have been reported (Ayyangar and Pandurangarao,1935) and it continues to be a point of concern for the scientists even these days (Parker *et al*, 2015). Micro pollen was considered to be any pollen grain that are less than half the diameter of the parental pollen grains. Increase in micro pollen is similar to the decrease in hybrid seed production (Trucco *et al*., 2005). Being the centre of origin, India is the place of great genetic variation and vast diversity of brinjal with regard to vegetative, floral, morphological and other reproductive characters particularly palinological characters, which are vital for improvement of any crop. With this background the present experiment was undertaken to study the *in vitro* germination, viability and morphology of pollen for some brinjal genotypes.

MATERIALS AND METHODS

The experiment was carried out at B.C.K.V., West Bengal, during autumn-winter season of 2014-2015 and 2015-2016 with twenty one brinjal genotypes (Table-1), evaluated in a randomized block design with three replications. Standard crop husbandry was followed to raise the crop but without any chemical protection as it

Email: arindamdasis01@gmail.com

may affect the pollen functions. Laboratory data were collected for standardization of pollen germinating medium (Table-2), pollen germination percentage in standard medium for all genotypes, pollen viability percentage, pollen diameter and micro pollen percentage (Table-3). Data on pollen characters were recorded from an average of five replications of each treatment. Anthers of all the genotypes were collected separately at the time of anthesis and kept in petri dish with moisture lined blotting paper for avoiding dehydration of pollen following the method as described by Franca *et al*. (2009). Fresh pollens are the best for fruit set. Pollens remain fairly viable at 20-22°C with 55% relative humidity (Daunay and Hazra, 2012). Fresh pollens from anthers of three genotypes namely BCB-1 (Round type), BCB-11 (Oval type) and BCB-12 (Slender type) were squeezed out and plotted in different germinating medium in groove slides. Pollen germination percentage was calculated under stereo-microscope (10 X magnification of Leica Laborlux K microscope with dedicated camera of model Leica EC3) for eleven germinating media after three hours of keeping in 25°C incubation. Beyond three hour of storage pollen used to start burst (Guler *et al*., 1995). Different germinating media used for this experiment were as follows: T_1=2% sucrose + 4 ppm boron; T_2 = 2% sucrose + 8ppm boron; T_3 = 5% sucrose+4ppm boron; T_4=5% sucrose+8ppm boron; T_5=10% sucrose+4ppm boron; T_6 = 10% sucrose + 8 ppm boron; T_7 = 15% sucrose+4ppm boron; T_8 = 15% sucrose + 8 ppm boron; T_9 = 20% sucrose + 4 ppm boron; T_{10} = 20% sucrose + 8 ppm boron; T_{11} = distilled water (control). Mean data for pollen germination percentage, after angular transformation, of three genotypes in eleven germination media were analyzed in two factors CRD (Gomez and Gomez, 1984) to optimize the standard germinating medium. Outer growth of the pollen tube beyond the diameter of the pollen was assumed as germinated.

For testing germination percentage, that standard solution has been used for all genotypes. Fresh pollens are collected and stained (2-3 drops) with aceto-orcein (Imanywoha *et al*., 1994) and studied under microscope for testing viability. The stained/viable, non-stained/non-viable and micro pollen (Non-viable but stained and smaller than actual pollen size) were counted (Figure-1). Pollen viability and micro pollen percentages of all genotypes were angular transformed before analysis. Pollen diameter was recorded in micron(ìm) unit (Figure-1) under 10X magnification using Carl Zeiss Vision Axio Vision software 4.8.2 (Aramendiz *et al.*, 2012). Nine microscopic fields in each genotype for 3 replications have been studied in complete randomized block design for all the experiments. Mean data pooled over the years except pollen germination percentage and ANOVA was statistically analyzed (Cochran and Cox, 1957; Gomez and Gomez, 1984) for all the characters.

Table 1: Symbol, name and source of the genotypes

Symbol	Name	Source
BCB- 1	Sada local	Banamalipara, Nadia, West Bengal
BCB- 2	Makra	Kanthaltala, Nadia, West Bengal
BCB- 3	Makra mid-long	Madanpur, Nadia, West Bengal
BCB- 4	Daab begun	Kalyani, Nadia, West Bengal
BCB- 5	Thubi	Kalyani, Nadia, West Bengal
BCB- 6	Bhangar	Ghoragacha, Nadia, West Bengal
BCB- 7	Gola	Chakdaha, Nadia, West Bengal
BCB- 8	Hajari	Tekkali, Andhra Pradesh
BCB- 9	Debgiri	Barasat, North 24 Parganas, West Bengal
BCB- 10	Simanta	Bhootta bazaar, Kalyani, Nadia, West Bengal
BCB- 11	Sada lamba	Bangaon, N-24 Pgs, West Bengal
BCB- 12	Kranti	New Delhi
BCB- 13	Anubhav	New Delhi
BCB- 14	Tara	New Delhi
BCB- 15	Purushottom	Raipur, Chattishgarh
BCB- 16	Purple round	New Delhi
BCB- 17	Kooli	Balagarh, Hooghly, West Bengal
BCB- 18	Utkal	Bhubeneswar, Odissa
BCB- 21	Jhuri	Basantapur, Nadia, West Bengal
BCB- 22	Makra mid	Haringhata, Nadia, West Bengal
BCB- 27	Kalyani	Kalyani market, West Bengal

RESULTS AND DISCUSSION

The data (Table-2) clearly show that among eleven treatments (10 media with varied concentrations of sucrose and boron and one control/distilled water), the best germination percentage was recorded in T_3 (5% sucrose+4 ppm boron) in all the three genotypes (BCB-1, BCB-11 and BCB-12), which is significantly higher than the other treatments (Figure-2). The interaction

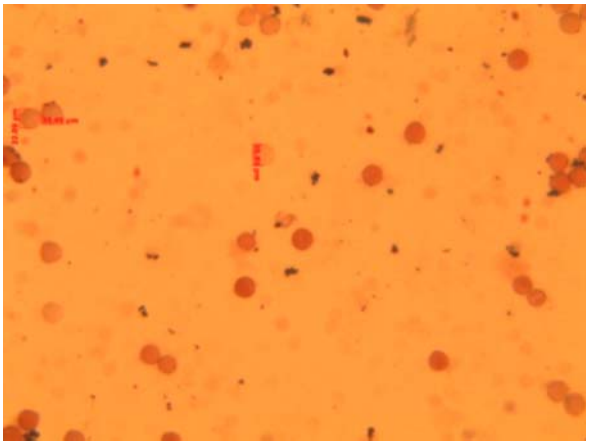

Fig.1: Stained (viable), non-stained(non-viable) pollen and stained small micro pollen

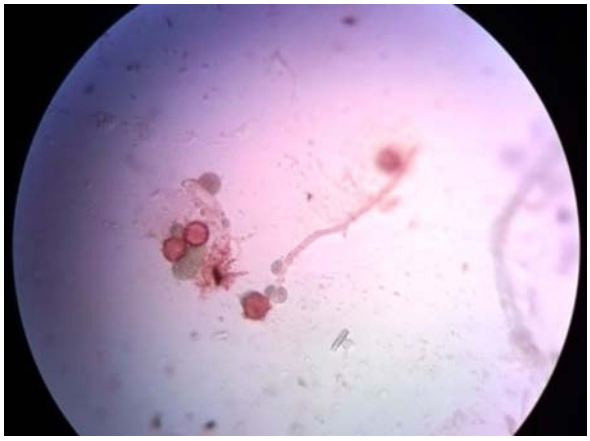

Fig.2: Germination and pollentube formation

Table 2*: In vitro* pollen germination in different media of three types of genotype (Round, Oval and Slender)

Treatment media	BCB-1	BCB-11	BCB-12
T_1 = 2%sucrose + 4ppm boron	38.94 (38. 65)	40.23 (39.35)	40.85 (39.87)
T_2 = 2%sucrose + 8ppm boron	30.84 (33.55)	30.80 (33.64)	23.62 (26.64)
T_3 = 5%sucrose + 4ppm boron	53.79 (47.17)	54.68 (49.20)	59.53 (50.52)
T_4 = 5%sucrose + 8ppm boron	30.80 (33.63)	31.85 (34.11)	31.79 (34.26)
T_5 = 10%sucrose + 4ppm boron	26.64 (31.00)	25.61 (30.40)	28.32 (32.15)
T_6 = 10%sucrose + 8ppm boron	17.83 (24.93)	18.07 (25.13)	18.21 (25.20)
T_7 = 15%sucrose + 4ppm boron	22.32 (28.01)	19.17 (25.95)	30.24 (33.35)
T_8 = 15%sucrose + 8ppm boron	12.41 (18 .55)	17.52 (24.55)	18.925 (25.75)
T_9 = 20%sucrose + 4ppm boron	20.12 (26.44)	19.395 (25.91)	22.90 (28.56)
T_{10} = 20%sucrose + 8ppm boron	16.89 (23.98)	15.72 (23.27)	21.70 (27.39)
T_{11} = Distilled water (control)	0	0	0
Significance (5%)			
Treatment	**4.36**		
Genotype	**7.54**		
T X G	**5.19**		

**Data were recorded after 3 hours of placing in the media.

***Figures out of parenthesis are angular transformed data used for calculation.

between treatments and genotypes was also found to be significant, which indicates that pollens of different genotypes have differential germinability in different media. The highest germination percentage was recorded in BCB-12 (50.52%). Findings derived from the table-2 shows that no germination took place in distilled water, indicating that sucrose and boron have important role for *in vitro* pollen germination of eggplant. A close perusal of the data in table-2, show that the lowest germination percentage was recorded in media with 20 per cent of sucrose and the media with high sucrose concentrations did not help in increasing germination percentage.

After standardization, pollen germination of the rest genotypes were tested using T_3 medium (Table-3). In the first year BCB-14 showed the highest pollen germination (57.44%) followed by BCB-7 (55.67%) and BCB-17 (55.30%). The least germination showed by BCB-5 (22.52%) followed by BCB-8 (23.00%). In both the cases, they were, however, statistically *at par*. For the second year, BCB-14 was at the top for pollen germination (60.05%), followed by BCB-15 (55.31%),

Table 3: Pollen characters of eggplant genotypes

Genotype	Pollen diameter (µm)	Pollen germination (%)		Pollen viability (%)	Micro pollen (%)	Micropollen diameter (µm)
		Year 1	Year 2			
BCB-1	30.44	47.17	52.70	57.25 (70.75)	32.56 (29.10)	13.21
BCB-2	27.84	51.52	51.66	63.46 (79.96)	22.08 (14.16)	10.58
BCB-3	28.42	49.89	49.38	63.69 (80.38)	14.85 (6.63)	13.23
BCB-4	31.64	42.91	43.42	69.78 (87.87)	25.70 (19.21)	14.74
BCB-5	30.65	22.52	25.06	70.00 (88.22)	16.41 (8.10)	9.93
BCB-6	29.88	34.97	34.76	66.14 (83.56)	17.30 (8.96)	13.37
BCB-7	29.63	55.67	53.34	70.06 (88.22)	18.87 (10.50)	13.38
BCB-8	31.24	23.00	22.07	67.84 (85.33)	23.71 (16.22)	13.45
BCB-9	22.84	29.39	30.60	71.63 (89.99)	15.84 (7.49)	10.39
BCB-10	25.93	29.58	30.13	76.59 (94.61)	15.00 (6.70)	10.83
BCB-11	29.11	49.20	50.51	65.73 (83.03)	15.17 (6.86)	13.54
BCB-12	33.72	50.52	48.55	72.77 (91.07)	17.11 (8.70)	13.21
BCB-13	31.33	51.62	52.35	71.96 (90.34)	14.24 (6.10)	12.63
BCB-14	25.27	57.44	60.05	72.02 (90.34)	10.03 (3.05)	11.80
BCB-15	28.35	53.05	55.31	75.04 (93.29)	12.08 (4.41)	9.60
BCB-16	31.36	36.10	34.44	57.14 (70.57)	17.42 (9.02)	13.95
BCB-17	30.54	55.30	53.82	73.00 (91.32)	14.74 (6.52)	10.42
BCB-18	25.64	41.70	42.90	59.30 (73.92)	19.69 (11.37)	9.84
BCB-21	29.39	47.27	48.03	76.29 (94.31)	23.31 (15.68)	9.11
BCB-22	29.34	45.68	48.47	65.25 (82.50)	16.26 (7.60)	8.37
BCB-27	34.00	49.88	51.89	71.61 (90.30)	22.95 (15.30)	8.53
SEm(±)	**0.60**	**2.45**	**1.62**	**1.55**	**1.17**	**0.76**
CD(0.05)	**1.68**	**6.99**	**4.63**	**4.46**	**3.35**	**2.17**

The range of germination % data was between 20-80, which required no transformation (Mead and Curnow, 1983).Figures in parentheses are angular transformed data used for calculation.

BCB-17 (53.82%) and BCB-7 (53.34%). Significant difference existed between BCB-14 and BCB-7. The minimum germination was noted in BCB-8 (22.07%) followed by BCB-5(25.06%). They were, however, statistically *at par*. Differences in germination percentage of same genotype over same seasons of two year may be due to environmental fluctuations. These findings are in tune with that of Aramendiz *et al.* (2012).

From the table-3, it is noted that BCB-10 had the highest pollen viability (76.59%) followed by BCB-21 (76.29%), BCB-15 (75.04%) and BCB-17 (73.00%) but they were, however, statistically *at par*. The minimum was observed in BCB 16 (57.14%), BCB-1 (57.25%), BCB-18 (59.30%) and BCB-2 (63.46%). BCB-16 is significantly different from BCB-2 but not with others. But some workers got different results of pollen viability like, 98% (Vijaylalitha and Ganesh, 2012), 83-93% (Vijay *et al*., 1997) and 85-86.5% (Srivastava and Bajpei, 1977). Pollen stainability does not always connote to proportional pollen germinability. As a rule, the percentage of pollen germination is always less than the pollen viability.Viable or non-viable pollens are often stained alike with aceto-orcein (Figure-1) because staining capacity of pollens depends on its contents and genetic combination, not on their germinability (Vasil and Johri, 1961).

The highest pollen diameter was observed (Table-3) in BCB-27 (34.0ìm), followed by BCB-12 (33.72 µm) and BCB-4 (31.64 µm). BCB-4 is significantly different from BCB-27 and BCB-12. BCB-9 recorded the minimum pollen diameter (22.84 µm) followed by BCB-14 (25.27 µm), BCB-18 (25.64 µm) and BCB-10 (25.93 µm). This result is very much close to the findings of Bokolial and Majumder (2003). Pollen shapes of different genotypes of eggplant were almost round (Figure-1).

Percentage of micro-pollen (Smaller than the actual pollen size and stained but not viable) was the lowest in BCB-14 (10.03%), followed by BCB-15 (12.08%) and BCB-13 (14.24%). Significant difference did exist between BCB-14 and BCB-13. The highest micro-pollen percentage was observed in the genotype BCB-1

(32.56%) followed by BCB-4 (25.70%), BCB-8 (23.71%) and BCB-21 (23.31%). BCB-1 is significantly different from rest of the genotypes (Table-3). The diameter of micro pollen of different genotypes ranging from 8.37 (BCB-22) to 14.74 µm (BCB-4), were less than half the diameter of actual pollen (Figure-1).

Medium containing 5 per cent Sucrose and 4ppm Boron recorded the highest germination of pollen in eggplant. For the first season the range of germination percentage of pollen was 57.44% (BCB-14) to 22.52% (BCB-5) and in the second it was 60.05% (BCB-14) to 22.07% (BCB-8). Genotype BCB-10 had the highest pollen viability (76.59%) and the minimum was observed in BCB-16 (57.14%). Pollen grains are generally round in shape. Pollen diameter was reported from 34 µm (BCB-27) to 22.84 µm (BCB-9). Percentage of micro-pollen was the lowest in BCB-14 (10.03%) and the highest was observed in the genotype BCB-1 (32.56%). The highest diameter of micro pollen was observed in BCB-4 (14.74 µm) and the lowest in BCB-22(8.37 µm).

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