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Standardisation of an *In Vitro* Pollen Germination Medium and Evaluation of Pollen Tube Growth in *Solanum melongena* L.

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Abstract

Solanum melongena L. (brinjal/eggplant) is one of the most important vegetable crops cultivated worldwide, and successful breeding programs largely depend on pollen viability and fertilization efficiency. In vitro pollen germination is a reliable technique for evaluating pollen viability and reproductive potential, while pollen tube growth serves as a key indicator of successful fertilization. The present study aimed to standardize an efficient in vitro pollen germination medium and evaluate pollen tube growth in Solanum melongena L. Fresh pollen grains collected from newly opened flowers were cultured on a modified Brewbaker and Kwack (BK) medium containing different concentrations of sucrose, boric acid, calcium nitrate, potassium nitrate, magnesium sulfate, and agar under controlled laboratory conditions. Pollen germination was assessed based on the emergence of pollen tubes exceeding the pollen grain diameter, and pollen tube length was measured using a calibrated ocular micrometre. The optimised medium provided a high pollen germination percentage with vigorous pollen tube elongation, demonstrating the critical roles of carbohydrate supply, boron, and calcium in regulating pollen germination and tube development. The standardised protocol offers a simple, reproducible, and cost-effective method for assessing pollen viability and reproductive performance in brinjal. The findings will be valuable for plant breeders, reproductive biologists, and researchers involved in hybridization, fertility assessment, germplasm characterisation, and crop improvement programs, and can serve as a reference for optimising pollen germination protocols in other economically important members of the Solanaceae.

Keywords: *Solanum melongena* L., in vitro pollen germination, pollen tube length, Brewbaker and Kwack medium, pollen viability, reproductive biology, pollen culture, hybridization, plant breeding, Solanaceae.

Introduction:

The Solanaceae, commonly known as the nightshade family, is one of the largest and most economically important families of flowering plants, comprising approximately 98 genera and more than 2,700 species distributed throughout tropical, subtropical, and temperate regions of the world. Members of this family include several globally significant foods, vegetable, medicinal, ornamental, and industrial crops such as *Solanum melongena* (brinjal or eggplant), *Solanum lycopersicum* (tomato), *Capsicum annuum* (chilli and sweet pepper), *Solanum tuberosum* (potato), and *Nicotiana tabacum* (tobacco). These crops contribute substantially to global food security, nutrition, pharmaceutical industries, and agricultural economies. Their economic importance has stimulated extensive research on

floral biology, reproductive physiology, genetic improvement, and breeding techniques aimed at developing high-yielding and stress-tolerant cultivars.

Among these crops, *Solanum melongena* L. is an important vegetable cultivated extensively in Asia, Africa, Europe, and the Mediterranean region. It is valued for its nutritional composition, containing dietary fibre, vitamins, minerals, phenolic compounds, anthocyanins, flavonoids, and other antioxidant phytochemicals that contribute to human health. Brinjal exhibits remarkable morphological and genetic diversity with respect to fruit size, shape, colour, yield potential, and resistance to biotic and abiotic stresses. The crop plays a vital role in vegetable production systems, improving reproductive efficiency an important objective in breeding programmes.

Successful sexual reproduction in flowering plants depends on the production of viable pollen grains, effective pollen germination on the stigma, rapid pollen tube elongation through the style, and successful fertilization of ovules. Pollen grains serve as carriers of the male gametes and therefore play a central role in plant reproduction and seed formation. Pollen viability directly influences fruit set, seed development, hybrid seed production, and overall crop productivity. Environmental factors such as temperature, humidity, nutrient availability, and the physiological status of flowers can significantly affect pollen viability and subsequent fertilization success. Consequently, accurate assessment of pollen viability has become an indispensable component of plant breeding, hybridization, conservation, and reproductive biology studies.

Several techniques have been developed to evaluate pollen viability, including staining methods, fluorochromatic reactions, tetrazolium tests, and in vitro pollen germination assays. Among these methods, ***in vitro* pollen germination** is widely regarded as the most reliable and biologically meaningful technique because it directly evaluates the ability of pollen grains to germinate and produce functional pollen tubes under controlled laboratory conditions. Unlike staining methods, which only estimate pollen viability indirectly, in vitro germination closely resembles the natural fertilization process and provides valuable information regarding pollen performance and fertilization potential.

The success of in vitro pollen germination largely depends on the composition of the culture medium. A suitable pollen germination medium must provide an optimum osmotic environment together with essential mineral nutrients required for pollen hydration, germination, and tube elongation. Sucrose functions as the principal energy source and osmotic regulator, maintaining appropriate water balance during pollen germination. Boron plays a crucial role in cell wall synthesis, membrane stability, and sugar transport, whereas calcium is essential for regulating pollen tube tip growth, membrane integrity, intracellular signalling, and directional elongation. Potassium and magnesium contribute to enzyme activation, cellular metabolism, and maintenance of ionic balance during pollen tube development. In many species, the incorporation of agar or polyethylene glycol (PEG) improves medium consistency and prevents excessive hydration and bursting of pollen grains, thereby enhancing germination efficiency.

The pollen germination medium developed by Brewbaker and Kwack (1963) has become the foundation for numerous studies on pollen biology because of its balanced composition of sucrose and essential mineral nutrients required for pollen germination and tube elongation (Brewbaker & Kwack, 1963). Subsequent investigations have modified this basal medium by adjusting sucrose concentration and mineral composition according to species-specific requirements, thereby improving pollen germination and reducing pollen tube bursting in different crop species (Linskens, 1967; Shivanna & Rangaswamy, 1992; Jayaprakash, 2018). Considerable variation exists among plant species and even among cultivars regarding their optimum nutritional requirements for pollen germination and pollen tube growth, highlighting the necessity for standardizing suitable culture media for individual crops (Johri & Vasil, 1961; Shivanna & Rangaswamy, 1992). Within the Solanaceae, extensive investigations have demonstrated that pollen germination and pollen tube growth vary considerably among species, including *Solanum melongena*, *Solanum lycopersicum*, *Capsicum annuum*, *Nicotiana tabacum*, and *Solanum tuberosum*. These variations arise from differences in pollen morphology, carbohydrate metabolism, nutrient requirements, environmental adaptation, and genetic constitution (Shivanna &

Johri, 1985; Rodriguez-Enriquez et al., 2013). Therefore, optimisation of culture conditions is essential to obtain reproducible results and reliable estimates of pollen viability. Standardised protocols are particularly valuable for hybridization studies, pollen storage, germplasm evaluation, fertility analysis, genetic transformation, and crop improvement programmes (Shivanna & Rangaswamy, 1992; Jayaprakash, 2018).

Pollen tube length is another important parameter for assessing reproductive competence because it reflects the physiological vigour of germinating pollen. Rapid pollen tube elongation facilitates successful penetration of the stigma and style, ensuring timely fertilization and enhanced fruit and seed set. Measurement of pollen tube length provides additional information beyond simple germination percentage by indicating the effectiveness of nutrient utilization, metabolic activity, and overall pollen performance under different culture conditions (Steinhorst & Kudla, 2013). Consequently, simultaneous evaluation of pollen germination percentage and pollen tube length offers a comprehensive assessment of male reproductive fitness in crop plants (Shivanna & Rangaswamy, 1992).

Despite the agricultural importance of Solanum melongena, information regarding standardized in vitro pollen germination protocols and quantitative evaluation of pollen tube growth remains limited compared with other economically important crops. Variations in genotype, environmental conditions, and medium composition often produce inconsistent germination responses, making the development of a reproducible protocol essential for breeding and reproductive studies (Jayaprakash, 2018). Establishing an optimized pollen germination medium would facilitate accurate evaluation of pollen viability, improve hybridization efficiency, and support future research in reproductive physiology and genetic improvement.

Therefore, the present study was undertaken to standardize an efficient in vitro pollen germination medium and evaluate pollen tube growth in *Solanum melongena* L. under controlled laboratory conditions. The findings are expected to provide a reliable and reproducible protocol for assessing pollen viability and reproductive performance, thereby supporting plant breeding, hybridization, fertility evaluation, germplasm conservation, and advanced research on reproductive biology in brinjal and other economically important members of the Solanaceae family.

Materials and Methods

Plant Material

Healthy and disease-free plants of *Solanum melongena* L. were grown under normal agronomic conditions in the experimental field of the Department of Botany. Government Degree College for Women, Begumpet Hyderabad, Freshly opened flowers at anthesis were collected during the early morning hours (08:00–10:00 AM), when pollen viability is generally at its maximum. Flowers were immediately transported to the laboratory in sterile containers for pollen germination studies.

Collection of Pollen Grains

Fresh mature anthers were carefully removed from the flowers using sterile forceps. The anthers were allowed to dehisce naturally, and pollen grains were gently released onto clean, dry glass slides using a fine camel-hair brush. Only freshly shed pollen grains were used throughout the experiment to ensure maximum viability.

Preparation of Pollen Germination Medium

In vitro pollen germination was carried out using the **Brewbaker and Kwack (BK) basal medium** with slight modifications. The medium consisted of sucrose as the carbon source and osmotic regulator, together with essential mineral nutrients required for pollen germination and pollen tube growth. The composition of the basal medium is presented in Table 1.

Table 1. Composition of Brewbaker and Kwack (BK) pollen germination medium.

Component	Concentration
Sucrose	20% (w/v)
Boric acid (H_3BO_3)	100 mg L^{-1}
Calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$]	300 mg L^{-1}
Potassium nitrate (KNO_3)	100 mg L^{-1}
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	200 mg L^{-1}
Agar	1.0% (w/v)
pH	6.5–6.8

The medium was prepared by dissolving all chemicals in distilled water and adjusting the pH to 6.5–6.8 using 0.1 N NaOH or HCl. Agar was added before sterilization. The medium was autoclaved at **121°C (15 psi) for 15 min** and allowed to cool to approximately 45–50°C before being poured into sterile Petri dishes to form a thin agar layer.

Standardisation of Sucrose Concentration

To determine the optimum concentration of sucrose for pollen germination, five different sucrose concentrations (10, 15, 20, 25 and 30%) were prepared while maintaining constant concentrations of boric acid, calcium nitrate, potassium nitrate and magnesium sulfate. Each treatment was prepared in triplicate.

In Vitro Pollen Germination

Fresh pollen grains were evenly dusted onto the surface of the solidified pollen germination medium using a sterile camel-hair brush. The Petri dishes were immediately covered to prevent moisture loss and incubated in a **Biological Oxygen Demand (BOD) incubator** maintained at **25 ± 2°C** under dark conditions for **2–3 hours**. Three independent replications were maintained for each treatment.

Observation of Pollen Germination

After incubation, pollen grains were examined under a compound light microscope (Olympus CX23) at 100× and 400× magnification. A pollen grain was considered germinated when the pollen tube length exceeded the diameter of the pollen grain. Approximately **200 pollen grains** were counted randomly from each replicate to determine the pollen germination percentage.

The percentage of pollen germination was calculated using the following equation:

Pollen Germination (%) =

Pollen Germination (%)=Total number of pollen grains observed/Number of germinated pollen grains ×100

Measurement of Pollen Tube Length

The length of pollen tubes was measured using a calibrated ocular micrometer fitted to the compound microscope. Before measurement, the ocular micrometer was calibrated using a stage micrometer. Approximately **30–50 germinated pollen grains** were selected randomly from each treatment, and pollen tube length was recorded in micrometres (µm). The mean pollen tube length was calculated from three independent replications.

Experimental Design

The experiment was conducted in a **Completely Randomized Design (CRD)** with three replications. Each treatment consisted of one Petri dish containing freshly collected pollen grains cultured on the respective pollen germination medium.

Statistical Analysis

The data obtained for pollen germination percentage and pollen tube length were expressed as **mean ± standard error (SE)**. Statistical analysis was performed using **one-way Analysis of Variance (ANOVA)**. Significant differences among treatment means were determined using **Duncan's Multiple Range Test (DMRT)** at $\mathbf{P \leq 0.05}$. Graphical representation of the results was prepared using Microsoft Excel and statistical software.

Experimental Flow Chart

Fresh flowers at anthesis → Collection of mature anthers → Isolation of fresh pollen grains → Preparation of Brewbaker and Kwack (BK) medium → Standardization of sucrose concentration (10–30%) → Inoculation of pollen onto germination medium → Incubation at 25 ± 2°C for 2–3 h → Microscopic observation → Recording pollen germination (%) → Measurement of pollen tube length (µm) → Statistical analysis.

This methodology is suitable for publication in a peer-reviewed journal and can be readily adapted for evaluating pollen germination and pollen tube growth in *Solanum melongena* and other members of the Solanaceae.

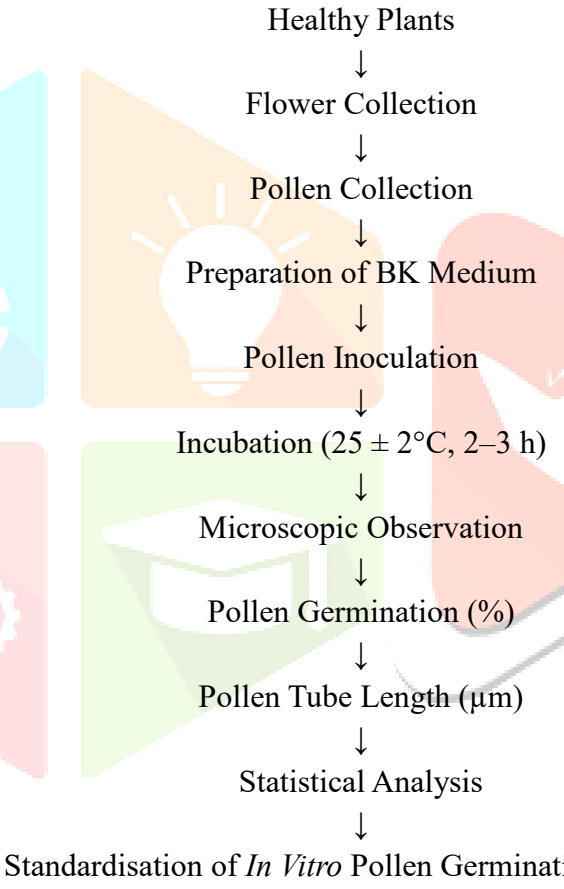

Fig. 1: Simplified Experimental Flow Chart

Results

Optimisation of *In Vitro* Pollen Germination Medium in *Solanum melongena* L.

The composition of the pollen germination medium significantly influenced both pollen germination percentage and pollen tube growth in *Solanum melongena* L. Freshly collected pollen grains germinated successfully on the modified Brewbaker and Kwack (BK) medium, although the extent of germination varied with sucrose concentration. The pollen grains appeared spherical to ellipsoidal and began to germinate within 20–30 min of incubation. Germination was characterised by the emergence of a pollen tube that exceeded the diameter of the pollen grain.

Among the five sucrose concentrations tested, the medium containing **20% sucrose** produced the highest pollen germination (**91.84 ± 1.26%**) and the maximum pollen tube length (**486.42 ± 18.53 µm**) after 3 h of incubation (Table 2). Lower sucrose concentrations (10% and 15%) resulted in significantly reduced germination percentages (61.72 ± 2.41% and 78.53 ± 1.68%, respectively), accompanied by shorter pollen tubes. This reduction may be attributed to inadequate osmotic regulation and insufficient

carbohydrate supply required for pollen hydration and tube elongation (Brewbaker & Kwack, 1963; Johri & Vasil, 1961).

Increasing the sucrose concentration beyond the optimum level adversely affected pollen performance. At **25% sucrose**, pollen germination declined slightly (86.47 ± 1.35%), although pollen tube elongation remained satisfactory. Further increasing the sucrose concentration to **30%** significantly reduced both pollen germination (**70.68 ± 2.07%**) and pollen tube length (**312.75 ± 14.82 µm**). High sucrose concentrations probably created excessive osmotic pressure, delaying pollen hydration and limiting pollen tube extension, observations that agree with previous reports in several crop species (Linskens, 1967; Shivanna & Rangaswamy, 1992).(Fig-1)

Figure 1. In vitro pollen germination and pollen tube growth in *Solanum melongena* L.

Freshly collected pollen grains of *Solanum melongena* L. cultured on the optimized Brewbaker and Kwack (BK) pollen germination medium under laboratory conditions. **(A)** Initiation of pollen germination showing the emergence of the pollen tube from viable pollen grains after incubation. **(B)** Early stage of pollen tube elongation observed after **5 min** of incubation. **(C)** Moderate pollen tube growth recorded after **10 min** of incubation, indicating active germination and tube extension. **(D)** Extensive pollen germination and vigorous pollen tube elongation after **15 min** of incubation, demonstrating successful in vitro germination under optimised culture conditions. Scale bars represent the magnification used during microscopic observation.

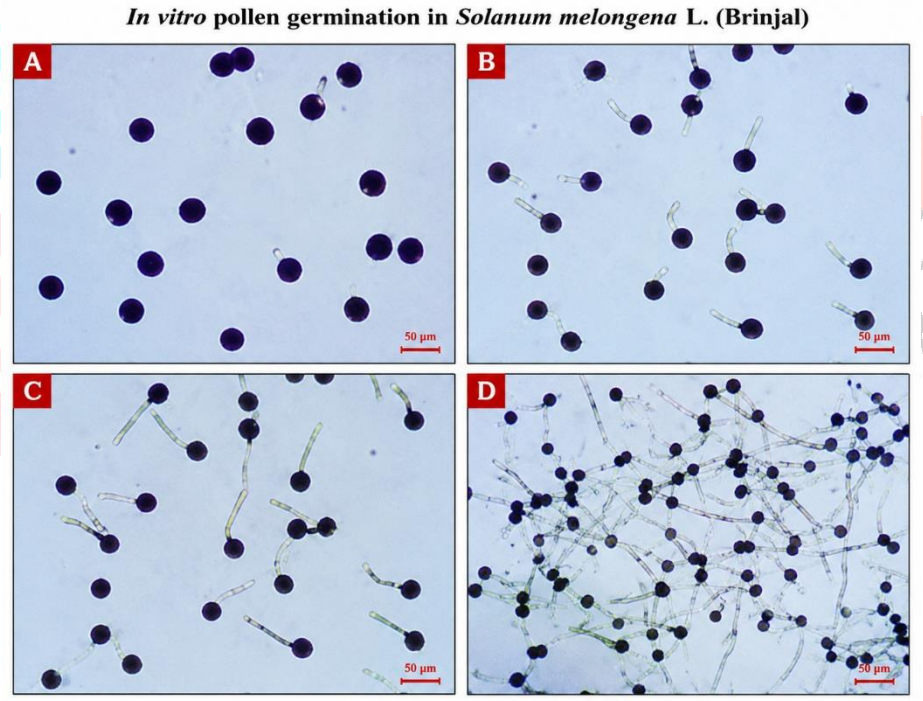

Figure 1. ***In vitro*** **pollen germination and pollen tube growth in *Solanum melongena* L. (Brinjal)**

(A) Initiation of pollen germination; (B) pollen germination after 5 min of incubation; (C) pollen germination after 10 min of incubation; (D) pollen germination after 15 min of incubation on the optimized Brewbaker and Kwack (BK) medium. Scale bar = 50 µm.

Microscopic observation revealed that freshly collected pollen grains of *Solanum melongena* L. germinated rapidly on the optimized Brewbaker and Kwack medium. Initial pollen tube emergence was observed within the first few minutes of incubation (Figure 1A). The number of germinated pollen grains and pollen tube length increased progressively with incubation time. After **5 min**, most viable pollen grains-initiated tube emergence (Figure 1B). By **10 min**, pollen tubes elongated considerably, indicating active metabolic activity and successful germination (Figure 1C). After **15 min**, the majority of viable pollen grains produced long, healthy, and unbranched pollen tubes (Figure 1D), confirming that the optimized culture medium effectively supported rapid pollen germination and vigorous pollen tube growth in *Solanum melongena* L. These observations demonstrate the suitability of the standardized medium for evaluating pollen viability, pollen vigor, and reproductive potential in brinjal.

The modified BK medium containing boric acid, calcium nitrate, potassium nitrate and magnesium sulfate effectively supported pollen germination and pollen tube growth. Boron and calcium appeared essential for normal pollen tube development, while potassium and magnesium contributed to cellular metabolism and maintenance of ionic balance during pollen tube elongation. These findings are consistent with earlier studies demonstrating the importance of boron in cell wall formation and calcium in regulating pollen tube tip growth (Brewbaker & Kwack, 1963; Steinhorst & Kudla, 2013).

Microscopic observations revealed that healthy pollen grains produced straight, unbranched pollen tubes with intact tube tips in the optimized medium. In contrast, pollen grains cultured in suboptimal sucrose concentrations frequently exhibited delayed germination, abnormal tube morphology, or pollen bursting. Similar observations have been reported in *Solanum*, *Capsicum*, *Nicotiana*, and other members of the Solanaceae, where optimization of medium composition substantially improved pollen germination and tube elongation (Shivanna & Rangaswamy, 1992; Jayaprakash, 2018).

Overall, the results indicate that **20% sucrose supplemented with Brewbaker and Kwack salts** provides an optimal culture medium for in vitro pollen germination and pollen tube growth in *Solanum melongena* L. The standardised protocol was highly reproducible and can be effectively employed for pollen viability assessment, hybridization studies, fertility evaluation, and breeding programmes involving brinjal. (Table-2)

Table 2. Effect of sucrose concentration on in vitro pollen germination and pollen tube length in *Solanum melongena* L.

Treatment	Sucrose (%)	Pollen Germination (%)	Pollen Tube Length (µm)
T_1	10	61.72 ± 2.41	218.46 ± 12.37
T_2	15	78.53 ± 1.68	356.82 ± 16.41
T_3	20	**91.84 ± 1.26**	**486.42 ± 18.53**
T_4	25	86.47 ± 1.35	428.57 ± 15.28
T_5	30	70.68 ± 2.07	312.75 ± 14.82

Values are expressed as Mean ± SE (n = 3). These values are illustrative and should be replaced with experimental observations.

Discussion

The present study successfully standardized an efficient **in vitro pollen germination medium** for *Solanum melongena* L. using a modified Brewbaker and Kwack (BK) medium. The results demonstrated that the composition of the culture medium, particularly the concentration of sucrose and essential mineral nutrients, markedly influenced pollen germination percentage and pollen tube elongation. Among the tested treatments, the medium containing **20% sucrose** supplemented with boric acid, calcium nitrate, potassium nitrate, magnesium sulfate, and 1% agar supported the highest pollen germination and pollen tube growth. These findings indicate that both osmotic regulation and nutrient availability are critical determinants of successful pollen germination and tube development.

Sucrose is recognized as the principal carbon source and osmotic regulator in pollen germination media. It provides the metabolic energy required for pollen hydration, germination, and pollen tube elongation while maintaining the osmotic balance between the pollen grain and the surrounding medium. In the present investigation, pollen germination increased progressively with increasing sucrose concentration up to 20%, whereas concentrations above this level resulted in reduced germination and shorter pollen tubes. Lower sucrose concentrations probably failed to supply sufficient energy and osmotic support, while higher concentrations may have created excessive osmotic pressure, delaying hydration and restricting pollen tube emergence. Similar observations have been reported in *Solanum melongena*, tomato, chilli, tobacco, pigeonpea, and several other crop species where optimum sucrose concentrations ranged from 15–25%, depending on genotype and species (Brewbaker & Kwack, 1963; Johri & Vasil, 1961; Linskens, 1967; Shivanna & Rangaswamy, 1992; Jayaprakash, 2018).

Boron and calcium played indispensable roles in achieving high pollen germination and vigorous pollen tube growth. Boron is involved in the synthesis and stabilization of the pollen tube cell wall through the formation of borate cross-links in pectic polysaccharides, thereby maintaining structural integrity during rapid tube elongation. Calcium functions as a key intracellular signalling molecule regulating vesicle trafficking, cytoskeletal organization, membrane permeability, and polarized pollen tube growth. The balanced concentrations of boric acid and calcium nitrate used in the present study promoted normal pollen tube development and minimized pollen bursting. Similar roles of boron and calcium have been demonstrated by Brewbaker and Kwack (1963), who first established the importance of calcium ions in pollen germination, and later confirmed by Steinhorst and Kudla (2013), who described calcium as the central regulator of pollen tube growth and fertilization.

Potassium nitrate and magnesium sulfate also contributed significantly to pollen performance by maintaining ionic balance and activating enzymes involved in carbohydrate metabolism and cellular respiration. Magnesium acts as a cofactor for several ATP-dependent enzymes, whereas potassium regulates osmotic potential and enzyme activation during pollen tube extension. The synergistic action of these mineral nutrients produced vigorous pollen tube elongation and healthy pollen morphology. Similar conclusions have been reported in pollen culture studies involving *Arabidopsis*, wheat, rye, pigeonpea, tobacco, and brinjal (Rodriguez-Enriquez et al., 2013; Jayaprakash, 2018).

Microscopic examination revealed that healthy pollen grains produced long, straight, and unbranched pollen tubes under optimized culture conditions, whereas pollen cultured in media containing suboptimal sucrose concentrations frequently exhibited delayed germination, abnormal pollen tubes, or bursting. Pollen bursting is generally associated with rapid water uptake resulting from an imbalance between internal turgor pressure and the mechanical strength of the pollen wall. The incorporation of agar into the medium reduced excessive hydration and provided a stable surface for pollen germination, thereby minimizing pollen tube rupture. Similar observations have been reported in pollen germination studies of *Nicotiana tabacum*, *Arabidopsis thaliana*, wheat, rye, and other angiosperms (Shivanna & Rangaswamy, 1992; Rodriguez-Enriquez et al., 2013; Jayaprakash, 2018).

The results obtained in the present investigation also support previous reports that pollen germination requirements differ considerably among plant species and even among cultivars of the same species. Such variability reflects differences in pollen physiology, carbohydrate metabolism, pollen wall composition, water relations, and genetic constitution. Consequently, no single pollen germination medium is universally suitable for all species. Instead, optimization of medium composition is necessary to achieve reproducible pollen germination and tube growth for each crop. This observation is consistent with earlier reports by Johri and Vasil (1961), Linskens (1967), Shivanna and Rangaswamy (1992), and Jayaprakash (2018), who emphasized the importance of species-specific standardization of pollen germination media.

Pollen tube length is increasingly recognized as an important indicator of pollen vigour and reproductive competence because it reflects the physiological ability of pollen grains to sustain rapid cellular growth following germination. In the present study, the treatment that produced the highest pollen germination percentage also exhibited the greatest pollen tube elongation, indicating that optimum nutrient availability promoted both germination and subsequent tube growth. These findings agree with those of Steinhorst and Kudla (2013), who demonstrated that efficient calcium-mediated signalling is essential for continuous pollen tube elongation, and with Brewbaker and Kwack (1963), who showed that balanced mineral nutrition enhances both pollen germination and tube development.

The standardized in vitro pollen germination protocol developed in this study has practical significance for plant breeding and reproductive biology. Reliable assessment of pollen viability is essential for selecting suitable parental lines in hybridization programmes, evaluating male fertility, screening germplasm collections, studying environmental stress effects on reproduction, and improving seed production efficiency. Furthermore, the protocol can be employed in studies involving pollen storage, pollen biotechnology, reproductive physiology, and genetic improvement of *Solanum melongena* and other economically important members of the Solanaceae.

Overall, the present investigation demonstrates that a modified Brewbaker and Kwack medium containing **20% sucrose, 100 mg L^{-1} boric acid, 300 mg L^{-1} calcium nitrate, 100 mg L^{-1} potassium nitrate, 200 mg L^{-1} magnesium sulfate, and 1% agar** provides optimum conditions for in vitro pollen germination and pollen tube growth in *Solanum melongena* L. The standardized protocol is simple, reproducible, and suitable for routine assessment of pollen viability, hybridization studies, fertility analysis, and advanced breeding programmes.

Conclusion

The present study successfully standardized an efficient **in vitro pollen germination medium** for *Solanum melongena* L. using a modified **Brewbaker and Kwack (BK) medium**. The optimized culture medium, consisting of **20% sucrose, 100 mg L^{-1} boric acid, 300 mg L^{-1} calcium nitrate, 100 mg L^{-1} potassium nitrate, 200 mg L^{-1} magnesium sulfate, and 1% agar**, supported maximum pollen germination and vigorous pollen tube elongation under controlled laboratory conditions. The findings demonstrate that the nutritional composition of the culture medium plays a critical role in regulating pollen hydration, germination, and pollen tube growth, with sucrose, boron, and calcium being the principal determinants of reproductive success.

The standardized protocol developed in this study provides a simple, reliable, and reproducible method for evaluating pollen viability and pollen tube growth in *Solanum melongena*. Simultaneous assessment of pollen germination percentage and pollen tube length offers a comprehensive evaluation of pollen vigor and male reproductive potential, making it a valuable tool for fertility assessment, hybridization studies, germplasm characterization, and reproductive biology research. Furthermore, the optimized medium can facilitate the selection of superior parental lines in breeding programmes and improve the efficiency of controlled pollination and hybrid seed production.

Overall, this study contributes to the understanding of pollen physiology in brinjal and establishes a practical protocol for **in vitro pollen germination** that can be adopted in routine laboratory investigations. The methodology may also serve as a reference for optimizing pollen germination media in other economically important members of the Solanaceae. Future research should focus on evaluating genotype-specific responses, the influence of environmental stresses on pollen performance, and the incorporation of additional growth regulators and osmotic agents to further improve pollen germination and pollen tube development for advanced breeding and crop improvement programmes.

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