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In vitro studies on the pollen viability and germination of *Impatiens cordata* Wight. an endemic species of the Southern-Western Ghats

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Abstract

Impatiens cordata is an endemic balsam in the southern Western Ghats. The present work aimed to study pollen morphology, fertility, viability, cytology, and *in vitro* pollen germination studies of *I. cordata*. The study also focused on finding a relationship between pollen viability and germination. The results revealed that the *I. cordata* pollen grains are elliptical and quadrangular. The pollen grains are bicellular, mainly tetracolpate, with some tricolpate occurrences, and have reticulate exine ornamentation. Brew baker's medium supplemented with 5% sucrose had the most remarkable ability to germinate *in vitro*. The stain and the number of days significantly affect germination. The peak of pollen viability was observed on the day of anthesis. The results of the Alexander stain were more strongly correlated with *in vitro* germination and suggested that for pollen viability studies, the Alexander stain is considered the best stain compared to others.

Keywords

Impatiens cordata ; southern Western Ghats; endemic; pollen cytology; pollen fertility; pollen germination

Introduction

Pollen grains are critical components of male gametophytes and play a fundamental role in sexual reproduction. Studying pollen characteristics is crucial for various research endeavours, including examining pollen storage, exploring interactions between pollen and pistils, gaining insights into incompatibility and fertility, and advancing breeding for crop improvement. The seed industry can also benefit significantly from pollen research (1). The quality of pollen is determined by its viability and vigour (2). Pollen fertility and viability are essential in the hybridization programme. Effective pollination is necessary for plants to produce seeds. Pollination allows for the transfer of pollen grains from the anther to the stigma, leading to fertilization. This process is vital for most plant species, directly impacting their ability to reproduce and propagate. Without adequate pollination, plants would struggle to produce the seeds necessary for survival. Thus, a comprehensive understanding of pollen biology, including pollen viability, germination, and pollen tube growth, is indispensable for developing a well-rounded strategy to enhance productivity significantly (1, 3).

Pollen viability encompasses various aspects of pollen behaviour, including its ability to fertilize, germinate, and stain (4). Commonly used

techniques for evaluating pollen viability include staining methods, *in vitro* germination assays, seed development assessment, and observing germination on excised stigmas, often called stigmatic germination. Staining pollen is done to emphasize particular compounds, contents, or cellular components linked to the viability of pollen. Starch, callose, and chromatin were visualized using potassium iodide, aniline blue, and acetocarmine stains. A lack of colouration indicates the nonviability of the pollen (5). The Alexander stain is a distinguishing agent, effectively separating aborted pollen grains from their nonaborted counterparts. In this procedure, the cytoplasm within the cells appears red, while the cell walls are green. In instances where the cytoplasm is not present, the green colouration of the cell walls becomes apparent, serving as a visual indicator of the absence of cellular activity or function (6). Heslop-Harrison devised a viability assay utilizing a fluorochromatic reaction, encompassing membrane integrity and enzyme activity assessments. Fluorescein diacetate (FDA) is an excellent dye that can penetrate the semipermeable membrane despite being nonfluorescent and polar. Upon entering the cell, the FDA undergoes hydrolysis by nonspecific esterases, accumulating fluorescein in the cytoplasm and producing a striking green fluorescence. Fluorescein can exit pollen grains through flaws in the plasmalemma, and its presence indicates the nonviability of the grains (7). Though widely employed to differentiate between viable and non-viable fresh pollen, staining methods might not effectively discern the degrees of viability. Accurate assessment of pollen viability is crucial for successful plant reproduction, and further research is needed to identify more effective techniques (8).

Examining pollen morphology involves employing light microscopy (LM) and scanning electron microscopy (SEM) to observe fundamental characteristics such as symmetry, shape, size, aperture count and position, and surface ornamentation. Transmission electron microscopy (TEM) was utilized to evaluate the stratification of the pollen wall layers and reveal distinctive cytoplasmic features (9).

Schnarf studied mature pollen grains in angiosperms, noting that they exist in binucleate or trinucleate forms. In his investigation of 143 flowering plant species, Schnarf found 41 families with binucleate species, 9 with trinucleate species, and 9 with both types (10). The Balsaminaceae family includes annual and perennial herbs with distinctive and impressive floral features. This family consists of 2 widespread genera: *Hydrocera*, a monotypic genus represented by a single species, and *Impatiens*, a genus encompassing numerous species. *Hydrocera* species can be recognized by their 5 separate petals and a capsular berry, whereas *Impatiens* species are distinguished by their 4 petals fused to form 2 lateral petals and a 5-valved capsule. *Hydrocera triflora* (L.) Wight & Arn., a semiaquatic herb, is found in the Indomalesian region (11).

Impatiens comprises more than 1,000 species (12–14). The species is distributed across tropical and subtropical areas of the Old World, and in the northern temperate regions (15, 16). This genus is recognized for its 5 distinct diversity hotspots, namely, tropical Africa, Madagascar, southern India, Sri Lanka, the Eastern Himalayas, and Southeast Asia (17). Many plants of this particular genus are deliberately cultivated for their ornamental value, while others are commonly employed in medicinal and cosmetic applications. These plants, known colloquially as 'balsams' or 'jewel weeds', are widely distributed throughout India, with approximately 210 species in the region. Interestingly, the Eastern Himalayas and the Western Ghats serve as 2 major centers of diversity for this genus in the country (18).

Our study focused on the following questions: 1. What are the morphological characteristics of *Impatiens cordata* pollen grains, and which medium is best for *in vitro* pollen germination? 2. Which staining method accurately determines the viability of *I. cordata* pollen, and is there a correlation between viability estimates obtained through staining and germination?. Pollen micromorphology is essential for accurately classifying *Impatiens species*. The findings from this research can serve as a valuable resource for upcoming studies in palynology and offer essential support for implementing artificial pollination techniques in breeding programs for *Impatiens*. The findings of this research will also help increase our understanding of the reproductive biology of this genus.

Materials and Methods

Study species

Impatiens cordata Wight is found near the banks of the Montane streams in the Mankulam forest areas of the Idukki district, which range in altitude from 1100 m above sea level. The total population available in the study area was 3, and the number of individuals per population ranges from 100 to 200.

The plants are annual herbs with decumbent to slightly erect growth habits, typically reaching heights of 15–30 cm (Fig. 1A). Stems mainly prostrate, rooting at the nodes. Leaves are arranged alternately, ovate-cordate in shape, with a glossy surface, obscurely crenate, measuring 4–7 cm in length and 3–4.5 cm in width, each with a distinct petiole. Flowers are either solitary or occur in pairs, exhibiting lilac hues with 2 purple markings at their centers, or they may be white with red centers (Fig. 1B). The lateral sepals are notable for their large, ovate shape, in contrast, the lower sepals are funnel-shaped, tapering into slender, inwardly curved spurs. The dorsal petal is small and round, while the lateral united petals are approximately 2.5 cm long, with the upper petals being minute and incurved and the lower petals significantly larger and spreading wider. The seed capsule is spindle-shaped, with a slight beak, measuring about 1 cm long.

Pollen morphology

Anthers were collected during anthesis and promptly preserved in vials with either 70% ethanol or glacial acetic acid to conduct pollen morphological studies. The acetolysis method was employed to prepare pollen samples as proposed by Erdtman (19) and Nair (20). The preserved

Fig.1. *Impatiens cordata.* (**A**) Habit (**B**) Flower and Fruit.

materials were crushed with a glass rod in a tube, and the resulting dispersion was filtered through a brass mesh (48 divisions/cm^2), collected in a glass centrifuge tube, and then subjected to centrifugation. After removing the supernatant, the pollen grains were washed with glacial acetic acid and treated with acetolysis (acetic anhydride and concentrated H2SO4 at a 9:1 ratio) in a centrifuge tube. The glass rod was used to position the tubes within a water bath heated to 70–100°C for 3–5 min until the medium adopted a brown colour. The supernatant was removed following centrifugation, and glacial acetic acid was added to the sediment. After another round of centrifugation and acid removal, the prepared pollen grains were ready for analysis. After acetolysis, the pollen grains were carefully placed in clear glycerin jelly and examined using a light microscope (LM). Using a Leica microscope (model DM750) at a magnification of 40 × 100, measurements were taken for 10 mature pollen grains per species. The acetolysed pollen grains were meticulously prepared by mounting them on gold-palladium-coated stubs and subsequently examined under a scanning electron microscope (SEM S, 2400, Hitachi). SEM micrographs were utilized primarily to analyze the general shape, size, type of ornamentation, and aperture characteristics of the pollen grains, in addition to obtaining more comprehensive information on sculpturing.

Pollen cytology

To examine the pollen cytology of I. cordata, pollen grains were extracted from the flowers when they opened. These pollen grains were then spread in a solution of 4′,6-diamidino-2-phenylindole (DAPI) on a slide. DAPI is a staining agent that enables the visualization of DNA under an ultraviolet microscope. The samples were studied at 400× magnification to analyse their cellular structures.

DAPI staining and mounting

The DAPI staining solution was prepared by dissolving 1 mg of 4′,6-diamidino-2-phenylindole (DAPI; Sigma #D9542) in 1 mL of water. The solution was sterilized by filtration and stored in 100 µL aliquots at -20°C, ensuring protection from direct light. For fresh use, on the day, 1.5 µL of a 1 mg/mL DAPI stock solution was added to 1 mL of sterile distilled water to create the DAPI staining solution. Coverslips (20 mm × 20 mm) were used. The exact size or model is not of vital importance (21).

Nail varnish

A cost-effective clear varnish is adequate for sealing the edges of the coverslip.

Scheme

A microscope slide was prepared with a fixed or fresh pollen sample, and a few drops of a working solution of 4′,6-diamidino-2-phenylindole (DAPI) were applied. A cover glass was gently placed over the sample, after which the sample was subjected to light pressure. The preparation was examined under a fluorescence microscope using a UV filter combination, and the nuclei were shown to exhibit blue fluorescence. To preserve the sample for future observation, the preparation was sealed with nail varnish and stored in a dark environment in a refrigerator.

Pollen viability tests

A comprehensive examination of the literature, specifically the work of Dafni and Firmage, was conducted to identify historical approaches to assessing pollen viability and to ascertain any challenges associated with these methods (21). The literature overviews various tests, outlining their respective advantages and disadvantages. After thoroughly reviewing the literature and laboratory resources, we have chosen 5 staining techniques to conduct our pollen viability experiment.

Acetocarmine test

According to research conducted by McKellar and Quesenberry and Marutani et al., using aceto-carmine staining for

pollen can provide valuable insights into the cytoplasmic presence of carmine. Research has suggested that viable pollen with a chromatin-rich nucleus will exhibit a pink to deep red stain. In contrast, sterile and mostly shrivelled pollen will remain nearly transparent and white, indicating a lack of stain absorption. These findings can potentially have significant implications in various fields of study and are worth considering when analysing pollen samples (22, 23).

Scheme

2 g of carmine powder were dissolved in 95 mL of 45% glacial acetic acid and diluted to 100 mL with distilled water. After boiling and cooling, the solution was filtered and refrigerated. 2–3 drops of the stain were applied to a slide, onto which pollen grains were dusted. Covering the slide with a coverslip, pollen viability was assessed after 5 to 10 min, with dark red-coloured grains indicating fertility and being counted.

Modified Alexander method

This staining method, a variant of Alexander's stain, effectively distinguishes between aborted and nonaborted pollen (6). The inclusion of acid fuchsin in the dye facilitates the staining of the protoplasm, while malachite green targets cellulose in the pollen walls. Viable pollen appears dark purple, while aborted pollen is stained green. Specifically, non-aborted pollen grains exhibit a magenta-red colour, whereas aborted pollen grains exhibit a blue-green hue (24).

Scheme

The stain was created by sequentially adding the following components: 10 mL of 95% ethanol, 1 mL of malachite green (1% solution in 95% alcohol), 50 mL of distilled water, 25 mL of glycerol, 5 mL of acid fuchsin (1% solution in water), 0.5 mL of orange G (1% solution in water), and 4 mL of glacial acetic acid. To reach a total volume of 100 mL, 4.5 mL of distilled water was added. During slide preparation, 2–3 drops of the stain were applied, and pollen grains were scattered onto the slide. The slide was then gently heated over an alcohol burner until the stain solution approached boiling, typically lasting about 30 sec. Employing a more controlled heating rate improves the penetration of the dye into the pollen's cellulose and protoplasm. Finally, coverslips were placed on the slides, and images were captured using a light microscope.

MTT (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test

This test revealed the presence of dehydrogenase (25).

Scheme

The test solution was prepared by dissolving a 1% concentration of the substrate 2,5-diphenyl tetrazolium bromide (MTT or thiazolyl blue) in 5% sucrose. The pollen grains were deemed viable if it turned a deep pink color or if it remained colorless but displayed irregular black lines on its surface.

FCR test

The fluorochromatic reaction (FCR) is a commonly employed method for assessing esterase activity and the integrity of the plasma membrane (7).

Scheme

As per the FDA protocol, the FDA standard solution was made by mixing 10 mg of fluorescein diacetate in 5 mL of acetone. The test solution was created by introducing a few drops of the stock solution into 1 mL of a 10% sucrose solution until the resulting mixture displayed consistent turbidity. A sufficient quantity of pollen grains was then immersed in a drop of FDA solution to ensure even distribution, and the mixture was incubated in a humidity chamber with a humidity exceeding 90% for 5–10 min. Subsequently, the samples were examined using a fluorescence microscope (Leica DME, Germany). Pollen grains exhibiting a bright yellowish-green fluorescence were considered viable (1).

Iodine-potassium iodide (IKI) test

The method outlined assesses the potential and starch concentration of pollen grains. When iodine is dissolved in a watery potassium iodide solution, it interacts with starch, forming a blue-black colour due to the breakdown of tri-iodide-anion complexes (5).

Scheme

1 g of potassium iodide and 0.5 g of iodine were combined in distilled water to create a solution with a total volume of 100 mL. 1 or 2 drops of the resulting dye were applied to the pollen, ensuring thorough mixing. The sample was placed under a cover slip and allowed to sit for 5 to 10 min. Subsequently, the microscope slides were examined to determine the quantity of darkly stained (indicating viability) pollen grains (39).

In vitro pollen germination

The experiments were carried out using various sucrose concentrations (1%, 3%, 4%, 5%, 6%, 7%, and 10%) independently and in combination with Brewbacker medium. Fresh pollen samples were collected on the day of anthesis and subsequently distributed onto microscopic slides containing a solution of sucrose, either alone or in combination with Brew Backer, at varying concentrations. The slides were then placed inside Petri plates with moist filter paper. The slides were observed at different time intervals using the prescribed method. Pollen grains were deemed to have germinated once their pollen tubes extended to a length twice that of the original pollen size within the surrounding medium. 4 hrs after the initiation of germination in a humid chamber, the lengths of the pollen tubes at different concentrations were measured, and the pollen germination percentage was calculated. The lengths of the pollen tubes were subsequently determined by calculating their average.

Data analysis

A two-way analysis of variance was used to compare the germination rates of plants on different days using SPSS version 25. A simple linear regression investigated the relationship between different stains' pollen viability and the Brewbaker medium's germination rate.

Results

Pollen morphology

Under light microscopic observation, 60% of pollen grains are peroblate, and 40% are euoblate. The average size of pollen measures from 39.9 ± 1.17 × 51.3 ± 0.83 µm. The polar outline shape shows variation, and it may be quadrangular or acute convex. The equatorial outline is elliptic. Aperture analysis indicated that the nature of the pollen grains' aperture was either tricolpate or tetracolpate. (Fig. 2B and 2C). The exine had an average thickness of 1.7 ± 0.07 µm. SEM observation also revealed that the exine ornamentation is reticulate (Fig. 2A).

Pollen Cytology

Microscopic observation of matured pollen grains from *Impatiens cordata* before pollination indicated that their cytological nature was bicellular (Fig. 3).

Pollen viability studies

Acetocarmine-glycerin staining demonstrated that 80.39 ± 1.52% of the pollen grains were fertile on the day of anthesis (Fig. 4F). The modified Alexander stain test revealed that 88.11 ± 1.16% of the pollen grains were viable (Fig. 4A). Also, the MTT test indicated that 61.44 ± 0.68% of the pollen grains were viable on the day of anthesis (Fig. 4E). Viability was also confirmed by the fluorochromatic reaction test (FCR), which revealed that 74.08 ± 1.17% of the pollen grains were viable on the day of anthesis, with viability decreasing on successive days (Fig. 4B). The iodine-potassium iodide (IKI) test indicated that 81.30 ± 0.93 pollen grains were viable (Fig. 4C). The Alexander staining method revealed, 81.7 ± 2.47% of pollen grains were viable. All the viability tests revealed that pollen grains were maximally viable on the day of anthesis. The results of different viability tests performed at different anthesis stages are depicted in Fig. 5.

In vitro pollen germination studies

The investigation of *in vitro* pollen germination with varying sucrose concentrations demonstrated that 80 ± 1.23% of the pollen grains were successfully germinated, with an average pollen tube length of 911 ± 2.5 µm in 5% sucrose media. Notably, the germination percentage markedly decreased in media with higher sucrose concentrations (Fig. 6). Brew baker medium supplemented with 5% sucrose was the best medium (81.6 ± 2.63 %). After 4 hrs of incubation, the pollen tube reached an average length of 1016 ± 4.6 µm in this medium (Fig. 4D).

Staining versus in vitro pollen germination at different floral stages

Two-way analysis was conducted to investigate whether there was an interaction effect between two independent variables (day and stain) on the continuous dependent variable (germination). The examination demonstrated a notable variance in the average rates of pollen germination across various staining techniques ($F=2084$, $p<0.05$) and different stages of flower maturation ($F=47930$, $p<0.05$). Furthermore, there was a significant interaction effect between stain and days on the mean values of plant germination ($F=252$, $p<0.05$). In the case of *I. cordata*, the correlation between *in vitro* germination and the 5 stains used did not show any significant difference. These results are depicted in Fig. 7–11. Among the different stains, the alexander stain exhibited the strongest correlation ($R=0.139$) with *in vitro* germination.

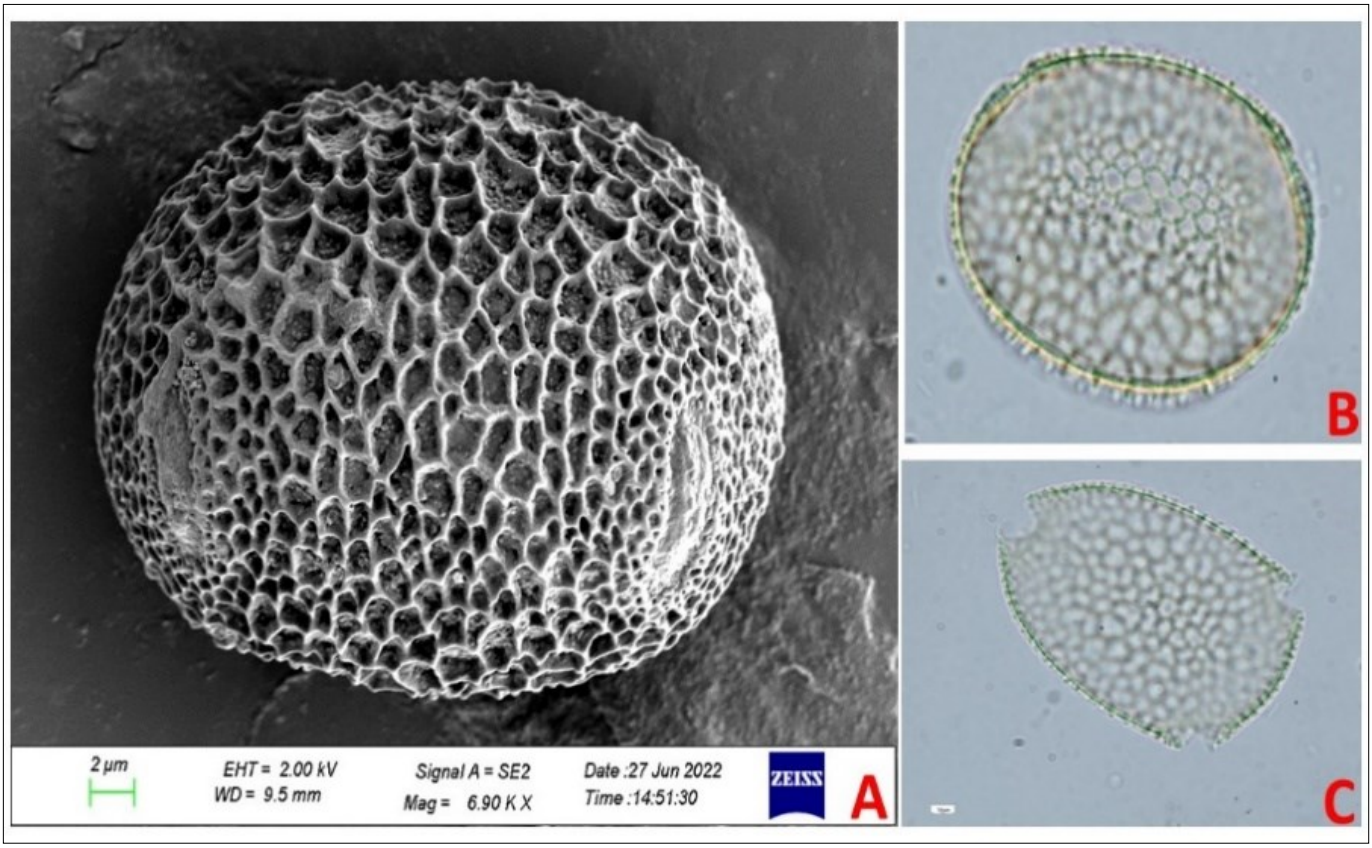

Fig. 2. Pollen morphology. (**A**) SEM image of pollen, (**B**) Tetracolpate pollen light microscopic view, (**C**) Tricolpate pollen light microscopic view.

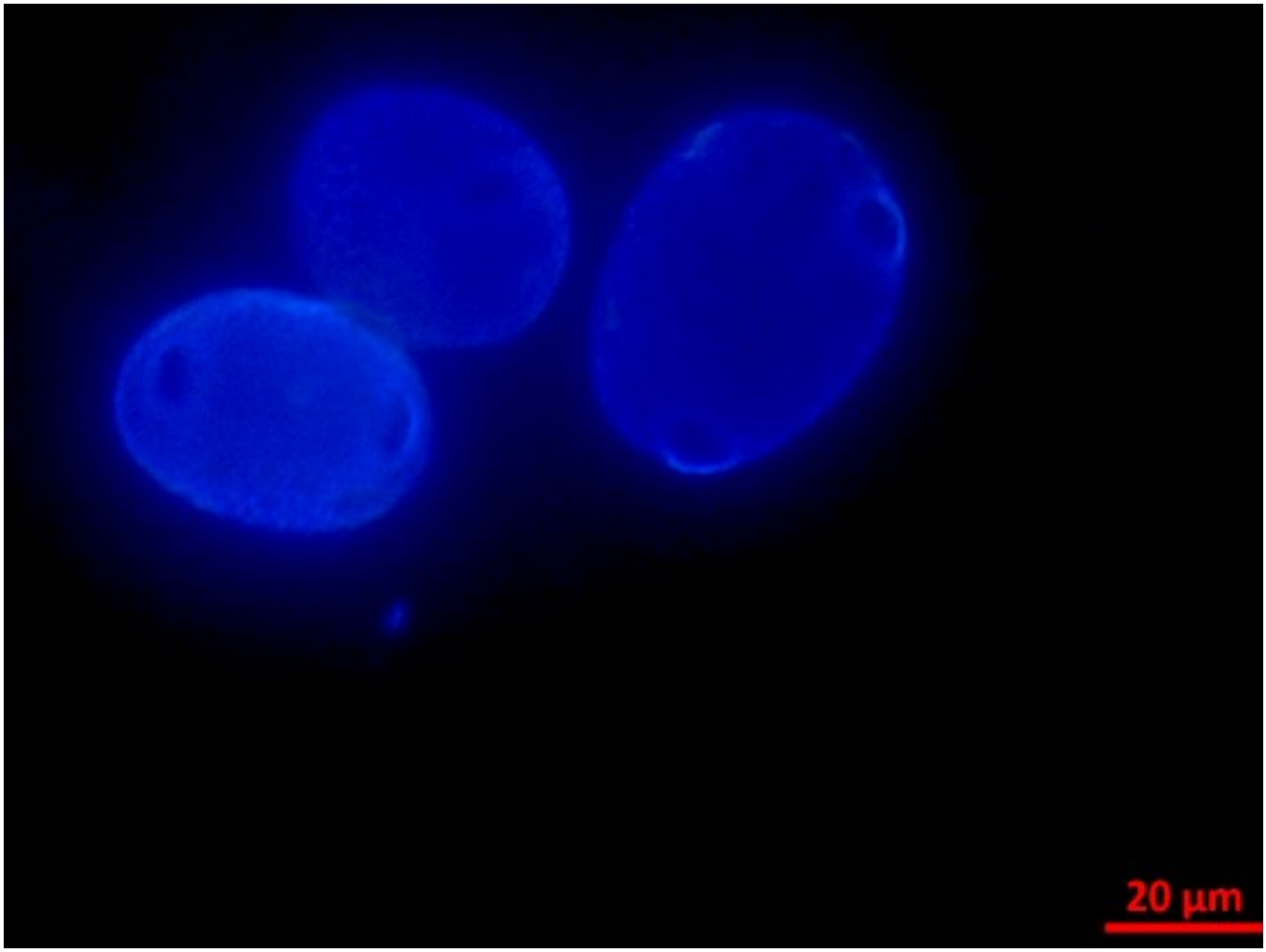

Fig. 3. Pollen cytology of *Impatiens cordata.*

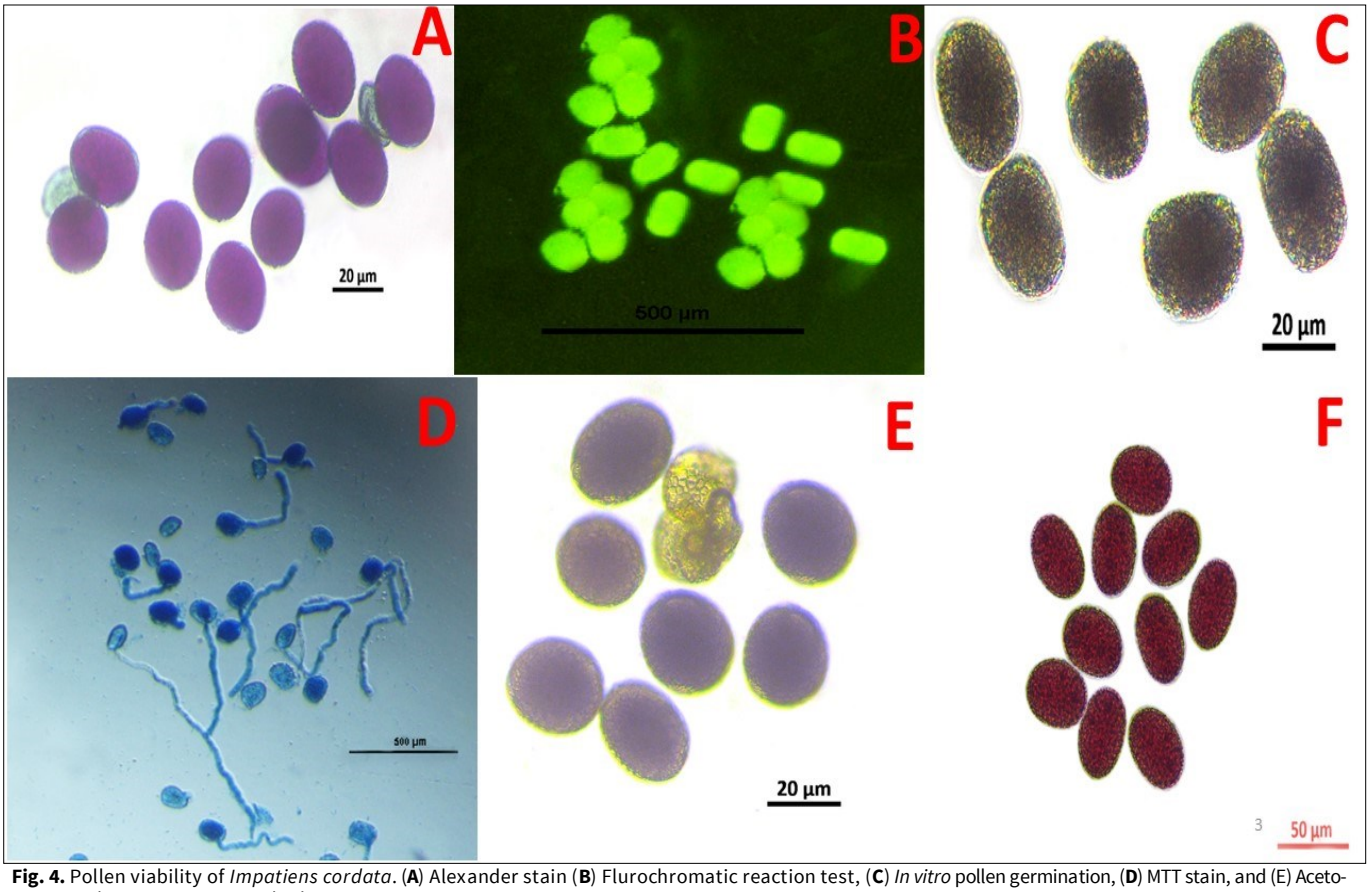

Fig. 4. Pollen viability of *Impatiens cordata*. (**A**) Alexander stain (**B**) Flurochromatic reaction test, (**C**) *In vitro* pollen germination, (**D**) MTT stain, and (E) Aceto-carmine-glycerine staining method.

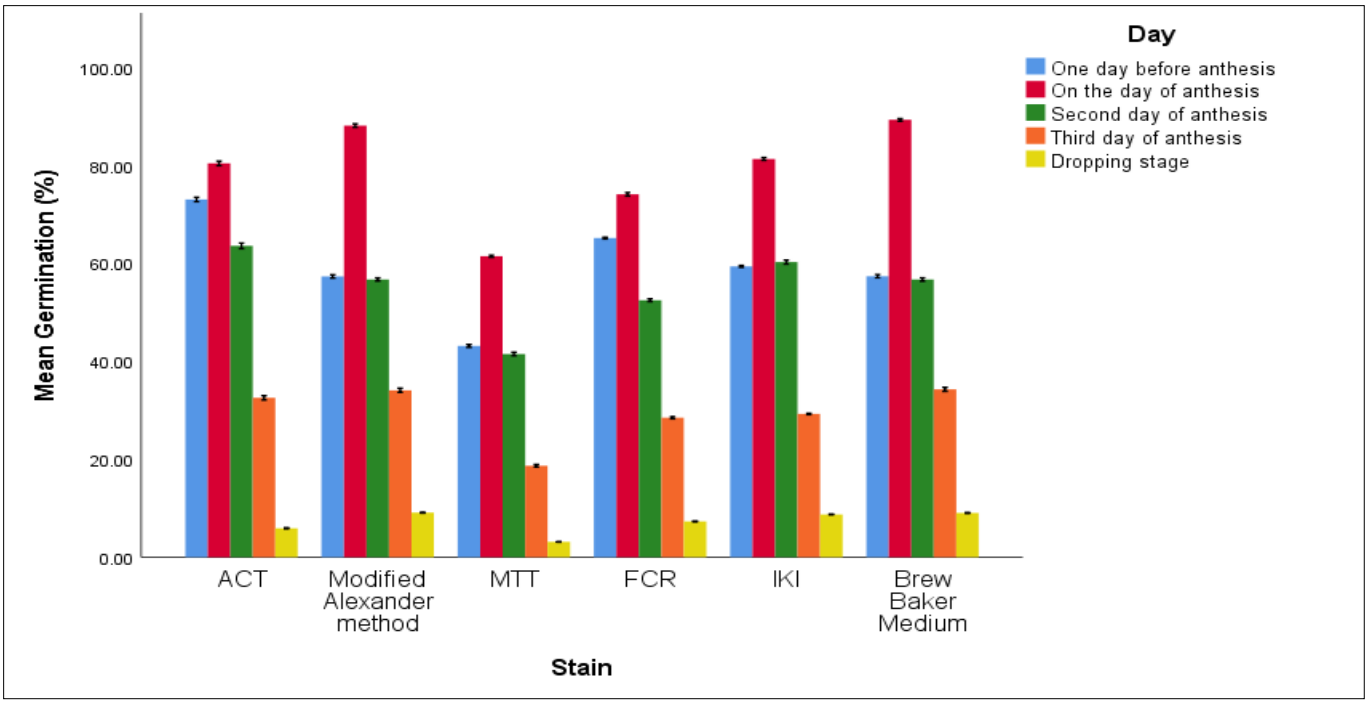

Fig. 5. Pollen viability of *Impatiens cordata* at different anthesis stages.

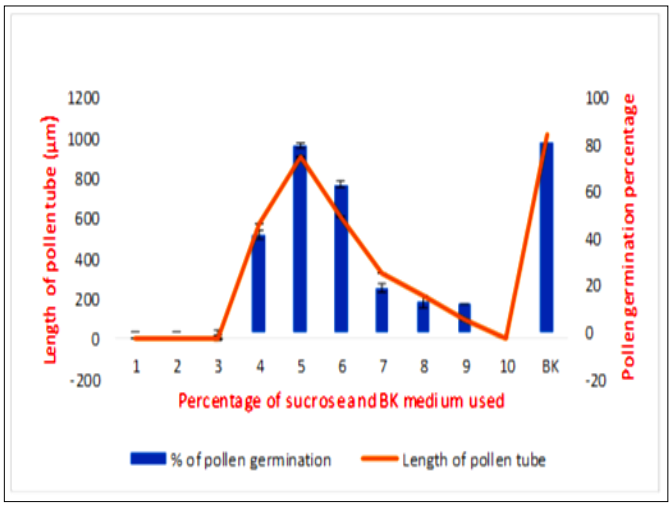

Fig. 6. *In vitro* pollen germination of *Impatiens cordata* in germination media.

Discussion

Pollen grain morphology is typically examined to observe the distinctive features of *Impatiens cordata*. Scanning electron microscopy (SEM) was used without altering the characteristic morphological traits of the plants. In this analysis, particular attention was given to pollen shape and exine ornamentation, as these features play a significant role in identifying the studied genus *Impatiens.*

Consequently, to highlight potential morphological distinctions between closely related species, researchers have employed SEM for micromorphological analysis of pollen grains (26). The size of the pollen grains of *I. cordata* was approximately 39.9 ± 1.17 × 51.3 ± 0.83 µm, while those of *Impatiens gardneriana* and *Impatiens cuspidate* were 33.16 µm and 28.24 µm in diameter, respectively (27,

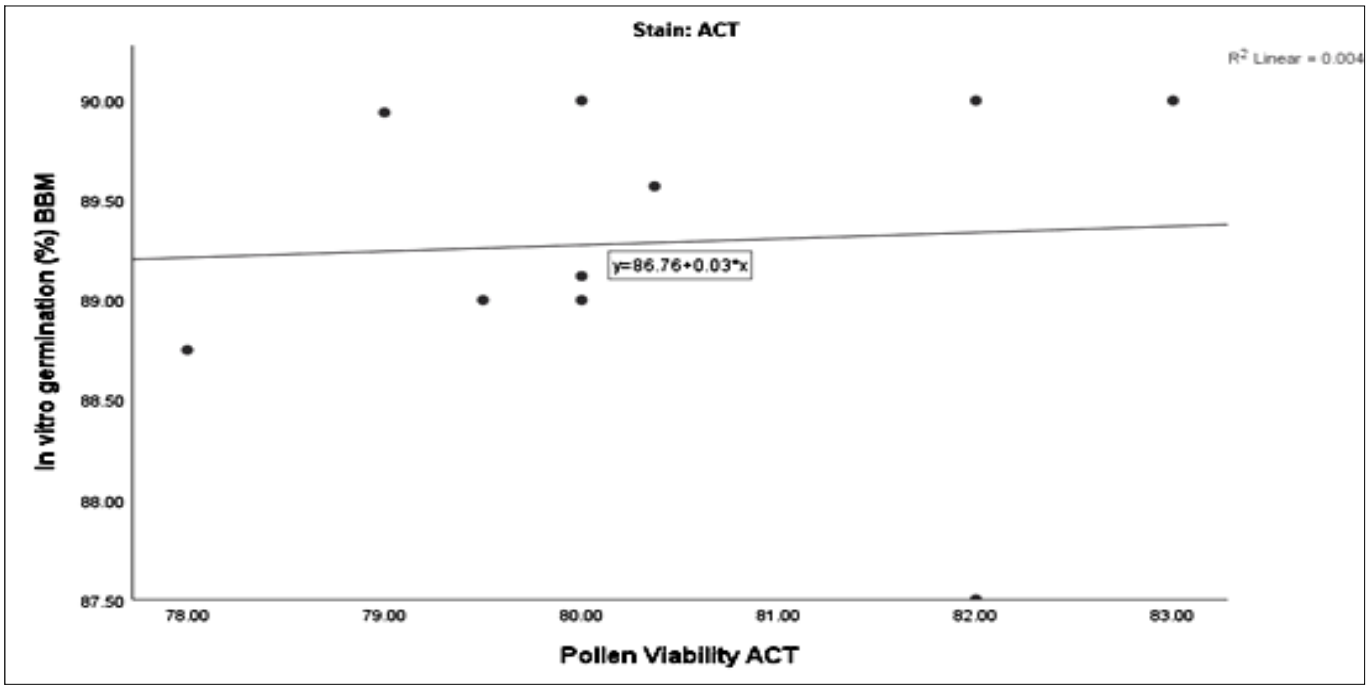

Fig. 7. Scatter plot depicting the relationship between pollen viability in ACT and *in vitro* germination percentage.

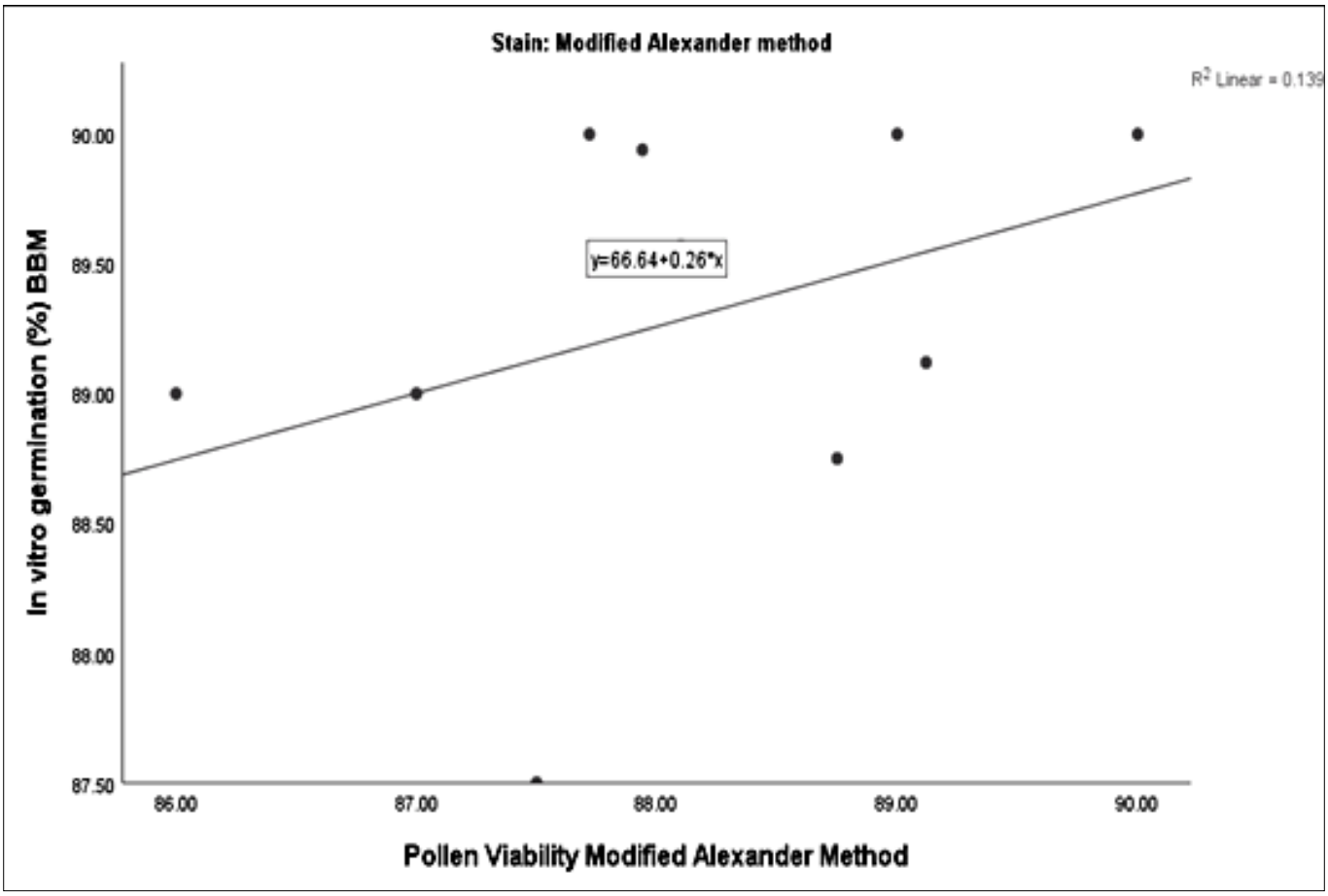

Fig. 8. Scatter plot depicting the relationship between pollen viability according to the modified alexander method and *in vitro* germination percentage.

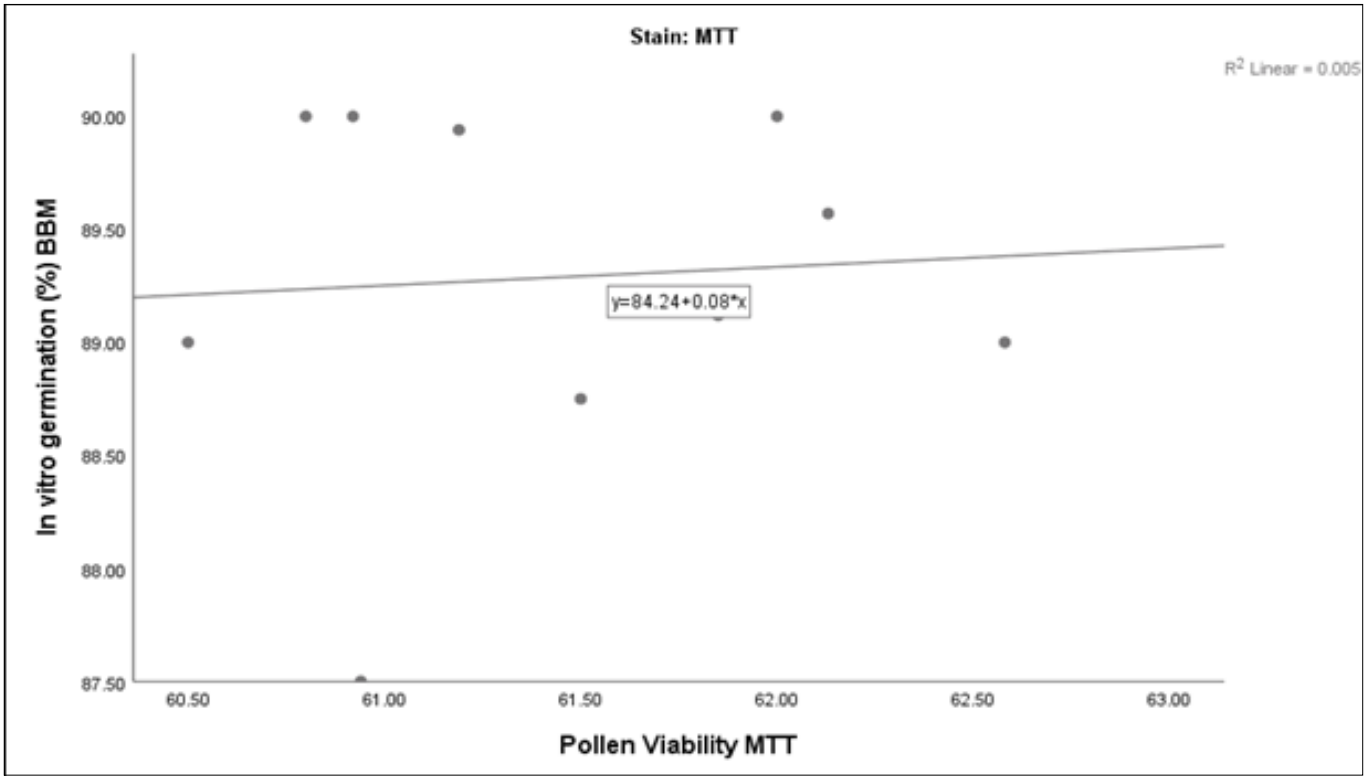

Fig. 9. Scatter plot depicting the relationship between pollen viability according to MTT and *in vitro* germination percentage.

28). We observed that the pollen grains of *I. cordata* were elliptical in equatorial view and quadrangular, acute, and convex in polar view. Other *Impatiens* species in the Nilgiris region display a diverse range of shapes and appearances (29). The *Impatiens* genera collected from Niligiris are prolate, subprolate, and prolate-spheroidal according to the equatorial view; in the polar view, they are circular, rectangular, triangular, quinquangular, elliptic, and quadrangular. Additionally, the pollen grains of *I. cordata* was found to be mainly tetracolpate, with sporadic tricolpate occurrences, and feature reticulate exine ornamentation, in line with the findings of Pechimuthu's research (29). Pechimuthu found that the pollen apertures of *Impatiens* varied, and most species had a reticulate orna-

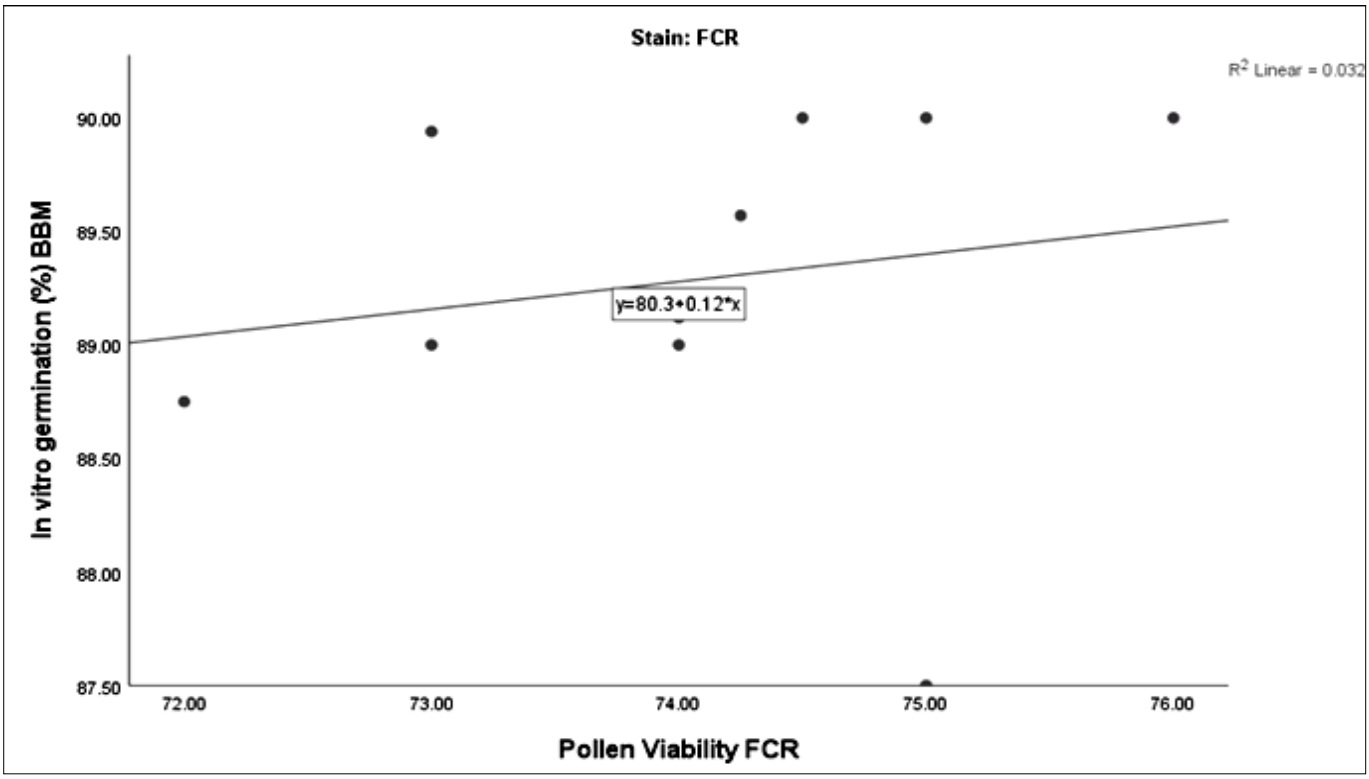

Fig. 10. Scatter plot depicting the relationship between pollen viability in FCR and *in vitro* germination percentage.

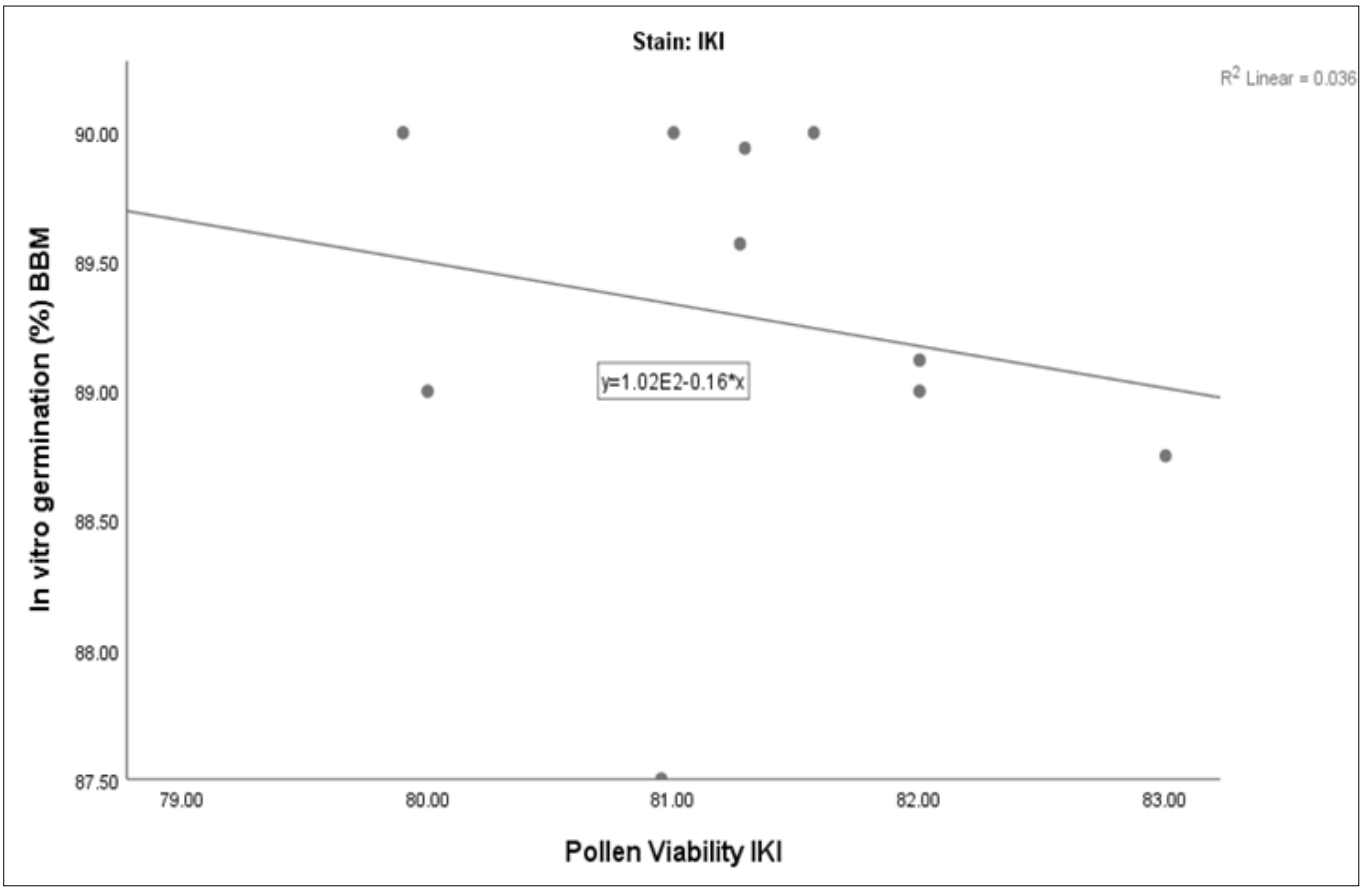

Fig. 11. Scatter plot depicting the relationship between pollen viability in the IKI and *in vitro* germination percentage.

mentation except for *Impatiens fruticosa*, which had an echinate ornamentation (29). Reticulation is crucial in pollination and fertilization and enhances pollen grains' structural integrity. This pattern facilitates interaction with insects and pollen transport (30). The study by He et al. showed that pollen micromorphology is important for classifying *Impatiens* species accurately (31).

Cytological investigations of *I. cordata* revealed that its pollen type is bicellular, and its stigmatic surface is wet. Our observations also revealed the presence of exudates on the stigma surface of *I. cordata* flowers. These findings align with the outcomes documented by Van Went & Willemse and Zhang et al., indicating a relationship between binucleate pollen and both moist and arid stigmas (32, 33).

In contrast, trinucleate pollen tends to favour dry stigmas. A microscopic analysis of mature borage pollen grains (34), revealed a tricellular cytological type. In contrast, borage flowers lack any exudate, a key characteristic defining their stigma as dry (35). The DAPI staining method was used to determine the pollen nucleus status. Upon shedding, angiosperm pollen grains contain either 2 cells (a large vegetative cell enclosing the generative cell) or 3 cells (a vegetative cell enclosing the 2 male gametes formed by the division of the generative cell). The cytology of pollen at the time of shedding (2 or 3 cells) has important correlations with several physiological features, including viability, storage, and *in vitro* germination. Binucleate pollen has longer viability than trinucleate pollen and can be used for pollen conservation. Pollen conservation is an important device in the management of plant genetic resources. Generally, 2-celled pollen exhibits the gametophytic type of self-incompatibility, while those with 3-celled pollen show sporophytic incompatibility (36). Most flowering plants release pollen grains at a binucleate stage before sperm formation (37). According to the Schuroff-Brewbaker law, which states that binucleate is primitive and trinucleate is advanced, the derived condition of angiosperm pollen is irreversible Brewbaker (38).

Assessing pollen viability is crucial, and staining has become widely used. By examining cell integrity, enzyme activities, and nutrient contents, staining provides valuable insights into the viability of pollen. However, it is essential to note that different staining methods are suitable only for assessing pollen viability in specific plants. Therefore, understanding the appropriate staining method for each plant is critical (39, 40). In the present study, among the 5 staining techniques employed, compared to other stains, the modified Alexander method was effective at discerning viable and nonviable pollen grains. According to Peterson et al*.,* viable pollen grains exhibit violet staining in this modified method, while nonviable pollen grains exhibit blue-green staining (41). However, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) staining method yields various colour tones, and distinguishing very dark pink pollen from black pollen is sometimes challenging.

Moreover, MTT rarely stained nonviable pollen; when it did, the stain was consistently lighter than fresh pollen. Therefore, caution is advised when using MTT, and consideration should be given to the specific species under examination. Parfitt and Ganeshan reported that, in certain *Prunus* species, heat-killed pollen was strongly stained using MTT (40). Other staining methods employed in this study generally struggled to distinguish between viable and nonviable pollen. Most of these methods resulted in minimal unstained pollen grains, suggesting high overall pollen viability. Barrow cautioned that some staining techniques might fail to distinguish between aborted and non-aborted pollen grains, emphasizing the importance of supplementing such assessments with *in vitro* pollen germination studies (41). The pollen germination results do not accurately resemble the pollen viability results obtained *in vitro*. In this study, we conducted several viability tests and confirmed that pollen viability peaks on the day of anthesis. However, as time progresses after anthesis, the viability gradually decreases. These findings align with similar observations in previous studies of *I. gardeneria . Cuspidate* (27, 28). In the case of *in vitro* germination*,* the Brew Baker medium supplemented with 5% sucrose demonstrated the best results, achieving a germination rate of 89.29 ± 0.80%. After 4 hrs of incubation, the pollen tube reached an impressive length of 1016 µm. Similarly, in *I. gardneriana* and *I. cuspidata*, the BK medium proved to be the most effective, yielding a 74% germination rate with a pollen tube length of 801 µm and an outstanding 96% germination rate with a remarkable pollen tube length of 1636 µm after 4 hr of incubation (27, 28). In the process of *in vitro* pollen germination and tube growth, it was determined that the presence and concentration of sucrose in the medium were crucial. A study conducted by Patel & Mankad (42) in *Impatiens balsamina,* an important horticultural plant, stated that in the various concentrations tested, it was found that 0.8% sucrose resulted in a good percentage of germination. Carbohydrates, mainly sucrose, play a vital role as a significant component of pollen tube reserves in the germination and growth process. Sucrose is the best carbohydrate source for pollen germination and tube growth in most systems studied. Among the different stains, the Alexander stain exhibited the strongest correlation (R=0.139) with *in vitro* germination.

Conclusion

Impatiens cordata pollen grains are bicellular, elliptical and quadrangular, mainly tetracolpate, with reticulate exine ornamentation. Modified Alexander stain was more effective for distinguishing between aborted and non-aborted pollen in *I. cordata*. Brew baker's medium with 5% sucrose had best germination. Day of anthesis affects pollen viability, with better correlation to Alexander stain for *in vitro* germination.

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Authors' contributions

AS and SAK conceived the idea and planned experiments. AS performed the experiments under the supervision of SAK. All authors analysed the research data and drafted the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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