EFFECTS OF TEMPERATURE ON POLLEN GERMINATION OF 'SABARA' JABOTICABA (*Plinia cauliflora* (Mart.) Kausel) *IN VITRO*

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ABSTRACT

Jaboticaba (*Plinia cauliflora* (Mart.) Kausel) is a sub-tropical fruit native to Brazil. It is cultivated in South America and Southeast Asia. 'Sabara' jaboticaba can produce fruit all year round under favorable conditions. However, sometimes fruits drop under high temperatures. This study was carried out in Tokyo University of Agriculture from 2018 to 2021. Sucrose concentration and thermal response on pollen germination *in vitro* were investigated. The favorable sucrose concentration for pollen germination was 10% while, pollen germination percentage and pollen tube length were highest at 25℃. The favorable temperatures for pollen germination were 20-30 ℃. In addition, low (10-15℃) temperatures exposure time did not affect pollen germination. However, in the high temperature treatments, pollen tube length decreased at 35℃ for 2 h and over, or 40℃ for 1 h and over. Fruit drop in hot summer might be due to the inhibition of pollen germination under high temperatures. It is desirable to use devices such as an electric fan under high temperatures to manage temperature under greenhouse conditions and in the field when the trees are in bloom.

Key words: artificial medium, jabuticaba, pollen germination percentage, pollen tube elongation, thermal response

INTRODUCTION

Jaboticaba (*Plinia cauliflora* (Mart.) Kausel) belonging to the family Myrtaceae is native to Brazil (Wu et al. 2013; Shinohara et al. 2021; TPL 2020) and cultivated in tropical, subtropical, and temperate climates, such as South America, the USA, China, and Southeast Asia (Marica et al. 2018; Salomão et al. 2018; Mitra 2010). The fruit is consumed mainly fresh (Teixeira et al. 2011). Several processed products such as jams, jelly, juice, and wine are also known (Montes et al. 2005). 'Sabara'jaboticaba strain are the most cultivated in Brazil (Freitas et al. 2020). The fruit is dark purple, with a thin and smooth skin and a very sweet flavor. 'Sabara' can be produced throughout the year under favorable conditions. However, it has been observed that fruit dropped in hot summer in Japan. Thermal responses on reproductive physiology in many plants have been reported (Thakur et al. 2010; Zinn et al. 2010; Matsuda et al. 2015). Some studies reported fruit drop under low or high temperatures due to the inhibition of pollen germination, such as peach (Kozai 2014), loquat (Yahata and Nakai 1994), mango (Sukhvibul et al. 2000), and cherry (Hagihara et al. 2018). Previous studies on pollen

germination have been conducted on an artificial medium and in pistils in various fruits, such as persimmon (Fukui et al. 1990), cherimoya (Lora et al. 2006), salak palm (Matsuda et al. 2020), peach and durian (Kozai 2014). Pollen germination test in pistils to determine whether the pistil or pollen causes the reaction is not possible. In addition, pollen germination of each cultivar might be a factor in determining the cultivation area of cultivars in lychee (Matsuda and Higuchi 2013).

There have been no reports on the thermal response of pollen germination in jaboticaba. Jaboticaba flowers bloom early in the morning, at which time pollen are attached to the stigma. After that, pollen germinate in the pistil. Therefore, pollen were exposed to high temperatures on the stigma during the daytime in summer. Thus, temperature treatments were conducted after placing the pollen onto the medium. In this study, the optimal temperature and tolerance of low and high temperatures on pollen germination were investigated in jaboticaba.

MATERIALS AND METHODS

Plant material. 'Sabara' strain of jaboticaba (*Plinia cauliflora* (Mart.) Kausel.) were cultivated under greenhouse conditions (winter minimum temperature > 10°C) in Tokyo University of Agriculture (35.6° N, 129.6° E) in Japan. Although 'Sabara' was propagated from seed, its characteristics are stable due to polyembryonic seed.

Sample collection. Pollen were collected from at least 30 full bloom flowers early in the morning because jaboticaba flowers open early in the morning. The flowers were collected from at least three trees, ranging from 15 to 35 years of age.

Pollen germination and pollen tube length. The number of germinated pollen grains was counted from 200 pollen grains and was replicated 4 times. In all experiments, pollen germination percentage was measured using this method. Pollen possessing pollen tube longer than the pollen grain were recorded as germinated. Pollen germination was observed with a fluorescence microscope (BX53, Olympus, Tokyo, Japan). The images were obtained with an attached camera (DP70-SET-A, Olympus, Tokyo, Japan) and software (CellSens, Olympus, Tokyo, Japan). After collection of images, pollen tube length was calculated using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Time-course of pollen germination. Pollen of 'Sabara' were placed onto the medium, consisting of 1 % agar and 10 % sucrose. The set-up was then incubated at 25°C under dark conditions. Pollen germination percentage and pollen tube length were measured at 3, 6, 9, 12, and 24 h after incubation. Pollen tube length was measured from 90 pollen grains.

Effects of sucrose concentration. Pollen of 'Sabara' were placed onto the medium, consisting of 1% agar with 0, 1, 10, 20, or 30% sucrose. The set-ups were then incubated at 25°C under dark conditions. Pollen germination percentage and pollen tube length were measured at 24 h after incubation. These treatments were conducted on the same day. Pollen tube length was measured from 90 pollen grains in all treatments.

Effects of temperature. Pollen of 'Sabara' were placed onto the medium, consisting of 1 % agar and 10 % sucrose. The set-up was incubated at 10, 15, 20, 25, 30, 35, or 40°C under dark conditions. Pollen germination percentage and pollen tube length were measured at 24 h after incubation. Pollen tube length was measured from 30 pollen grains at 10°C, 10 pollen grains at 40°C, and 90 pollen grains for the other treatments. Each temperature treatment was conducted on the same day.

Low and high temperature exposure time. Pollen of 'Sabara' were placed onto the medium, consisting of 1 % agar and 10 % sucrose. In the low temperature treatments (10 and 15°C), the set-up

was incubated for 0.5, 1.0, 2.0, or 3.0 h. In the high temperature treatments (35 and 40°C), the set-up was incubated for 1.5 h at 25°C after placement. These were then incubated for 0.5, 1.0, 2.0, or 3.0 h at 35 or 40°C. These were cultivated for 24 h at 25°C after low and high temperature treatments. Pollen germination percentage and pollen tube length were measured. As a control, pollen were incubated for 24 h at 25°C after placement without treatment. Pollen tube length was measured from 90 pollen grains in all treatments. These experiments were conducted because 'Sabara' flowers open early in the morning. The low temperature treatments (10 and 15°C) sought to simulate low temperatures in the early morning, while the high temperature treatments (35 and 40°C) simulated high temperatures during daytime. The low and high temperature treatments with control were conducted on the same day.

Statistical analysis. One-way ANOVA was conducted for statistical analysis of pollen germination and pollen tube length followed by Tukey test ($P < 0.01$) in the open-source statistical language R environment (Version 4.0.0., R Development Core Team 2020). Pollen germination percentage was analyzed with language R after arcsin transformation. For the effects of low and high temperatures exposure time, we conducted statistical analysis compared each temperature with control.

RESULTS AND DISCUSSION

Time-course of pollen germination. Pollen germination rate and pollen tube elongation determines the incubation time of pollen in 'Sabara' jaboticaba. The pollen germination rate reached a maximum at 6 h incubation (Fig.1). On the other hand, pollen tube elongation continued to be observed until 9 h. There were no significant changes observed in pollen germination and pollen tube elongation until 24 h. Incubation for more than 9 h therefore was sufficient for pollen germination in 'Sabara' jaboticaba.

Compared to other fruits, the time required for pollen germination in 'Sabara' jaboticaba was showed no noticeable difference. Maximum pollen germination rate and pollen tube elongation were reached at 2 h and 6 h in cherry (Beppu and Kataoka 1999) and 9 h and 18 h in lychee (Matsuda and Higuchi 2013). However, the favorable sucrose concentration to germinate pollen is differs depending on the kind of fruits (Iwanami 1980). Therefore, the favorable sucrose concentration in 'Sabara' jaboticaba was investigated.

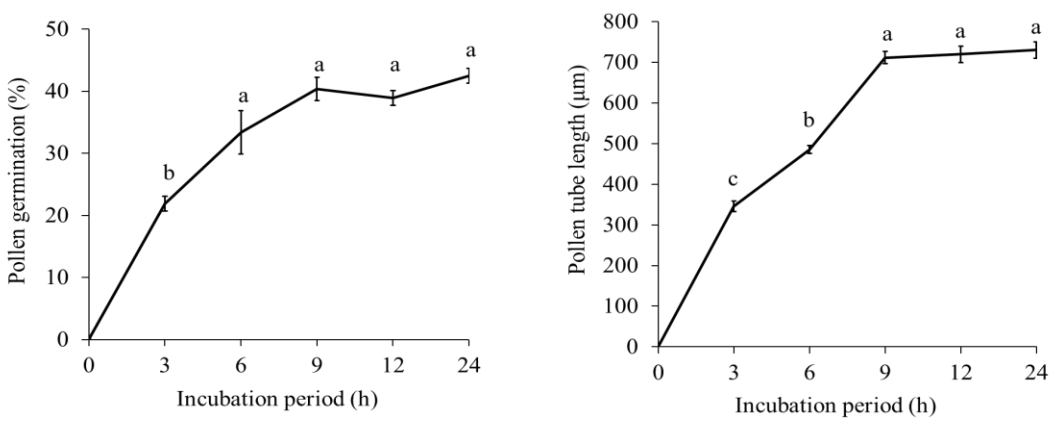

Fig. 1. Time-course of pollen germination percentage (left) and pollen tube elongation (right) in 'Sabara' jaboticaba. Pollen were incubated at 25 °C for 3, 6, 9, 12 or 24 h on the medium, consisted of 1% agar and 10% sucrose under dark condition. Vertical bars indicate S.E. Different letters indicate significant difference using Tukey test at $P < 0.01$.

Sucrose concentration effects. Sucrose concentration affected pollen germination percentage and pollen tube elongation (Fig. 2). These parameters were highest at 10 % sucrose, followed by 1 % and

20 % sucrose. Pollen germination percentage and pollen tube length were 48.1% and 656 ㎛, respectively for 10 % sucrose. In 30 % sucrose, these were 2.9 % and 106.1µm, respectively, while in 0 % sucrose, these were 9.1 % and 188.7µm, respectively.

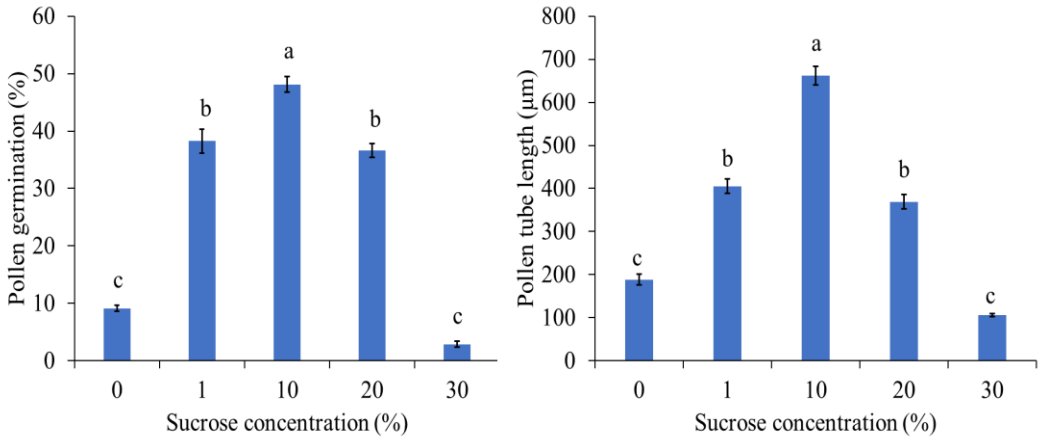

Fig. 2. Effects of sucrose concentration on pollen germination percentage (left) and pollen tube elongation (right) in 'Sabara' jaboticaba. Pollens were incubated at 25 ℃ for 24 h in medium supplemented with 1 % agar and each sucrose concentration under dark conditions. Different letters indicate significant difference using Tukey test at $P < 0.01$.

Temperature effects. Pollen germination percentage and pollen tube length were evaluated under different temperatures (Fig. 3). Pollen germination percentage and pollen tube length were linked in each treatment. These were highest at 25 ℃. Furthermore, these were decreased to less than half at below 15 ℃ or above 35 ℃, compared to 25 ℃. In 10 ℃, these were 1.75 % and 79.2µm, respectively. In 40 ℃, these were 0.5 % and 88.0µm, respectively. The favorable temperatures of pollen germination in jaboticaba were 20-30 ℃. Therefore, we considered that jaboticaba can produce fruit throughout the year in tropical and sub-tropical regions. Favorable temperatures for pollen germination have been reported in other tropical and sub-tropical fruits, *i.e*., 25-27℃ in avocado (Loupassaki et al. 1997), 22-25 ℃ in cherimoya (Yonemoto et al. 1999), 25-30 ℃ in lychee (Matsuda and Higuchi 2013), 20-30℃ in white sapote (Yonemoto et al. 2000), 30 ℃ in longan (Pham et al. 2015), and 30-40℃ in pitaya (Macha et al. 2006). However, pollen germination percentage and pollen tube length decreased below 15 ℃ or above 35 ℃ in 'Sabara' jaboticaba. Temperatures can be below 15 ℃ or above 35 ℃ in temperate, tropical, and subtropical regions such as South America and South Asia countries. Recently, it is over 40 ℃, event in temperate regions.

Based on the results, it was suggested that jaboticaba production should be careful about low temperatures in winter and high temperatures in summer at the daytime in temperate, tropical, and subtropical regions. From practical point of view, it is desirable to use black cheesecloth or use an electric fan in cultivated areas under high temperature. Under low temperature, it is advisable to turn a heater. However, it is not known how long pollen can withstand temperatures below 15℃ or above 35℃. Therefore, we investigated the effects of exposure time on pollen germination under low and high temperature conditions.

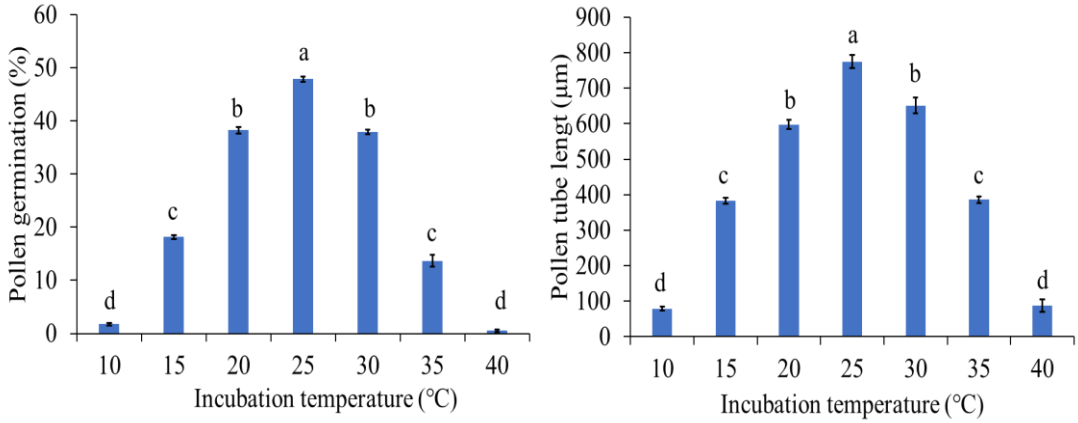

Fig. 3. Effects of temperatures on pollen germination percentage (right) and pollen tube elongation (left) in 'Sabara' jaboticaba. Pollen were incubated at each temperature for 24 h in the media supplemented with 1 % agar and 10 % sucrose under dark condition. Different letters indicate significant difference using Tukey test at $P < 0.01$.

Low and high temperatures exposure time. Although collected pollen was treated on agar medium at 10°C or 15°C for 3 h before incubation at 25°C, pollen germinated as well as those incubated at 25°C immediately after placement on the medium (Table 1). In addition to germination percentage, pollen tube length was not affected by the low temperature treatments. These results indicate that pollen of 'Sabara' can tolerate low temperatures of 10°C and 15°C for 3 h.

Table 1. Effects of exposure time in low temperatures on pollen germination and pollen tube in 'Sabara' jaboticaba.

Incubation temperature	Exposure time (h)	Pollen germination (%)	Pollen tube length (µm)
25°C	0	50.3 ± 0.5 a	802.7 ± 25.6 a
10°C	0.5	51.1 ± 2.9 a	782.9 ± 17.5 a
	1.0	53.3 ± 1.8 a	805.0 ± 16.4 a
	2.0	54.9 ± 0.7 a	802.1 ± 17.1 a
	3.0	55.9 ± 0.7 a	789.4 ± 16.8 a
15°C	0.5	51.0 ± 1.8 a	751.0 ± 13.0 a
	1.0	55.6 ± 0.9 a	770.1 ± 15.2 a
	2.0	51.7 ± 1.7 a	754.9 ± 20.0 a
	3.0	50.8 ± 2.0 a	760.0 ± 13.3 a

Different letters within a column indicate significant difference using Tukey test at $P < 0.01$.

In the high temperature treatments, pollen germination percentage was not affected by the high temperature treatments at 35 or 40 °C for 3 h (Table 2). However, pollen tube length decreased for treatments at 35°C for 2 and 3 h or at 40 °C for 1, 2 and 3 h incubation compared to control.

Table 2. Effects of exposure time at high temperatures on pollen germination and pollen tube in 'Sabara' jaboticaba.

Incubation temperature	Exposure time (h)	Pollen germination (%)	Pollen tube length (µm)
25℃	0	50.3 ± 0.5 a	802.7 ± 25.6 a
35℃	0.5	50.4 ± 1.1 a	782.8 ± 20.3 a
	1.0	53.3 ± 1.4 a	695.4 ± 21.4 a
	2.0	50.9 ± 1.0 a	647.7 ± 26.8 b
	3.0	50.3 ± 1.9 a	632.5 ± 26.3 b
40℃	0.5	52.1 ± 1.5 a	826.9 ± 19.7 a
	1.0	50.1 ± 1.2 a	606.6 ± 24.5 b
	2.0	57.0 ± 2.9 a	451.8 ± 24.1 c
	3.0	54.8 ± 2.7 a	319.2 ± 11.0 d

Different letters within a column indicate significant difference using Tukey test at $P < 0.01$. The control is the same as Table 1.

Pollen exine is composed of sporopollenin, an organic biopolymer of extremely high stability (Wiermann and Gubatz 1992). Therefore, pollen is extremely durable. Pollen from tens of thousands of years ago has been found deposited in lakes or land (Berthou and Leereveld 1990; Karrow and Anderson 1975; Nakagawa et al. 1996). It was considered that pollen germination percentage not affected in the treatments because pollen is protected by sporopollenin. On the other hand, pollen tubes are composed of cell walls containing pectin, callose, and cellulose (Aloisi et al. 2017; Mascarenhas 1993). Therefore, pollen tubes might have been more sensitive to thermal changes than pollen grains. It is therefore necessary to proactively manage temperature by using black cheesecloth roof or an electric fan, when the temperature in the cultivated area is at 35℃ for less than 2 h or at 40℃ for 1 h in summer at daytime.

CONCLUSION

Pollen germination percentage and pollen tube elongation *in vitro* were affected by sucrose concentration and temperature. The sucrose concentration (10%) was favorable, and pollen germination decreased at lower (0–1 %) and higher (20-30 %) sucrose concentrations. The favorable temperatures for pollen germination ranged from 20-30℃ and decreased at temperatures below 15°C and above 35°C. Pollen germination was not affected by low temperatures (10-15℃). Pollen tube elongation was inferior to that at 25°C as pollen was exposed to higher temperatures (35-40℃) for a long time. Jaboticaba fruit drop in hot summer days might be due to the inhibition of pollen germination under high temperatures. It is desirable to use black cheesecloth or an electric fan in the cultivated area under high temperature and a heater under low temperature. It is not clear how pollen reacts to temperatures before anther dehiscence. Future studies on the thermal responses on pollen germination during anther dehiscence and in the pistil may reveal more details about the thermal response mechanism and improved productivity.

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