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Original article

Sucrose and boric acid effects on *in vitro* pollen viability of *Durio zibethinus*

Muhammad Raihan Akbar[1], Amin Retnoningsih[1*]

[1] Department of Biology, Universitas Negeri Semarang, Kampus UNNES Sekaran, Gunungpati, Semarang, 50229, Indonesia

Abstract

Durian is a highly valued tropical fruit, but fruit set and seed development remain variable in mixed orchards where cross-pollination depends on viable pollen. *In vitro* germination provides a functional estimate of pollen viability and can guide controlled pollination and breeding. This study evaluated the effects of sucrose and boric acid concentrations in Brewbaker and Kwack medium on pollen germination and pollen tube growth of normal-seeded Duri Hitam durian and described its basic pollen morphology. Pollen collected from flowers at anthesis was tested in a 4 × 4 factorial completely randomized design with four sucrose concentrations and four boric acid concentrations, with five replications. Pollen morphology was observed by light microscopy, whereas germination percentage and pollen tube length were measured after dark incubation. Sucrose, boric acid, and their interaction significantly affected germination percentage. The highest mean germination occurred at 16% sucrose and 0.01% boric acid, but this treatment was statistically comparable with several neighboring combinations. For pollen tube length, sucrose and boric acid had significant main effects, whereas their interaction was not significant; therefore, the longest combination-level mean observed at 16% sucrose and 0.01% boric acid is descriptive rather than evidence of a unique optimum. Pollen grains were oblate spheroidal, psilate, and tricolporate. These results indicate an interactive effect of sucrose and boric acid on *in vitro* pollen germination but independent effects on pollen tube length under the tested conditions. Controlled pollination and multi-genotype validation are needed before these findings can be extrapolated to reproductive success *in vivo*.

Keywords: boric acid, *Durio zibethinus*, *in vitro* germination, pollen morphology, sucrose, viability

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Introduction

Durian (*Durio zibethinus* L.) is a tropical fruit tree with high economic and cultural value in Southeast Asia. Its reproductive biology is strongly related to fruit set, seed development, and the stability of edible aril production. Durian flowers are generally associated with cross-pollination and animal-mediated pollen transfer, including bat and insect visitation, while cultivar compatibility can influence the final fruit set (Baqi *et al*., 2022; Ei & Ismail, 2022; Ketsa *et al*., 2020; Zulfahmi *et al*., 2022). In mixed orchards, pollen may originate from several genotypes, so pollen quality and compatibility become important components of successful fertilization.

Pollen viability is commonly defined as the capacity of pollen to germinate and produce a functional pollen tube (Su *et al*., 2024; Wani *et al*., 2020). In this study, pollen viability was evaluated using *in vitro* pollen germination and pollen tube growth as functional indicators. These parameters indicate the capacity of pollen to initiate germination and tube elongation under controlled conditions, but they do not directly confirm fertilization capacity or reproductive success *in vivo*. After compatible pollen lands on the stigma, hydration, metabolic activation, tube emergence, and polarized tube elongation are required to deliver sperm cells toward the embryo sac (Adhikari *et al*., 2020; Li *et al*., 2023). Pollen quality can be estimated by staining, stigma-based observations, or *in vitro* germination. Staining methods are rapid, but they may overestimate functional viability because stained pollen is not always able to produce a normal pollen tube (Impe *et al*., 2020; Langedijk *et al*., 2023).

In vitro pollen germination provides controlled conditions for evaluating pollen germination and tube growth and facilitates observation of changes in tube emergence and elongation (Li *et al*., 2023; Tushabe & Rosbakh, 2021). Pollen germination medium is species-specific and depends on osmotic balance, available carbon, pH, minerals, and incubation conditions (Tushabe & Rosbakh, 2021; Weng *et al*., 2023). Brewbaker and Kwack medium is widely used because calcium, magnesium, potassium, boron, and sugar contribute to pollen tube emergence and tip growth (Brewbaker & Kwack, 1963; Fragallah *et al*., 2019). Sucrose provides osmotic support and carbon for respiration and wall synthesis, whereas boron contributes to cell wall stability and calcium-related regulation during tube elongation (Fang *et al*., 2019; Seitz *et al*., 2023; Zhang *et al*., 2022). However, excessive or insufficient sucrose and boric acid can suppress germination because pollen tubes are sensitive to both osmotic stress and ion imbalance (Bodhipadma *et al*., 2022; Su *et al*., 2024; Yohana *et al*., 2025).

Previous studies have investigated the effects of sucrose and boric acid on *in vitro* pollen germination by optimizing these components under specific conditions. For example, Castillo *et al*. (2022) tested different sucrose concentrations while maintaining a fixed boric acid concentration and subsequently tested different boric acid

* Corresponding Author:
Amin Retnoningsih
Department of Biology, Universitas Negeri Semarang, Kampus UNNES Sekaran, Gunungpati, Semarang, 50229, Indonesia
Phone: 081316535512
E-mail: aminretnoningsih2016@mail.unnes.ac.id

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concentrations while maintaining a fixed sucrose concentration. Although this approach provides information on the response to each component, it does not directly evaluate whether the response to sucrose changes across different boric acid concentrations, or vice versa.

Information on the *in vitro* germination requirements of pollen from local durian cultivars grown in mixed orchards remains limited, particularly regarding the combined effects of sucrose and boric acid concentrations. A factorial approach may provide additional information by evaluating the individual effects of these components and determining whether their effects interact under different concentration combinations. In addition, pollen morphology such as grain shape, exine surface, and aperture type may influence hydration and tube emergence because apertures serve as preferential sites for water exchange and pollen tube initiation (Halbritter *et al.*, 2018; Pacini & Franchi, 2020). This study aimed to evaluate the effects of sucrose and boric acid combinations in Brewbaker and Kwack medium on *in vitro* pollen germination and pollen tube length of normal-seeded durian, and to describe its basic pollen morphology.

Methods

Research design and pollen source

The study combined descriptive pollen morphology observation with an experimental *in vitro* germination assay. The germination assay used a 4 × 4 factorial completely randomized design. The first factor was sucrose concentration at 8, 16, 24, and 32% (w/v), coded as S1, S2, S3, and S4. The second factor was boric acid concentration at 0, 0.01, 0.02, and 0.03% (w/v), coded as B1, B2, B3, and B4. Sixteen treatment combinations were prepared, each with five replications. One petri dish represented one experimental unit, and 100 pollen grains were counted per unit.

Pollen was collected from 20 fully opened flowers of one normal-seeded Duri Hitam durian tree in Brongkol Village, Jambu District, Semarang Regency, Central Java. Flowers were sampled at anthesis in the evening because durian pollen release and pollination activity occur mainly at night (Zulfahmi *et al*., 2022). Pollen was removed from the anthers using a sterile brush, pooled, distributed as uniformly as possible into sterile petri dishes, air-dried at 25–30 °C for two hours, transported in a cool box, and stored at −20 to −22 °C until analysis. The use of one donor tree was treated as a practical limitation of the study and the findings are interpreted as cultivar-level preliminary evidence rather than broad species-wide inference.

Preparation of germination medium

The basal medium followed Brewbaker and Kwack medium with 300 mg/L $Ca(NO_3)_2{\cdot}4H_2O$, 200 mg/L $MgSO_4{\cdot}7H_2O$, and 100 mg/L KNO_3 (Brewbaker & Kwack, 1963). Sucrose and boric acid were added according to the treatment combinations. The components were dissolved in distilled water, adjusted to pH 6.0 using HCl or NaOH, and sterilized by autoclaving at 121 °C for 20 min. The medium was cooled before use and stored under refrigerated conditions to maintain sterility.

Pollen inoculation, incubation, and observation

Stored pollen was equilibrated at 25 °C for 30 min before inoculation to reduce osmotic shock during rehydration. Inoculation was performed aseptically. Each petri dish received 500 µL of liquid medium, and a sterile filter paper moistened with 200 µL of distilled water was placed on the lid to maintain humidity. The dishes were sealed and incubated in the dark for 36 h at 25 °C.

Pollen germination was observed with a Nikon ECLIPSE 80i light microscope. Pollen grains were considered germinated when the pollen tube emerged from an aperture and reached at least the pollen grain diameter (Langedijk *et al.*, 2023; Mendez & Acma, 2018). Germination percentage was calculated as the number of germinated pollen grains divided by the total observed pollen grains multiplied by 100. Pollen tube length was measured from calibrated micrographs using ImageJ. Pollen morphology was described qualitatively based on shape, exine ornamentation, and aperture type using standard pollen terminology (Halbritter *et al.*, 2018).

Data analysis

Germination percentage was converted to a proportion and square-root transformed before analysis, while pollen tube length was square-root transformed. Normality and homogeneity of variance were evaluated before two-way analysis of variance (Two-Way ANOVA). When a significant interaction was detected, treatment-combination means were compared using Duncan's multiple range test (DMRT); when only main effects were significant, means were compared within the corresponding factor using DMRT at $\alpha = 0.05$.

Results

Pollen germination percentage

Sucrose, boric acid, and their interaction significantly affected pollen germination percentage (Table 1). The significant interaction indicates that the response to sucrose depended on boric acid concentration and vice versa.

Table 1. Two-way ANOVA for durian pollen germination percentage

Variable	Source	df	SS	MS	F	P
Germination percentage	Sucrose	3	0.32	0.107	8.117	<0.001
	Boric acid	3	0.209	0.07	5.288	0.003
	Interaction	9	0.28	0.031	2.365	0.022

The highest germination percentage was recorded in S2B2, containing 16% sucrose and 0.01% boric acid, with a mean of 53.00%. This value was statistically similar to S1B2, S1B3, S2B1, and S2B3, indicating that the optimum range was not restricted to a single exact combination (Table 2). The lowest germination occurred in S1B4, suggesting that low sucrose combined with the highest boric acid concentration was unfavorable for pollen germination.

Table 2. Duncan's multiple range test for the interaction effect on durian pollen germination percentage. Values are mean ± SD. Means followed by the same letter are not significantly different at α = 0.05

Treatment	Sucrose (%)	Boric acid (%)	Germination (%)
S1B1	8	0	27.40 ± 10.78^{cde}
S1B2	8	0.01	51.40 ± 31.50^{ab}
S1B3	8	0.02	46.00 ± 21.92^{abc}
S1B4	8	0.03	13.80 ± 2.77^{e}
S2B1	16	0	50.60 ± 17.86^{a}
S2B2	16	0.01	53.00 ± 21.94^{a}
S2B3	16	0.02	39.40 ± 8.62^{abcd}
S2B4	16	0.03	27.20 ± 9.39^{cde}
S3B1	24	0	21.60 ± 7.63^{de}
S3B2	24	0.01	26.20 ± 14.18^{cde}
S3B3	24	0.02	26.80 ± 9.78^{cde}
S3B4	24	0.03	28.80 ± 5.93^{bcde}
S4B1	32	0	20.00 ± 8.27^{e}
S4B2	32	0.01	28.40 ± 10.57^{cde}
S4B3	32	0.02	23.60 ± 6.30^{de}
S4B4	32	0.03	21.80 ± 8.87^{de}

Pollen tube length

Sucrose and boric acid significantly affected pollen tube length as individual factors, but their interaction was not significant (Table 3). Therefore, the main effects were interpreted statistically, while the treatment-combination means were used only to describe the observed tendency.

Table 3. Two-way ANOVA for pollen tube length of durian pollen

Variable	Source	df	SS	MS	F	P
Pollen tube length	Sucrose	3	78.354	26.118	19.828	<0.001
	Boric acid	3	18.912	6.304	4.786	0.005
	Interaction	9	9.219	1.024	0.778	0.637

The 16% sucrose treatment produced the longest mean pollen tube among sucrose levels and was not significantly different from 8% sucrose. For boric acid, 0.01% produced the longest mean pollen tube and was not significantly different from 0.02% (Table 4). Descriptively, S2B2 produced the longest tube length at 106.36 µm, followed by S2B3, but these combination-level differences were not statistically tested further because the interaction was not significant.

Table 4. Main effects of sucrose and boric acid on pollen tube length. Values are mean ± SD. Means within the same factor followed by the same letter are not significantly different at α = 0.05

Factor	Level	Pollen tube length (µm)
Sucrose	8%	78.85 ± 20.17^{a}
	16%	92.30 ± 23.39^{a}
	24%	58.69 ± 19.34^{b}
	32%	51.07 ± 18.50^{b}
Boric acid	0%	58.59 ± 18.68^{b}
	0.01%	82.01 ± 28.38^{a}
	0.02%	71.96 ± 28.64^{ab}
	0.03%	68.33 ± 22.74^{b}

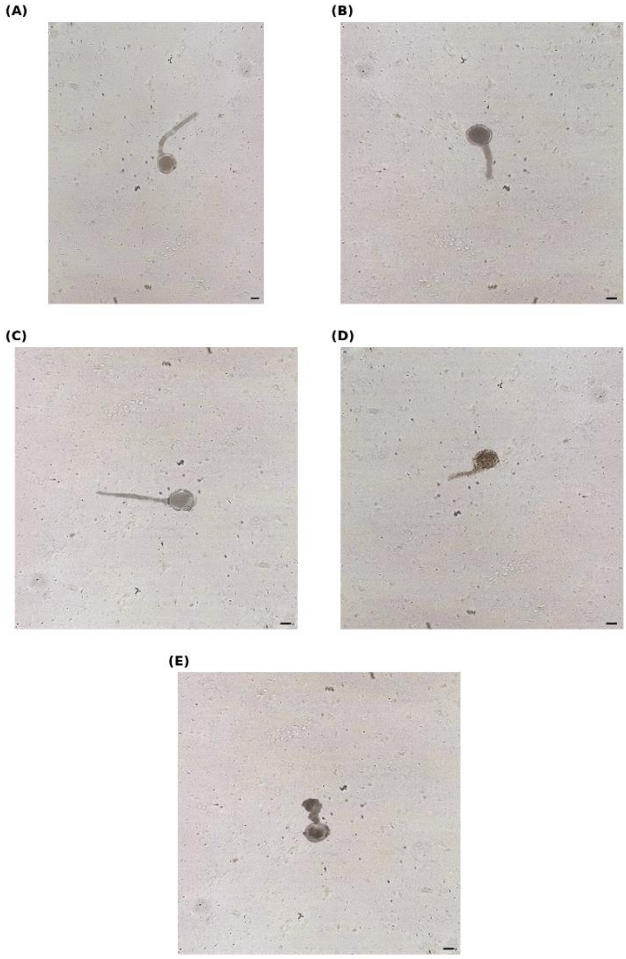

Fig. 1. Representative germinated pollen grains and pollen tubes from the S2B2 treatment after 36 h of incubation. Scale bars are shown in the micrographs. Scale bar for A–E = 50 µm

Pollen morphology

Light microscopy showed that the pollen grains of normal-seeded Duri Hitam durian were oblate spheroidal, with a smooth psilate exine and tricolporate apertures (Figure 2). The visible apertures were the main sites associated with tube emergence during germination observation.

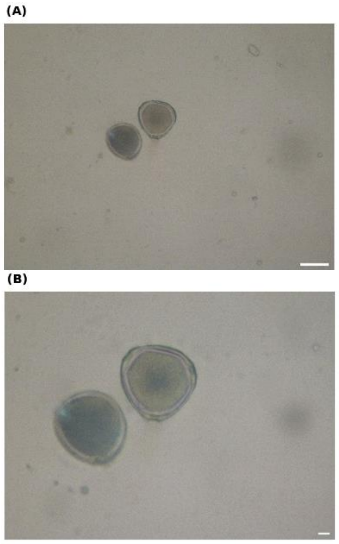

Fig. 2. Morphology of Duri Hitam durian pollen observed by light microscopy: (A) general pollen grains and (B) detailed pollen surface and aperture position. Scale bars: A = 50 µm; B = 20 µm

Discussion

The significant interaction between sucrose and boric acid for germination percentage indicates that the effect of sucrose on *in vitro* germination depended on boric acid concentration, and vice versa. The highest germination occurred at 16% sucrose plus 0.01% boric acid, but several neighboring combinations were statistically comparable. This pattern suggests a relatively broad favorable range around moderate sucrose and low boric acid. Similar species-specific optima have been reported in other crops, such as quinoa, foxtail millet, sweet potato, and rice, in which different sugar and boron ranges were required to support pollen germination (Castillo *et al*., 2022; Su *et al*., 2024; Weng *et al*., 2023; Yohana *et al*., 2025).

Sucrose likely supported germination by maintaining osmotic balance and supplying carbon for respiration, membrane activity, and new wall formation during tube emergence (Su *et al*., 2024). When sucrose is too low, excessive water influx may disturb cell integrity and reduce available energy. When sucrose is too high, water uptake may be restricted and metabolic activity may decline under osmotic stress (Bodhipadma *et al*., 2022; Seitz *et al*., 2023; Winship *et al*., 2021). The lower germination observed at 24-32% sucrose in several combinations is consistent with this osmotic interpretation.

Boric acid contributed positively at low concentration, particularly 0.01%, but the highest boric acid level did not improve germination. Boron supports pollen tube growth by stabilizing pectic components of the cell wall and interacting with calcium-dependent processes at the tube apex (Fang *et al*., 2019; Zhang *et al*., 2022; Zhou *et al*., 2022). However, excessive boron may disturb ion balance, membrane stability, and enzyme activity (Su *et al*., 2024). This possible adverse effect at higher boron availability may help explain the low germination observed at 8% sucrose plus 0.03% boric acid; however, the present experiment did not directly measure boron toxicity or its underlying physiological mechanisms.

Unlike germination percentage, pollen tube length was affected by sucrose and boric acid as separate factors rather than by their interaction. Previous studies have shown that pollen tube growth involves complex processes related to sugar metabolism, ion dynamics, and cell wall regulation (Seitz *et al*., 2023; Zhang *et al*., 2022; Zhou *et al*., 2022). However, these processes were not directly measured in the present study; therefore, the observed differences in pollen tube length should not be attributed to a specific physiological mechanism. The absence of a significant interaction indicates that the effects of sucrose and boric acid on pollen tube length were not statistically dependent on their combination under the experimental conditions used.

Brewbaker and Kwack medium already contains calcium, magnesium, and potassium salts, which can maintain ionic conditions for polarized growth (Brewbaker & Kwack, 1963; Tushabe & Rosbakh, 2021). The descriptive superiority of S2B2 for tube length should therefore be interpreted cautiously because the statistical interaction was not significant.

Furthermore, the observed oblate spheroidal shape, psilate exine, and tricolporate apertures provide a descriptive morphological baseline for Duri Hitam durian pollen. Pollen aperture areas commonly function as sites for water uptake, volume regulation, and pollen tube initiation, while exine structure and pollen coat properties contribute to protection, adhesion, and water interaction (Halbritter *et al*., 2018; Huth *et al*., 2022; Lin *et al*., 2015; Sutthinon *et al*., 2022). These observations should be interpreted descriptively because the present study did not experimentally test relationships between pollen morphology and germination performance.

The study has several limitations. Pollen was obtained from one donor tree and from a limited number of flowers, so genotype and tree-level variation could not be separated. The *in vitro* response also does not necessarily predict fruit set under orchard conditions, where stigma receptivity, pollen compatibility, humidity, temperature, pollinator behavior, and post-fertilization processes interact. Future work should include multiple durian genotypes, compare *in vitro* germination with controlled pollination *in vivo*, and evaluate pollen storage time because storage conditions can alter pollen function (Li *et al*., 2023; Wang *et al*., 2021; Wu *et al*., 2022).

Conclusion

Sucrose, boric acid, and their interaction significantly influenced *in vitro* germination percentage of Duri Hitam durian pollen. The 16% sucrose plus 0.01% boric acid treatment produced the highest mean germination, but it was statistically comparable with several neighboring combinations. Sucrose and boric acid significantly affected pollen tube length as individual factors, whereas their interaction was not significant; therefore, the longest combination-level mean observed in S2B2 should be interpreted descriptively. The pollen grains were oblate spheroidal, psilate, and tricolporate. Overall, sucrose and boric acid showed an interactive effect on *in vitro* pollen germination but independent effects on pollen tube length under the tested conditions. Controlled pollination and multi-genotype validation are needed before these findings are applied to orchard-level reproductive management.

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