NBUJPS
NBU Journal of Plant Sciences

ISSN No. 0974-6927
Vol. 15 (2023), pp. 65-73

Research Article

Standardization of *In Vitro* Pollen Germination Medium of Two Economically Important Plants: *Brassica juncea* (L.) Czern. and *Lens esculenta* M.

Nasrin Banu Khan, Soumya Chatterjee, Ganapati Basak, Purba Sarkar, Chandan Barman*

Reproductive Ecology of Angiosperms Laboratory, Department of Botany, University of Gour Banga, P.O. Mokdumpur, Malda, 732103, West Bengal, India

Abstract

Pollen fertility and viability are essential for fruit and seed set in angiosperms, as well as for the successful development of hybrid plants and storage of germplasms. Our study focused on identifying the optimal culture medium for *in vitro* pollen grain germination and growth of pollen tube for two commercially important crops *Brassica juncea* and *Lens esculenta*. Percentage pollen fertility was analyzed using 1% acetocarmine solution. After ten days, pollen fertility decreased from 100% to 75.14±2.69 % and 72.31±2.52% for *B. juncea* and *L. esculenta*, respectively. SGM for pollen germination and tube growth in *B. juncea* contain boric acid (300 mg/L), calcium nitrate (400 mg/L), magnesium sulphate (300 mg/L), potassium nitrate (400 mg/L) along with 15% sucrose. Addition of 15% PEG in SGM gave the highest value for the percentage of pollen germination (52.90±0.72%) and resulted in the maximum length of pollen tube (45.2±4.93µm). SGM for *L. esculenta* composed of boric acid (200 mg/L), calcium nitrate (400 mg/L), magnesium sulphate (300 mg/L) and potassium nitrate (200 mg/L) supplemented with 15% sucrose. Among the polyamines, 10^{-4} M spermine gave the best results for the percentage of pollen germination (42.22±1.36%), while 10^{-3} M spermine showed highest pollen tube growth (80.92±16.57µm). This study will help to establish protocols for *in vitro* pollen germination and tube growth of several species of plants closely related to *Brassica* sp. and *Lens* sp.

Article info

Received **08 September 2023**
Revised **17 December 2023**
Accepted **11 January 2024**

Keywords: Pollen germination, Pollen tube length, Pollen fertility, BK medium, Standardized germination medium (SGM)

DOI: *https://doi.org/10.55734/nbujps.2023.v15i01.010*

Introduction

Pollen, the male gametophyte, facilitates successful genetic exchange between distinct individuals of the same species as well as contributes in species' survival (Pacini and Dolferus 2016). Pollen grains are crucial for successful seed set (Shivanna and Rangaswamy 1992; Dafni and Firmage, 2000; Rosbakh et al. 2018) among flowering plants as pollen germination play crucial role in the fertilization process of siphonogamous angiosperms where germinated pollen grains release male gametes through its' protruding pollen tube which intrude the ovule through the micropyle or chalaza region (Biasi et al. 1999). A viable and fertile pollen grain is required during fertilization events, because it has the potential to enter inside the embryo sac and perform fertilization events upon releasing male gametes from pollen tube (Qureshi et al. 2009). Studies about germination of pollen grains under *in vivo* conditions is much struggled because it enter through the massive tissues of female gametophyte (Dresselhaus and Franklin-Tong 2013) to start the process of fertilization but under *in vitro* conditions pollen grains germinate within short period of time by consuming nutrients from supplied germination medium.

Pollen grains are an excellent model for observing fundamental physiological as well as biochemical events during germination (Taylor and Heplar 1997; Williams and Reese 2019). Detailed knowledge of pollen viability is crucial for the success of experimental breeding programs aimed at producing hybrids (Benko et al. 2020; Guclu et al. 2020; Luo et al. 2020; Impe et al. 2020; Silva et al. 2020) and

* ***Correspondence*** - chandan.botanyugb@gmail.com

aides in selecting different stress-resistant varieties that show high yield, possess other superior qualities necessary for agriculture (Pereira et al. 2019; Lima et al. 2020). *In vitro* germination helps to assist the germinability of both fresh and stored pollen grains, supporting future cryopreservation efforts (Shivanna and Rangawamy 1992).

In vitro approach of pollen germination is quite faster as the pollen grains utilize energy from the externally supplied nutrient media under laboratory conditions. Therefore, study of pollen germination as well as pollen tube growth become easier (Taylor and Helper 1997) although some groups of scientists have been already criticized this approach (Rodriguez-Enriquez et al. 2013). To analyze the rate of pollen germination, BK medium (Brew baker and Kwack 1963) is commonly used but it doesn't always give optimum results for pollen germination of all the species of angiosperms and hence modifications of the components of BK medium is needed often (Brew baker and Kwack 1964). Hence, media for optimum pollen germination varies from different concentrations of sugar, boric acid, etc (Stanley and Linskens 1964) to complex mediums supplemented with Poly Ethylene Glycol (PEG) (Shivanna et al. 1997; Tandon et al. 1999) and polyamines (Vikas et al. 2012).

Indian mustard [*Brassica juncea* (L.) Czern.] is one of the most important oilseed crops in terms of its economic importance from past 30 years (Zhu et al. 2016). *Brassica* sp. is responsible for 24.2% of the total oil seed production in India and grows at 23.5% of the total agricultural land of the country. After Canada and China, India has occupied the position of third largest (11.3%) oil seeds producing country in the World but still India have to import (~57% of the total demand for edible oils) oilseeds to endure the demands of edible oil across this large country, because of the low productivity of *Brassica* sp. due to drought, unseasonal rains, climate fluctuations, pest-pathogens, abiotic and biotic stress factors including the issues of poor potentiality of the varieties in terms of their genotypes (Jat et al. 2019).

Lentil (*Lens esculenta* M.) is an another economically important food crop considered in this study. Lentils serve as the second largest producing pulses (after chick pea or *Cicer* sp.), grow in the period of winter across the India. Lentils have food value of 24-26% proteins, 57-60% carbohydrate, 1.3% fat, 3.2% fiber, 300 mg/100 g phosphorus, 7 mg/100 g iron, 10-15 mg/100 g VitC, 69 mg/100 g calcium, calories 343, Vit A and riboflavin. In terms of lentil production, India used to stand first among the countries, but from 2014 onwards, Canada have snitched the position because of decrease (16.2%) in the production of lentils due to water-deficit conditions in Bihar which used to serve as one of the largest lentils producing state of India during 2014-16 (Ahmad et al. 2018). Our present study aims to establish a standardized germination medium (SGM) for pollen grains of two commercially important species *B. juncea* and *L. esculenta*.

Materials and Methods

Study site and sample collection

In this present study, two different plant species *B. juncea* and *L. esculenta* were marked in the campus of University of Gour Banga (24°58′60′′N and 88°8′19′′E) and other nearby areas named Aam Bazar (24°98′19′′N and 88°13′55′′E) and Sasraj (24°98′05′′N and 88°13′34′′E). During flowering season, between 0600 to 0700 hrs, freshly dehisced flowers were collected and brought to the laboratory for further analysis.

Pollen fertility

1% acetocarmine was used to test fertility of pollen grains for both plant species. Pollen grains from freshly dehisced anthers were uniformly suspended on a clean glass slide. A drop of acetocarmine was taken on it, pollen grains were mixed gently with the help of a needle and a coverslip was laid gently over it. The fertile grains appear deep red in colour (Figure 2). Fertility (%) was deduced by counting the number of fertile pollen grains from random microscopic field. The fertility was assessed for 10 days after anther dehiscence (anthers stored in room temperature).

Media standardization

Pollen grains were gently separated from the anthers of freshly dehisced flowers buds (n = 5) using forceps and a needle. Only pollen grains were kept on a clean glass slide by removing debris like tissues of anther-wall. Then, pre-hydration was done for 45 min within an improvised humidity chamber, a set of petri plates with moist tissue papers. The original compositions of BK medium (Brew baker and Kwack 1963) contain 100 mg/L of boric acid (H_3BO_3), 300 mg/L of calcium nitrate [$Ca(NO_3)_2$], 200 mg/L of magnesium sulphate ($MgSO_4$), 100 mg/L of potassium nitrate (KNO_3) and 15% of sucrose. Compositions of BK medium was manipulated and tested in order to standardize the media compositions for getting an optimal result for pollen germination. At first (Experiment 1), five

different concentrations of sucrose (5%, 10%, 15%, 20% and 25%) were used to establish the optimal concentration of sucrose for the maximum percentage of pollen germination and pollen tube growth. In Experiment 2, four different concentration gradients (100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L) of H_3BO_3 were taken, keeping the other medial compositions at constant amount as per original media. The new standardized value for H_3BO_3 which was obtained from previous test, maintained to standardize the next constituent of media following the same procedure and this technique was carried out to standardize each of the components of BK medium. In Experiment 3, three different concentration gradients (300 mg/L, 400 mg/L and 500 mg/L) of $Ca(NO_3)_2$, three different concentration gradients (Experiment 4: 200 mg/L, 300 mg/L and 400 mg/L) of $Mg(SO_4)$ and four different concentration gradients (Experiment 5: 100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L) of KNO_3 were tested separately to get optimum results for pollen germination. Five different concentration gradients (10^{-2} M, 10^{-3} M,10^{-4} M, 10^{-5} M and 10^{-6} M) of each type of three mentioned polyamines and four different concentration gradients (5%, 10%, 15% and 20%) of PEG were tested with SGM to obtain standardized values of those constituents, for pollen germination and pollen tube growth.

Results

Analysis of pollen fertility

Fertility tests of pollen grains of *B. juncea* and *L. esculenta* were performed starting from date of collection Day 1 to Day 10 (Figure 1; Figure 2). Fertility of fresh pollen grains was found to be 100 % from the date of sample collection (Day 1). 100% fertility was observed in both the plant species on Day 4 from the date of collection. From Day 5 fertility of pollen grains of both *B. juncea* and *L. esculenta* tend to decrease. For *B. juncea*, the average values of fertility of pollen grains on Day 5 were found to be 97.19±1.32 %. In case of *L. esculenta*, the fertility of pollen grains on Day 5 was 85.39±2.22%. On Day 10, the fertility of pollen grains declined to 75.14±2.69% in case of *B. juncea* and 72.31±2.52% in case of *L. esculenta*.

Media standardization

In *B. juncea*, BK medium along with 15% sucrose solution resulted for the maximum value of percentage of pollen germination (35.84±3.29%) with a tube length of 54.72±4.95µm (Table 1, Figure 2). Maximum value of pollen tube length (PTL, 130.8±22.27µm) was obtained at 5% sucrose solution along with BK medium, although the percentage of pollen germination was significantly low (16.0±1.27%) (Table 1). Experiments were performed to standardize the various compositions of pollen germination medium. BK medium supplemented (15% sucrose) with 300 mg/L of H_3BO_3 gave the best results for the percentage of pollen germination (41.21±1.51%) and the value of pollen tube length was moderately high (51.48±2.26µm). The length of pollen tube reached its highest value (69.84 ± 3.19 µm) at a concentration of 200 mg/L boric acid. Positive responses were not observed in the values of percentage pollen germination with other concentrations (100 mg/L, 200 mg/L and 400 mg/L) of boric acid when compared with BK medium. The lowest value for PTL was 20.16±2.21µm at a concentration of 400 mg/L of H_3BO_3 in Bk medium (Table 1). Other compositions of BK medium $Ca(NO_3)_2$ (400 mg/L), $MgSO_4$ (300 mg/L) and KNO_3 (400 mg/L) gave highest values for percentage of germination of pollens (47.96±1.57%, 41.56±0.88% and 37.73±1.53% respectively) and length of pollen tube (25.6±2.82µm, 42.8±4.22µm and 50±2.51µm respectively). For $Ca(NO_3)_2$ (500 mg/L) lowest values of pollen-germination percentage (14.59±0.68%) and pollen tube length (19.6±2.27µm) were observed. The lowest value of percentage of pollen germination (10.76±0.77%) and the lowest value for PTL (19.2±2.40µm) was obtained at a concentration of 400 mg/L of $MgSO_4$. KNO_3 at a concentration of 100 mg/L and 300 mg/L resulted in the lowest value for the percentage of pollen germination (15.83±0.64%) and pollen tube length (24±2.36µm), respectively (Table 1). Therefore, SGM for pollen germination and tube growth in *B. juncea* contain H_3BO_3 (300 mg/L), $Ca(NO_3)_2$ (400 mg/L), $MgSO_4$ (300 mg/L), KNO_3 (400 mg/L) along with 15% sucrose. Application of this SGM medium showed 50.48±1.35 % germination and pollen tube length of 70.4±2.54 µm. Addition of 15% PEG in SGM gave the highest value for percentage of pollen germination (52.90±0.72%) and resulted for maximum length of pollen tube (45.2±4.93µm). Addition of various concentrations of polyamines (*viz*. spermine, spermidine & putrescine) in SGM does not show any positive response in terms of germination of pollen grain and pollen tube growth in *B. juncea* (Table 2) with respect to SGM. Out of the three polyamines, addition of 10^{-3} M concentration of putrescine significantly increased the PTL of *B. juncea* (154.2±32.85 µm) when compared with standardized pollen germination medium. But there

was no positive response for germination after the addition of these polyamines in SGM.

Table 1: Effects of different concentrations of Sucrose, Boric Acid, Calcium Nitrate, Magensium Sulphate, Potassium Nitrate on pollen germination (%) and pollen tube growth (µm) of *Brassica juncea* and *Lens esculenta*

	Brassica juncea		*Lens esculenta*	
Experiment 1: BK medium with different concentrations of sucrose	Germination (%)	Pollen tube length (µm)	Germination (%)	Pollen tube length (µm)
a) 5 % Sucrose	16.0±1.27	**130.8±22.27**	10.91±1.11	46.41±11.15
b) 10 % Sucrose	29.86±5.13	61.92±6.68	14.61±2.13	**53.55±10.87**
c) 15 % Sucrose	**35.84±3.29**	54.72±4.95	**32.52±1.89**	47.46±9.99
d) 20 % Sucrose	13.65±1.93	57.6±4.25	7.7±1.52	40.69±6.06
e) 25 % Sucrose	10.63±0.805	51.12±3.10	8.34±2.16	39.74±8.50
Experiment 2: BK medium (15% sucrose) with different concentrations of Boric Acid				
a) 100 mg/L	3.21±1.49	45.6±3.14	17.86±1.39	19.04±3.88
b) 200 mg/L	29.21±1.50	**69.84±3.19**	**38.12±0.93**	**30.94±8.03**
c) 300 mg/L	**41.21±1.51**	51.48±2.26	16.55±0.83	26.65±5.60
d) 400 mg/L	13.21±1.52	20.16±2.21	6.22±1.02	26.65±7.03
Experiment 3: BK medium (15% sucrose) with different concentrations of Calcium nitrate				
a) 300 mg/L	18.44±1.15	**39.2±3.14**	5.94±1.21	**29.98±4.23**
b) 400 mg/L	**47.96±1.57**	25.6±2.82	**34.91±0.80**	**39.99±4.50**
c) 500 mg/L	14.59±0.68	19.6±2.27	5.36±1.12	21.89±3.13
Experiment 4: BK medium (15% sucrose) with different concentrations of Magnesium sulphate				
a) 200 mg/L	13.17±1.07	22±1.57	24.65±0.86	21.89±3.94
b) 300 mg/L	**41.56±0.88**	**42.8±4.22**	**36.35±0.61**	**22.37±4.67**
c) 400 mg/L	10.76±0.77	19.2±2.40	15.82±0.86	19.99±3.41
Experiment 5: BK medium (15% sucrose) with different concentrations of Potassium nitrate				
a) 100 mg/L	15.83±0.64	30.8±2.09	23.55±0.93	**36.17±18.31**
b) 200 mg/L	21.05±0.26	35.6±3.36	**46.32±1.38**	35.32±7.85
c) 300 mg/L	24.95±1.41	24±2.36	25.20±1.15	23.8±5.79
d) 400 mg/L	**37.73±1.53**	**50±2.51**	15.30±1.47	30.94±13.65
Experiment 6: Standardized Germination medium (15 % Sucrose +300 mg/L Boric Acid + 400 mg/L Calcium nitrate + 300 mg/L Magnesium sulphate + 400 mg/L Potassium nitrate) for *Brassica juncea*	**50.48±1.35**	**70.4±2.54**		
Experiment 7: Standardized Germination medium (15 % Sucrose + 200 mg/L Boric Acid + 400 mg/L Calcium nitrate + 300mg/L Magnesium sulphate + 200mg/L Potassium nitrate) for *Lens esculenta*			**41.34 ± 2.43**	**52.02 ± 1.67**

In *L. esculenta*, BK medium supplemented with 15% sucrose solution showed best response of pollen germination (32.52±1.89%) with a pollen tube length of 47.46±9.99µm (Figure 2). Maximum pollen tube growth (53.55±10.87µm) was observed at 10% sucrose with a germination rate of 14.61±2.13% (Table 1). BK medium supplemented with H_3BO_3 (200 mg/L) resulted highest values of pollen germination (38.12±0.93%) and PTL (30.94±8.03µm). Lowest values of pollen germination (6.22±1.02%) and PTL (19.04±3.88µm) were observed at 400 mg/L and 100 mg/L of boric acid in BK medium, respectively (Table 1). Calcium nitrate (400 mg/L) showed maximum values of pollen germination (34.91±0.80%) and tube length (39.99±4.50µm). Lowest values of germination (5.36±1.12 %) and pollen tube length (21.89±3.13 µm) were observed at 500 mg/L of $Ca(NO_3)_2$ in the BK medium (Table 1). Magnesium sulphate (300 mg/L) gave best

results for pollen germination (36.35±0.61%) with maximum tube length of 22.37±4.67µm. $MgSO_4$ at 400 mg/L gave the lowest percentage of pollen grain germination (15.82±0.86%) and tube growth (19.99±3.41µm) (Table 1). KNO_3 (200 mg/L and 100 mg/L) gave best results for pollen germination (46.32±1.38%) and pollen tube growth

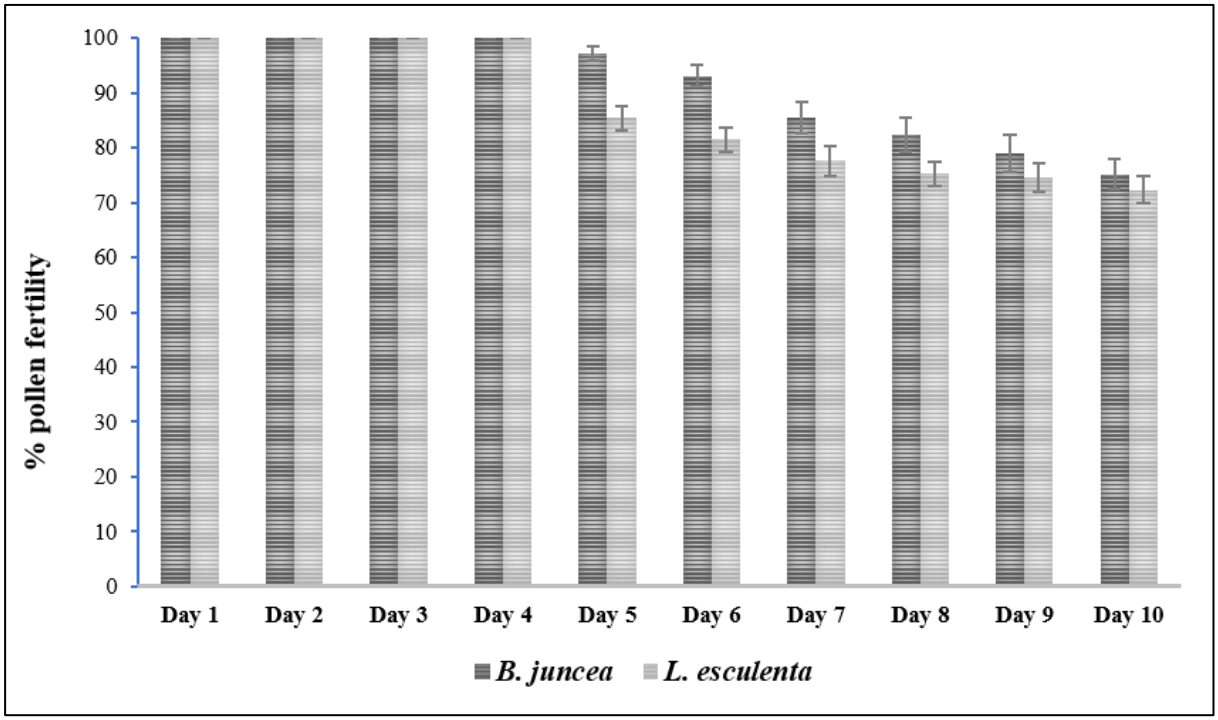

Figure 1. Percentage (mean ± SE) fertility of pollen grains at different time intervals (Day 1, Day of floral anthesis to Day 10, 10th day after floral anthesis) in *B. juncea* and *L. esculenta.*

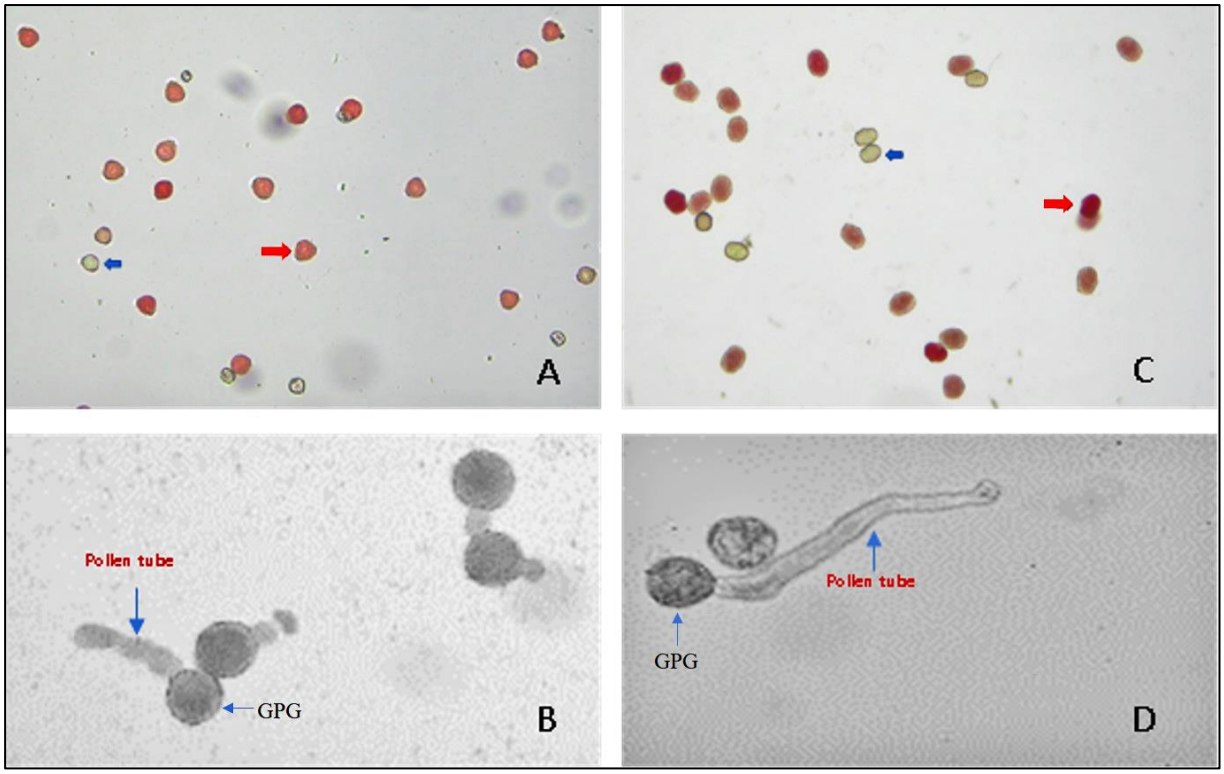

Figure 2. (A-B) Fertile pollen grains (black arrow), non-fertile pollen grain (blue arrow) and a germinated pollen grain (B) of *B. juncea*. (C-D) Fertile pollen grains (black arrow), non-fertile pollen grain (blue arrow) and a germinated pollen grain (D) of *L. esculenta*. GPG-Germinated pollen grain.

(36.17±18.31µm), respectively. KNO_3 (400 mg/L and 300 mg/L) gave the lowest value for germination of pollen grains (15.30±1.47%) and tube length (23.8±5.79µm), respectively (Table1). Composition of SGM for *L. esculenta* includes boric acid (200 mg/L), $Ca(NO_3)_2$ (400 mg/L), $MgSO_4$ (300 mg/L) and KNO_3 (200 mg/L) supplemented with 15% sucrose. When this SGM was applied in experiment, it showed 41.34±2.43% pollen germination and pollen tube length of 52.02±1.67 µm. The addition of different concentrations of PEG did not show any significant positive response when compared with SGM (Table 2). However, among all the polyamines, 10^{-4} M spermine gave the best results for the percentage of pollen germination (42.22±1.36%), while 10^{-3} M spermine showed the highest pollen tube growth (80.92±16.57µm) (Table 2).

Discussion

Both *B. juncea* and *L. esculenta* are monoecious. *B. juncea* is extremely cross-pollinated species with high level of self-incompatibility shown by its sporophyte (Hiscock and McInnis 2003). *L. esculenta* exhibits autogamous type of pollination service with very lower extent (<0.8%) of cross-pollination (Wilson and Law 1972).

In both the species, 5%, 20% & 25% of sucrose in the BK medium showed the lowest percentage of pollen germination. Moreover, higher concentrations (20% & 25%) of sucrose decreased the percentage of germination (10-13% in *B. juncea* and 7-8% in *L. esculenta*) rather than tube growth in both the species. This clearly indicates that the toxicity of the medium affects more the initiation of germination rather than tube-growth. Sucrose

Table 2: Effects of different concentrations of PEG and polyamines (spermine, spermidine & putrescine) on pollen germination (%) and pollen tube growth (µm) of *Brassica juncea* and *Lens esculenta*

Experiment 8: SGM with different concentrations of PEG	*Brassica juncea*		*Lens esculenta*	
	Germination (%)	Pollen tube length (µm)	Germination (%)	Pollen tube length (µm)
a) 5%	10.64±0.30	20.4±3.38	8.46±2.12	**50.32±10.02**
b) 10%	31.22±0.51	32.8±4.66	10.97±6.7	26.18±5.42
c) 15%	**52.90±0.72**	**45.2±4.93**	**19.32±4.07**	37.12±8.86
d) 20%	12.87±0.45	33.2±4.78	13.78±1.06	20.94±6.40
Experiment 9: SGM with different concentrations of spermine				
a) 10^{-2}M	3.01±0.26	27±5.16	28.28±2.26	35.7±7.52
b) 10^{-3}M	1.76±0.24	**63.4±14.65**	17.71±2.41	**80.92±16.57**
c) 10^{-4}M	2.67±0.25	19.4±5.28	**42.22±1.36**	68.54±19.17
d) 10^{-5}M	0.77±0.34	11±4.34	10.08±1.76	44.26±16.48
e) 10^{-6}M	**5.07±1.8**	27.2±3.43	9.80±0.77	79.49±23.02
Experiment 10: SGM with different concentrations of spermidine				
a) 10^{-2}M	**4.58±0.95**	**30.2±6.53**	5.22±0.45	6.66±1.38
b) 10^{-3}M	2.57±0.62	19±3.57	6.19±0.79	22.84±2.87
c) 10^{-4}M	1.25±0.40	11.8±4.18	7.09±0.74	28.08±7.15
d) 10^{-5}M	0.7±0.40	12.6±4.31	**8.47±0.99**	**46.17±8.82**
e) 10^{-6}M	1.60±0.09	25.4±6.17	4.50±0.76	10.94±2.45
Experiment 11: SGM with different concentrations of putrescine				
a) 10^{-2}M	2.2±0.36	18.6±3.84	7.18±0.65	**42.84±11.03**
b) 10^{-3}M	2.34±0.75	**154.2±32.85**	**11.61±3.01**	22.37±6.67
c) 10^{-4}M	6.53±2.03	22.6±5.8	10.13±3.03	35.7±12.79
d) 10^{-5}M	**8.69±4.28**	13.4±3.95	6.14±1.20	24.27±6.79
e) 10^{-6}M	7.09±1.25	12.2±4.16	4.84±1.26	5.71±1.93

maintains the osmotic balance in BK medium but sucrose at a higher concentration than the optimum level causes an inhibition of pollen germination and disruption of newly emerging small pollen tubes near the germ pore, as osmotic balance gets disrupted at very high concentrations (Vikas et al. 2012).

Sucrose at 15% in BK medium gave the optimum values of pollen germination in both the plants (35.84±3.29% in case of *B. juncea* and 32.52±1.89% in case of *L. esculenta*; Table 1). However, pollen tube growth was maximum at 5% sucrose for *B. juncea* (130.8±22.27 µm) and 10% sucrose for *L. esculenta* (3.55±10.87 µm). Sucrose is a disaccharide composed of monomers of glucose and fructose, help in germination of pollen grains and growth of pollen tubes. Most of the sucrose gets metabolized during tube growth after germination (Nygaard,1977). Our study showed that higher percentage (15%) of sucrose is needed for the optimum level of pollen germination rather than for tube growth, for both species. The amount of sucrose metabolized during pollen germination was not estimated in our study. Some fluctuations in the data were observed, this may be due to various environmental factors. At very high concentrations, sucrose creates toxicity in medium by increasing the rate of respiration which ultimately increases the permeability of the plasma membrane, thus causing toxic effects in the medium (Bair and Loomis 1941). In our study, lower concentrations of PEG gave poor responses for germination and tube growth. In *L. esculenta* PEG did not show any significant responses as compared to SGM. In *B. juncea*, 15% PEG enhances the germination percentage but decrease in pollen tube length was observed at 15% PEG. In order to avoid these negative effects of high concentrations of sucrose, PEG is used in germination medium. Function of PEG is to maintain osmotic balance in BK medium along with sucrose. Plasma membrane's permeability is controlled by PEG (Read et al. 1993). Therefore, it can also enhance the rate of pollen germination along with tube growth.

Very lower level of Boron is found to be present endogenously in pollens but it serves as an essential component for the formation of the wall of the pollen tube (Dickinson 1978; Obermeyer and Blatt 1995; Stanley and Loewus 1964) because it acts as a translocator of sucrose by forming ion-complexes with sucrose (Gauch and Dugger 1953; Sisler et al. 1956). The optimum level of boron for pollen germination was found to be 300 mg/ml and 200 mg/ml in *B. juncea* and *L. esculenta*, respectively.

Lower or higher than the optimum level of Boron in BK medium showed low germination and tube growth for both species. In our study, this clearly depicts the inhibitory responses of boron at concentrations higher than 300 mg/ml in *B. juncea* and 300 mg/ml in *L. esculenta*.

Calcium is another important component that helps in pollen tube growth (Pierson et al. 1996; Holdaway-Clarke and Helper 2003). Our study revealed that the maximum value of germination and tube growth (with exception of *B. juncea*, Table 1) was obtained at 400 mg/L concentration of $Ca(NO_3)_2$ in BK medium. Higher concentration (500 mg/L) of $Ca(NO_3)_2$, resulted in lower rate of germination of pollen grains and tube growth. MgSO4 (300 mg/L) gave optimum results of germination and tube growth in both the species. However, higher concentrations of MgSO4, resulted in significant decrease in the percentage of pollen germination and tube growth for both the species. Optimal concentrations of KNO_3 for germination and tube growth were found to be different in the two species. For *B. juncea*, 400 mg/ml of KNO_3 gave highest germination and pollen tube growth. This may be due to the presence of very low amount of endogenous K^+ ions in the pollen grains. Whereas pollen grains of *L. esculenta* need very little amount of exogenous KNO_3 (200 mg/L, 100 mg/L) for optimum germination and tube growth, respectively, suggesting that, an ample amount of endogenous K^+ is present in pollen grains of this species.

Polyamines are known for stabilizing the plasma membrane. By binding with proteins and phospholipid molecules, it stabilizes the plasma membrane (Martin-Tanguy 2001). In our study, various concentration gradients of three types of polyamines: spermine, spermidine and putrescine were added individually in SGM to observe their effects on pollen-germination and pollen tube growth. In *B. juncea*, none of the polyamines showed a positive response for germination. However, 10^{-3} M putrescine only enhanced the pollen tube growth in *B. juncea*. Thus, polyamines in *B. juncea* to some extent increase the pollen tube growth but not pollen gemination. Out of the three polyamines, only spermine (10^{-4} M) gave best responses for pollen germination in *L. esculenta*. However, pollen tube growth was observed highest in 10^{-3} M spermine in *L. esculenta*. These suggest that, higher amount of polyamine is necessary for pollen germination rather than for pollen tube growth.

Conclusion

Our current study has demonstrated the effects of different mineral salts at different concentrations in BK medium (Brew baker and Kwack 1963) augmented with various concentrations of sucrose, PEG and polyamines; for two economically important crop *B. juncea* and *L. esculenta*. The knowledge on in-vitro germination of pollen grains, suggested that compositions of BK medium need modifications to get optimum results of pollen germination and pollen tube growth. For *B. juncea*, SGM contains 15 % sucrose, 300 mg/L H_3BO_3, 400 mg/L $Ca(NO_3)_2$, 300 mg/L MgSO4 and 400 mg/L KNO_3. For *L. esculenta*, SGM contains 15 % sucrose, 200 mg/L H_3BO_3, 400 mg/L $Ca(NO_3)_2$, 300 mg/L MgSO4 and 200 mg/L KNO_3. Inclusion of PEG in SGM may increase pollen germination suggesting its role in maintaining osmotic potential of pollen grains. However, its effect may vary in other species. Addition of polyamines significantly increase pollen tube growth than control in both the species, indicate the importance of polyamines in fertilization process. All of these findings, give valuable suggestions for standardizing the pollen germination medium for *B. juncea* and *L. esculenta* which will enlighten the research in reproductive biology of angiosperms. Standardization of pollen-germination medium can be done for several other commercial crops which have not been focused yet in the area of research. So that, fruit set or seed set can occur even in the absence of pollinators; by manual pollination and fruit or seed set can be enhanced by manual cross pollination, using the in vitro germinated pollens.

Acknowledgement

Nasrin Banu Khan, Soumya Chatterjee, Ganapati Basak, Purba Sarkar and Chandan Barman acknowledge the assistance of the University of Gour Banga administration in Malda, India. Ganapati Basak and Nasrin Banu Khan sincerely thanks Swami Vivekananda Merit-Cum-Means Scholarship, Govt. of West Bengal for research grants. Soumya Chatterjee sincerely thanks Council of Scientific and Industrial Research (CSIR, New Delhi, Govt. of India) as he receives research grants in the form of Senior Research Fellowship.

Competing interest:

Authors declare that there is no competing interest regarding this study.

Reference

Ahmad N, Sinha DK, Singh KM (2018) Economic analysis of production and instability of lentil in major lentil growing states of India. Int J Pure Appl Biosci 6(1):593-8.

Aldahadha A, Sane KA, Bataineh A, Alloush AA, Hamouri Z (2019) Pollen viability and in vitro germination of six pistachio (Pistacia vera L.) cultivars grown in northern Jordan. Adv Hortic Sci 33(3):441-6.

Bair RA, Loomis WE (1941) The germination of maize pollen. Science 94(2433):168-9.

Biasi R, Franceschetti MM, Falasca G, Altamura MM, Bagni N (1999) Polyamines as markers in sexual reproduction of kiwifruit. Anther and Pollen: From Biology to Biotechnology.:31-43.

Brew baker JL (1967) The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. Am J Bot 54(9):1069-83.

Brew baker JL, Kwack BH (1963) The essential role of calcium ion in pollen germination and pollen tube growth. Am J Bot 50(9):859-65.

Brewbaker JL, Kwack BH (1964) The calcium ion and substances influencing pollen growth. North-Holland publishing company.

Dafni A, Firmage D (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. Pollen and pollination 113-32.

Dickinson DB (1978) Influence of Borate and Pentaerythritol concentrations on Germination and Tube Growth of Lilium longiflorum Pollen1. J Am Soc Hortic Sci 103(3):413-6.

Dresselhaus T, Franklin-Tong N (2013) Male–female crosstalk during pollen germination, tube growth and guidance, and double fertilization. Mol Plant 6(4):1018-36.

Gauch HG, Dugger Jr WM (1953) The role of boron in the translocation of sucrose. Plant Physiol 28(3):457.

Hiscock SJ, McInnis SM (2003) Pollen recognition and rejection during the sporophytic self-incompatibility response: Brassica and beyond. Trends Plant Sci 8(12):606-13.

Holdaway-Clarke TL, Hepler PK (2003) Control of pollen tube growth: role of ion gradients and fluxes. New Phytolo 159(3):539-63.

Hong-Qi Z, Croes AF (1982) A new medium for pollen germination in vitro. Acta Bot Neerl 31(1-2):113-9.

Jat L, Rai SK, Choudhary JR, Bawa V, Bharti R, Sharma M, Sharma M (2019) Phenotypic evaluation of genetic diversity of diverse Indian mustard (Brassica juncea L. Czern and Coss) genotypes using correlation and path analysis. International Journal of Bio-resource and Stress Management 10(5):467-71.

Martin-Tanguy J (2001) Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth Regul 34:135-48.

Nygaard PE (1977) Utilization of exogenous carbohydrates for tube growth and starch synthesis in pine pollen suspension cultures. Physiol Plant 39(3):206-10.

Obermeyer G, Blatt MR (1995) Electrical properties of intact pollen grains of Lilium longiflorum: characteristics of the non-germinating pollen grain. J Exp Bot 46(7):803-3.

Pacini E, Dolferus R (2016) The trials and tribulations of the plant male gametophyte—Understanding reproductive stage stress tolerance. In Abiotic and biotic stress in plants-recent advances and future perspectives. IntechOpen.

Pierson ES, Miller DD, Callaham D, Van Aken J, Hackett G, Hepler PK (1996) Tip-localized calcium entry fluctuates during pollen tube growth. Dev Biol 174(1):160-73.

Qureshi SJ, Khan MA, Arshad M, Rashid A, Ahmad M (2009) Pollen fertility (viability) status in Asteraceae species of Pakistan. Trakia J Sci 7(1):12-6.

Read SM, Clarke AE, Bacic A (1993) Stimulation of growth of cultured Nicotiana tabacum W 38 pollen tubes by poly (ethylene glycol) and Cu (II) salts. Protoplasma 177:1-4.

Rodriguez-Enriquez MJ, Mehdi S, Dickinson HG, Grant-Downton RT (2013) A novel method for efficient in vitro germination and tube growth of Arabidopsis thaliana pollen. New Phytol 197(2):668-79.

Rosbakh S, Pacini E, Nepi M, Poschlod P (2018) An unexplored side of regeneration niche: seed quantity and quality are determined by the effect of temperature on pollen performance. Front Plant Sci 9:384-748.

Shivanna KR, Rangaswamy NS (1992) Self-incompatibility. Springer.

Shivanna KR, Saxena NP, Seetharama N (1997) An improvised medium for in vitro pollen germination and pollen tube growth of chickpea. International Chickpea Newsletter 4: 28-29

Sisler EC, Dugger Jr WM, Gauch HG (1956) The role of boron in the translocation of organic compounds in plants. Plant Physiol 31(1):11.

Stanley RG, Linskens HF (1964) Enzyme activation in germinating Petunia pollen. Nature 203(4944):542-4.

Stanley RG, Loewus FA (1964) Boron and myo-inositol in pollen pectin biosynthesis. Pollen physiology and fertilization 1964:128-36.

Tandon R, Manohara TN, Nijalingappa BH, Shivanna KR (1999) Polyethylene glycol enhances in vitro germination and tube growth of oil palm pollen. Indian J Exp Biol 37: 169-172.

Taylor LP, Helper PK (1997) Pollen germination and tube growth. Annu Rev Plant Physiol Plant Mol Biol 48:461-491.

Vikas VK, Tandon R (2012) Polyethylene glycol and Polyamines promote Pollen germination and Tube growth in Azadirachta indica (Meliaceae). The International Journal of Plant Reproductive Biology 4:23-9.

Williams JH, Reese JB (2019) Evolution of development of pollen performance. Curr Top Dev Biol. 1;131: 299-336.

Wilson VE, Law AG (1972) Natural Crossing in Lens esculenta Moench1. J Am Soc Hortic Sci 97(1):142-3.

Zhu B, Tu Y, Zeng P, Ge X, Li Z (2016) Extraction of the constituent sub genomes of the natural allopolyploid rapeseed (Brassica napus L.). Genetics 204(3):1015-27.