Pollen Germination Ability of Acerola in Relation to Fruit Set©

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The fruit of acerola (*Malpighia*) are known for their extremely high vitamin C content. However, the rate of fruit set by open pollination is generally low. Therefore, factors that might affect pollen germination rate such as temperature, humidity, and time of flowering during the day were investigated using several cultivars. The pollen germination rate varied depending on the time sampled, while treatment with humid air after flowering was found not to influence the pollen germination rate. Flower buds were sampled from acid-type trees about 3 days before bloom. They were maintained in an incubator at 25, 30, 35, and 40°C. For buds that bloomed after 2 or 3 days of incubation, the pollen grains were sown on agar medium and maintained at 27°C for 5 h. The pollen germination rate decreased significantly when the buds were placed under high-temperature conditions at 35 or 40°C. It can be inferred that the nutritional conditions of the tree, such as photosynthetic products during flower bud development, affect the pollen germination rate at flowering time.

INTRODUCTION

Acerola, an evergreen shrub in the family *Malpighiaceae*, is regarded as having originated in Central America. The fruit is extremely rich in vitamin C. Their contents are almost 3,000-4,000 mg per 100 g for immature fruit or about 2,000 mg per 100 g for mature fruit. Therefore, the fruit has attracted interest not only for its nutritional function but also for medical applications. The trees continue growth at 15°C or more, and bloom 3-4 times each year. Nevertheless, the rate of fruit set is generally low (Ishihata and Ito, 1994; Yonemoto, 2009). Yonemoto (2009) noted that trees in the open field set fruit well by open pollination, but that the rate of fruit set by open pollination is low under greenhouse culture in Okinawa. Acerola is entomophilic, and can set fruit by either self-pollination or cross-pollination. Acerola anthers produce many imperfect pollen grains, which is regarded as one reason for its lower rate of fruit set. Reportedly, the pollen germination rate is low (Handa et al., 2003) because the anther does not dehiscence automatically in Japanese cultivars or in those recently introduced from overseas (Handa et al., 2005). However, more than 50% of open pollinated flowers reportedly set fruit in Jamaica (Raw, 1979), and 51.7% of hand-pollinated flowers set fruit in Hawaii (Yamane and Nakasone, 1961). Freitas et al. (1999) reported that 30% of open pollinated flowers, 23.8% of cross-pollinated flowers, and 17.3% of self-pollinated flowers set fruit in Brazil. Therefore, the rate of fruit set in acerola can vary greatly among cultivars and according to environmental conditions.

In this study, factors that might affect pollen germination rate such as temperature, humidity, and time of flowering each day were investigated using several acerola cultivars.

MATERIALS AND METHODS

Plant Material

Experiments were conducted in 2012 with trees growing in a greenhouse at Meiji University. The cultivars used in the experiments included two cultivars of sweet-type and one cultivar of acid-type (cv. unknown). One sweet-type cultivar was 'Hawaiian Queen' (Sweet type B). The other was unknown (sweet-type A). These trees were obtained from Kagoshima University in 2006 and were planted in 29 or 60-L pots with humus soil.

Fruit Set by Hand Pollination

Pollen of the acid-type cultivar was hand-pollinated to flowers of the sweet-type cultivars at 9:00, 12:00, and 15:00 on 20 July and 21 July. The anthers of acid-type cultivar were

collected one day before hand pollination, with dehiscence at 28°C for 24 h. Hand pollination of flowers of sweet-type cultivars was performed using a small paintbrush. The pollinated flowers were assigned a label to record the flowering date and time. The fruit set percentages were determined 2 weeks after pollination.

Pollen Germination Rate

The pollen germination rate was evaluated on agar medium containing 1% agar, 0.01% of H_3BO_3, and 20 or 30% of sucrose. In addition, the pH of the medium was adjusted to 6.0. The pollen germination percentage was determined under a digital microscope (VH-8000C; Keyence Co.) as the ratio of the number of germinated grains per field of view to the total number of grains per field of view. A pollen grain was regarded as germinated when the pollen tube length was greater than the grain diameter. On 20 July and 21 July, flowers of acid type were sampled at 9:30, 12:30, and 15:30. Then the pollen grains were sown on the agar medium immediately and were incubated under dark condition at 27°C for 24 h.

On 8 August flowers were sampled immediately after blooming from two sweet-type trees and one acid-type tree. Then anthers were collected from each flower. The anthers of each cultivar were divided into two groups. Those of one group were allowed to open in the usual manner after being put into a drying oven maintained at 28°C for 24 h. Another group of anthers was then wrapped with the paraffin paper and put into the 100 ml beaker with a small quantity of water for treatment with humid air for 24 h. The pollen grains were sown on the agar medium after treatment. They were then incubated at 27°C for 5 h.

Flower buds at 1-3 days before blooming were sampled from acid-type trees in August, September, and November. The floral axis of the bud was dipped into water (August) or properly diluted floral preservative solution (September and November) in a small container (ca. 50 ml). They were maintained at 25, 30, 35, and 40°C in an incubator (LH-30-8CT; Nippon Medical and Chemical Instruments Co. Ltd.). The day length in the incubator was fixed at 14.5 h. The buds bloomed after 1 or 2 days. Then the pollen grains collected from bloomed flowers and sown on the agar medium and maintained at 27°C for 5 h.

Pollen Morphology

The diameters of germinated and the non-germinated pollen were determined under a digital microscope.

RESULTS AND DISCUSSION

Fruit set by the cross pollination at different times after blooming are shown in Table 1. The flowers of 'Hawaiian Queen' set no fruit by pollination at any time. Although the sweet type B set fruit by pollination at 9:00 AM and 15:00 PM, the rates were 5% at both times. As noted also in previous reports (Handa et al., 2003; Yonemoto, 2009), the rate of fruit set of acerola trees used for this study was very low.

Table 1. Differences in the fruit set by cross-pollination among the times of pollination in sweet type cultivars.

Cultivar	Time of pollination	No. flower	No. fruit set	Rate of fruit set (%)
Sweet-type A	9:00	40	2	5.0
	12:00	20	0	0.0
	15:00	40	2	5.0
Sweet-type B	9:00	15	0	0.0
	12:00	10	0	0.0
	15:00	15	0	0.0

The pollen germination rate was generally higher in the acid-type cultivar than in the sweet-type cultivar in these experiments: the germination rate of acid-type cultivar was about 20%, although it was invariably less than 10% in sweet-type cultivars. However, the germination rates of the pollen collected from the flowers after bloom showed no consistent tendency, even in the acid-type cultivar. The pollen germination rate differed depending on the time at which the pollen was sampled (Fig. 1). Matsuda et al. (2009) reported that the pollen of passion fruit maintained germination ability for a longer period under humid conditions (about 80% RH) in comparison with dry condition (about 13% RH). However, in acerola, the relative humidity of the air was also considered not to influence the germination rate of pollen collected from the flowers after blooming (Fig. 2). Therefore, the daily variation in the pollen germination rate seemed to be unaffected by relative humidity of the air.

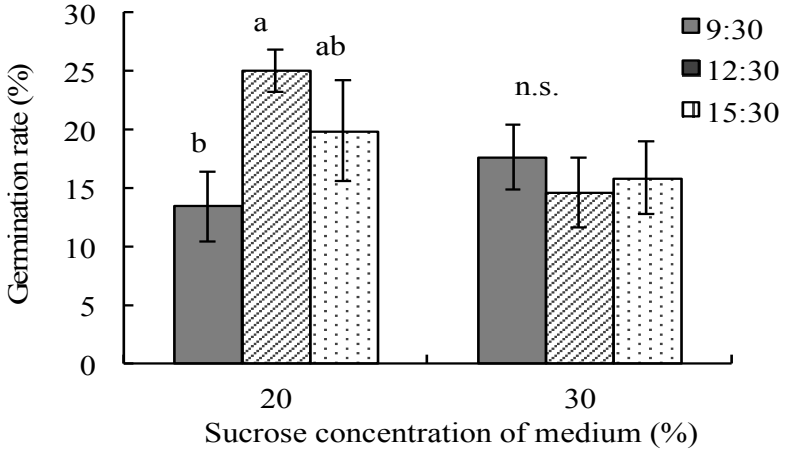

Fig. 1. Changes in the germination rates after flowering. Values with different letters are significantly different by Fisher's PLSD at 5% level.

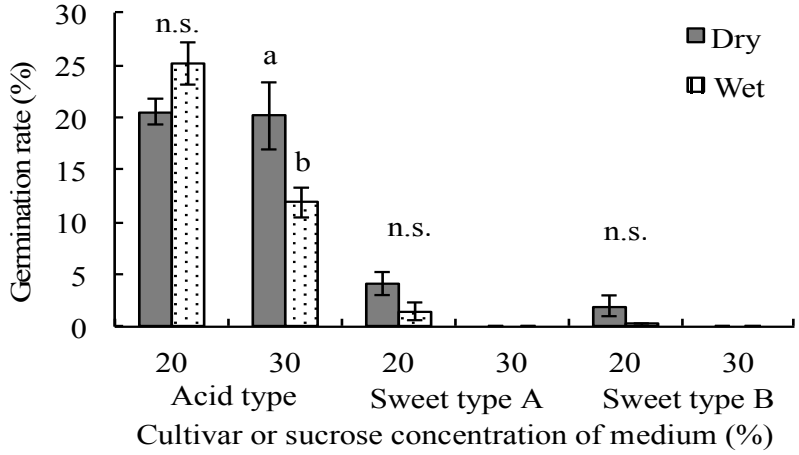

Fig. 2. Differences in germination rates of the pollen treated with dry or wet air. Values with different letters are significantly different by Fisher's PLSD at 5% level.

When buds before flowering were placed under high temperature conditions for 24-48 h, the pollen germination rate decreased significantly (Figs. 3 and 4). Decreased pollen germination rate with the rise of treatment temperatures was similar, but the sucrose

contents of the medium changed. Therefore, high temperatures immediately before flowering are thought to reduce pollen germination after flowering. The influence of high temperature was apparently greater in September than in November. In September, no difference was found in the pollen germination rate between 25 and 35°C, although it was significantly higher at 25°C than at 35°C in November. The effect of a difference in the temperature on pollen germination was apparently affected by the temperature in the greenhouse during flower-bud development (Table 2). Moreover, the germination rate of pollen treated with 25°C was highest in September and lowest in November, suggesting that the daily minimum temperature also affects the germination rate (Fig. 5).

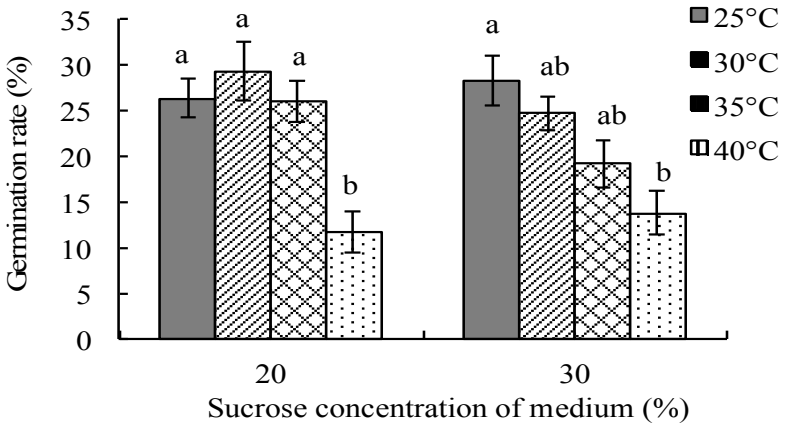

Fig. 3. Differences in the pollen germination rates of the flower treated with temperatures just before flowering (Sept.). Values with different letters are significantly different by Fisher's PLSD at 5% level.

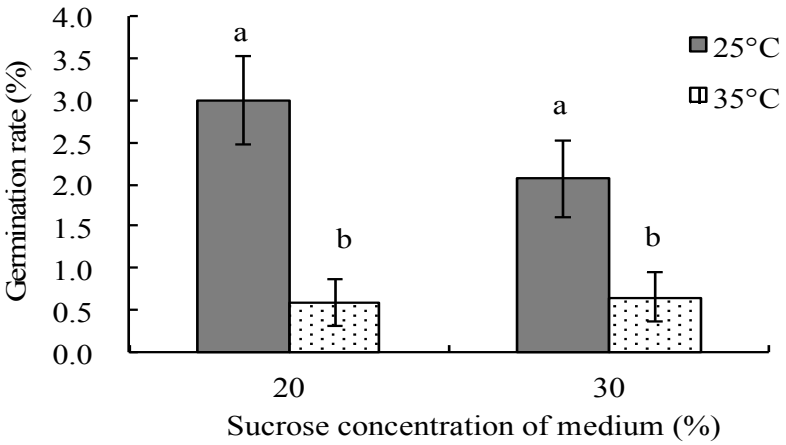

Fig. 4. Differences in the pollen germination rates of the flower treated with temperatures just before flowering (Nov.). Values with different letters are significantly different by Fisher's PLSD at 5% level.

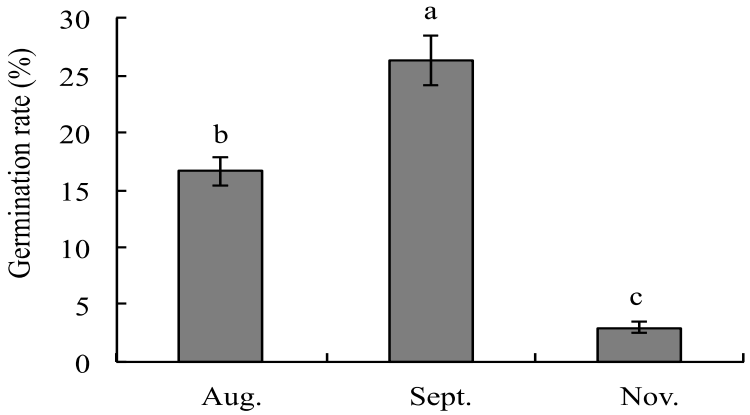

Fig. 5. Differences in the pollen germination rates of the flowers treated with 25°C just before flowering. Different letters indicate significant difference by Fisher's PLSD at 5% level.

Table 2. Maximum, mean, and minimum temperatures in the greenhouse during 5 days just before flower sampling in each month.

	Max. (°C)	Mean (°C)	Min. (°C)
Aug.	41.3	30.6	24.4
Sept.	37.4	27.7	23.1
Nov.	27.2	19.0	15.4

The germinated pollen diameter was significantly larger than that of non-germinated pollen (Fig. 6). Therefore, development of pollen was thought to be inferior at high temperatures and the number of germinated pollen decreased. In general, the rate of pollen germination decreases under high temperature conditions (Yasutomi, 1994; Yonemoto et al., 1999). Nevertheless, the results obtained in this study indicate clearly that high temperatures during pollen development also decrease the rate of germination. It can be inferred that the nutritional conditions of the trees, such as the photosynthetic products during flower bud development, affect the pollen germination rate at flowering time.

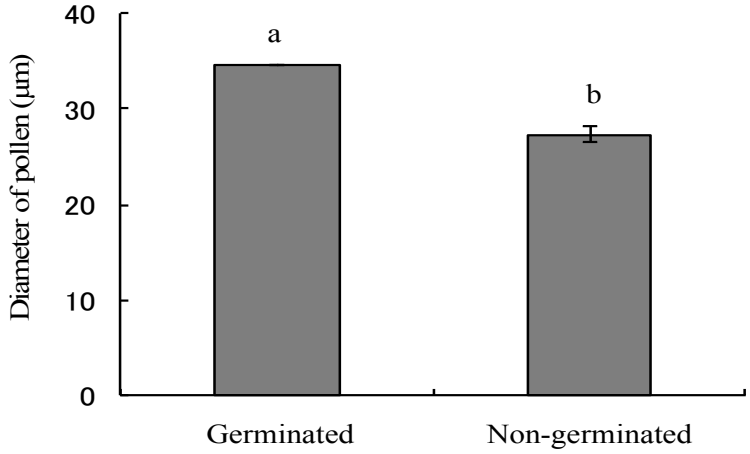

Fig. 6. Difference in diameters between germinated and non-germinated pollen. Different letters indicate significant difference by Fisher's PLSD at 5% level.

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