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RESEARCH ARTICLE

Pollen germination and hand pollination in pitaya *(Selenicereus spp.)*

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ABSTRACT

Hand pollination is a necessary assisting method for pitaya *(Selenicereus spp.)* production to achieve a high yield. With the cultivated area increasing at an exponential rate in recent years, a comprehensive study of the pollination process was conducted. We developed an ideal medium for pitaya pollen in vitro germination in this study, then tested the activity of pollen collected from or stored for various time periods. We discovered that those collected between 2 h before blooming and 6 h after blooming had the higher germination rates (27.2–65.1%), the highest activity of pollen occurred between 2 h and 4 h after blooming, and that storing them at 4°C for 24 h reduces their germination rate from 65.2 percent to 35.5 percent and their production to about 82%. As a result, pollinating plants with pollen that has been held for more than 24 h is not recommended unless a breakthrough in pollen storage is achieved. We also discovered that stigma receptivity and pollen activity are synchronized, which together determines the rate of fruit setting and the size of the fruit. Pollination within 6 h after flowering offers the optimum fruit setting percentage and size, while pollination at 6:00 pm, 2 h before blooming, is also a good alternative; however, pollination at 6:00 am the next morning is expected to result in a 23% drop in the fruit size. These findings will be beneficial for reproductive biology research, as well as laying the groundwork for hand pollination to boost pitaya output and breeding efficiency.

Keywords: Pitaya; Pollination; *In vitro* pollen germination; Stigma receptivity

INTRODUCTION

Pitaya (*Selenicereus spp.*) is a tropical and subtropical fruit tree native to Latin America that has attracted significant interest in the last decade for its potential as new exotic fruit crops and has been planted all over the world(Lichtenzveig et al., 2000). The planting area of which has risen tremendously in China in the previous 5 years due to its high market demand. Pitaya is a member of the Cactaceae family that blooms at night and is pollinated by nocturnal pollinators such as bats and moths in the native environment(Tran et al., 2015). However, because many pitaya varieties are self-incompatible, nocturnal pollinators are insufficient in production, hand pollination has become the most significant supplement for production.

Pitaya's florescence lasts about 5 days for one turn, and the bloom time of pollen source and mainly-planted varieties can differ by 1 or 2 days, making fresh pollen supply unseasonable, necessitating pollen storage. Pollen can remain viable from several minutes to tens of years, depending on the species(Shivanna, 2019), for pitaya, pollen sealed and stored below 4°C can be utilized for pollination the next day or even the day after the next day in production, but fruit setting percentage and size decreased. However, no data exists to date to illustrate the precise loss of fruit setting percentage and fruit size due to protracted pollen storage, and no more effective storage methods have been documented as of yet.

Kadri found the best fruit set for date palm (*Phoenix dactylifera L.*) was achieved by pollination between 12:00

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p.m. to 15:00 p.m. with fresh pollen performed on the same day of spathe cracking(Kadri et al., 2021), higher storage temperature and longer storage duration can decrease the fruit set rate to as 45% or less. Pitaya blooms at 8:00 p.m. and fades around 8:00 a.m. the next day. Neither the time-varying activity of pollen nor the time-varying stigma receptivity have been investigated. Such a short flowering period means that the pollination time needs to be more precisely timed to ensure fruit set and yield, a thorough research of the time-dependent pollen activity and stigma receptivity in pitaya is needed.

In vitro germination is one of the most essential approaches for understanding pollen properties, and research has revealed many types of media for pollen germination and pollen tube extension. Sucrose serves as the most common carbon source (O'Kelley, 1955; Nygaard, 1977), boric and/or calcium are essential for pollen tube growth (Brewbaker and Kwack, 1963; Potts and Marsden-Smedley, 1989). A conventional pollen germination medium consists of sucrose, boric acid, calcium nitrate, magnesium sulfate, and potassium nitrate (Brewbaker and Kwack, 1963; Roberts et al., 1983), media consist of those component with species differ concentration have been widely used. Aside from nutrition, pH and temperature are major environmental elements that influence *in vitro* pollen germination. Most of the media, for example, those of *Prunus laurocerasus* (Sulusoglu and Cavusoglu, 2014), *Garcinia mangostana* (Sutthinon et al., 2018), Triticum aestivum (Turan et al., 2018), and *Hydrangea macrophylla*, *Dichroa febrifuga* and their hybrids (Alexander, 2019) have been developed based on the Brewbaker and Kwack medium, which comprises 10% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulfate, and 100 ppm potassium nitrate (Brewbaker and Kwack, 1963). In fact, in the most essential ones, sucrose or/and boric acid is sufficient; (Sarkar et al., 2018); however, the presence of iron will improve germination or extension (Brewbaker and Kwack, 1963). An optimal medium for pitaya pollen germination is essential for an *in vitro* pollen study.

We are trying to produce an optimal pollen germination media to observe pollen activity utilizing *in vitro* germination and stigma receptivity by evaluating the fruit setting percentage and fruit size in order to better understand pollen activity and stigma receptivity. The ultimate goal is to find the time or stage when pollen activity is at its peak, collect the pollen, and use it to pollinate the stigma in the best possible state to boost yeild. The results will be a scientific guide for pitaya hand pollination.

MATERIALS AND METHODS

Plant material

'Red Crystall' and 'Big&Red' pitaya varieties were used in this study. 'Big&Red' was used as the pollen source variety for 'Red Crystall', and the plants were grown in a greenhouse at the Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou, China (23°09'14.9"N 113°22'22.0"E). All data were collected between June and October 2019 and 2020, during which the average low and high temperatures in the region were 24.9 and 32.1°C, respectively, and the average daytime was about 13 h.

METHODS

Medium for pollen germination

The Brewbaker and Kwack medium (pH 7.0) was used as control. We first investigated the time-varying germination rate in the control medium to estimate the time required to reach the highest germination rate at four different temperatures, 25, 28, 30, and 32°C (from ambient average low to high temperatures). We then investigated the time-varying germination rate at the optimum temperature for seven different concentrations (0, 50, 100, 200, 300, 500, and 800 ppm) of sucrose, boric acid, calcium nitrate, magnesium sulfate, potassium nitrate and varying pH (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5) values. After the single-factor experiment, combine those factors to test their interaction to get a best combination of concentration, temperature, and pH. To avoid the interference of the population effect of pollen, all the germination experiments were adjusted to about 10,000 pollen grains per milliliter medium. The germination rates were estimated using a microscope with at least 5 visual fields for one sample(Acar and Kakani, 2010).

Germination rates of pollen collected at different time points

Pollen from the 'Big&Red' plants were collected every 2 h, starting from 8:00 am on the first day (12 h before flower blooming) to 8:00 pm on the next day (12 h after flower withering), and germinated immediately using the optimal medium, and the germination rate was recorded for all the time points. The pollen collected from three plants was mixed as one sample.

Fruit setting percentage for pollination at different time points

The 'Red Crystall' plants were pollinated every 2 h starting from 8:00 am on the first day (12 h before flower blooming) to 8:00 pm on the next day (12 h after flower withering) with pollen collected at 8:00 pm before the first day from 'Big&Red' when the flowers just start blooming, and

the fruit setting percentage was recorded for all the time points. The uniformity of pollination is controlled using a sprinkling can, with 1 g pollen being dispersed in 50 ml germination medium and one punch per flower at 5 cm away from the stigma (0.7 ml per punch).

Pollen storage and activity

The fresh pollen collected from 'Big&Red' at 8:00 pm were divided into four parts. The 'Red Crystall' plants were pollinated using the first part immediately. The other three parts were stored under 4°C and used to pollinate the 'Red Crystall' plants at 24, 48, and 72 h (the second day, third day, and fourth day), respectively. For this experiment, both pollen collection and pollination were done at 10:00 pm, about 2 h after blooming. Each time, pollen were collected and mixed from at least 10 flowers, and 30 flowers were pollinated.

Fruit size and seed number description

Fruits from the treatment groups (at least 30 fruits from each treatment group) were weighed individually to determine their fruit sizes. The seed number per unit area was calculated from the transection of the fruits; the number of seeds at the center of the section, that is, in an area of 2 cm* 2 cm, were counted to obtain the seed intensity.

RESULTS

Pollen germination and pollen tube length in different temperatures and incubation times

For all treatment groups, the germination rate kept increasing from the 1st to the 10th h, after which it was almost constant till the 24th h (Figure 1a, Table S1). Although the germination rate at the 10th h was good enough as a representative final result for one of the treatment groups, we used the data of the 12th h as the representative result in order to be sure. On temperature experiment, 28°C is optimal for germination rate (Figure 1a), and the measurement of pollen tube length at 12th h point to the same result (Figure 1b, Figure S1, Table S2), so all the subsequent *in vitro* germination experiment will under 28°C.

Optimal *in vitro* pollen germination medium

By change one factor of the control medium under 28°C and estimated the germination rate after 12 h, it was seen that while the pollen were very sensitive to the change in the concentrations of sucrose and boric acid, and the pH (Figure 2a, Table S3), they were highly tolerant to the change in the concentrations of calcium nitrate, magnesium sulfate, and potassium nitrate (Figure 2b, Table S3). It was observed that, under conditions of pH below 6.5 or above 7.5, boric acid concentration below 100 ppm or above 500 ppm, and sucrose concentration below 10% or above 25%, the germination rates dropped rapidly. The optimal concentrations of sucrose and boric acid are 20% and 100 ppm, respectively, and the optimal pH is 7.5. Through the lack of calcium nitrate, magnesium sulfate, and potassium nitrate results in a drop in the germination rate, the change in their concentration from 50 to 800 ppm has very little influence on the germination rate. Therefore, we fix calcium nitrate, magnesium sulfate, and potassium nitrate at the optimal concentrations of 100, 300, and 300 ppm, respectively, and then explore the germination rates with different combinations of boric acid (100, 200, 300, and 500 ppm) and sucrose (10%, 15%, 20%, and 25%), and pH (6.5, 7.0, and 7.5; Figure 2c, Table S4). The optimal germination medium for pitaya pollen is 20% sucrose + 300 mg/L H_3BO_3 + 100mg/L Ca $(NO_3)_2 \cdot 4H_2O$ + 300mg/L KNO_3 + 300 mg/L $MgSO_4 \cdot 7H_2O$ under 28°C, pH 7.0 (Figure 2c, Table S4).

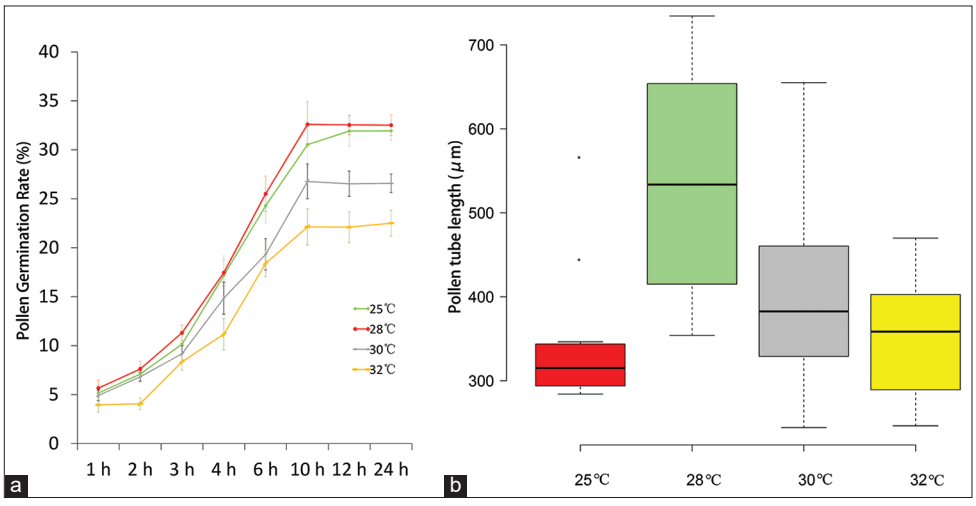

Fig 1. *In vitro* pollen germination rate and pollen tube length under different temperatures and incubation times. (a) Time-varying germination rate under different temperatures. (b) Pollen tube length statistics at the 12th h.

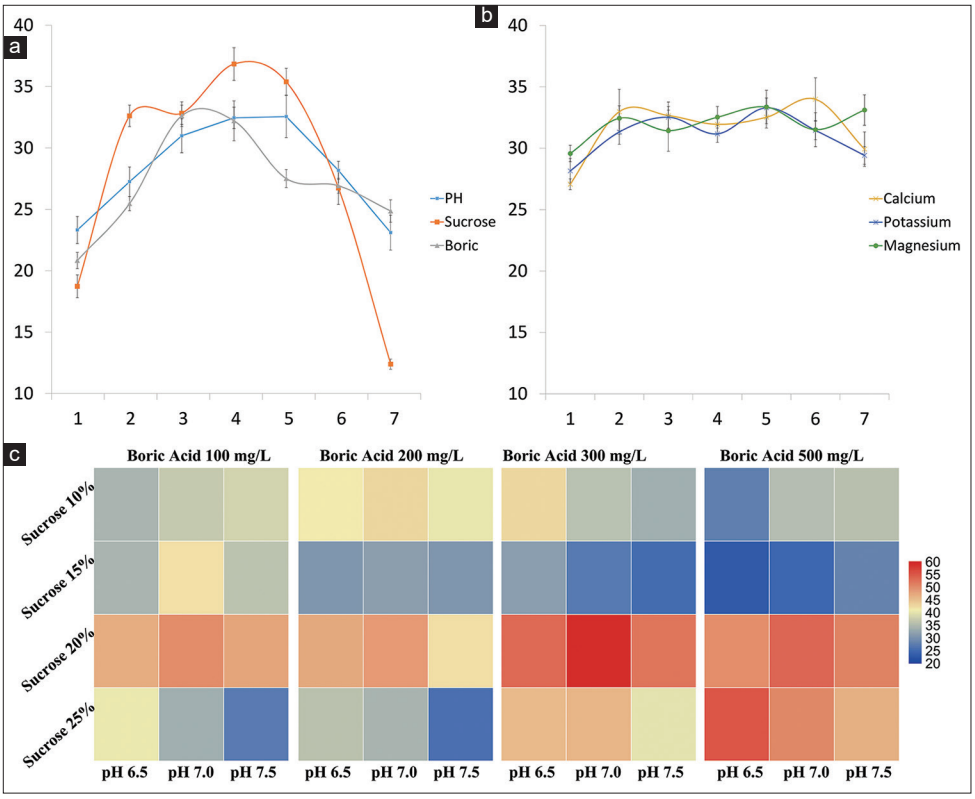

Fig 2. Germination rates under different media. (a) Germination rates under different concentrations of sucrose and boric acid, and varying pH. (b) Germination rate under different concentrations of iron, calcium, potassium, and magnesium. (c) Germination rates under different combinations of pH, sucrose, and boric acid. The number on the x-axis 1–7 means concentration 0, 50, 100, 200, 300, 500, 800 ppm or pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5.

Pollen storage and activity

The activity of pollen stored at 4°C or room temperature were estimated by *in vitro* germination every 2 h from 0 h (freshly collected pollen) to 48 h. At room temperature, the germination rate dropped to almost 0% in 18 h (Figure 3a, Table S5). For pollen stored at 4°C, the situation was much better because the germination rate was 29.5% at 24 h, and the pollen displayed some activity even at 48 h (Figure 3b, Table S6). When pollen stored at 4°C for 24, 48, and 72 h were used for hand pollination, the fruit setting rates were 92.5%, 16.7%, and 2.5% (Figure 3c, Table S7) and the fruit sizes were 283.6, 120.1, and 73.3 g (Figure 3d, Figure 4, Table S8), respectively, which were significantly lower than those when pollinated using fresh pollen (the fruit setting percentage was 99.2% and the average fruit size was 319.5 g). Therefore, it may be concluded that it is better to use fresh pollen for pollination as compared to pollen stored under 4°C for 24 h pollination because the latter leads to a 18% reduction (according to the reduction of fruit setting percentage and fruit size) in production.

The seed intensity was not different for fruits from different treatment groups (Figure 4, Table S9). In other words, it may be concluded that fruit size is positively related to the seed number and the pollen activity.

Time-varying pollen activity and stigma receptivity

To make the time point easier to understand, we define 8:00 pm, that is, the time flowers begin to bloom as 0 h, 2 to 12 h before blooming as –2h to –12h, 2 to 12 h after blooming as 2 h to 12 h, 2 to 12 h after the flowers withered as +2 h to +12 h. The pollen collected at –12 h or –10 h have almost no activity; the activity keeps increasing after –10 h, with the germination rate reaching the highest value of 65.1% at 2h. The germination rate starts dropping after 2 h, reaching 0% at +8 h (Figure 5a, Table S10). Similar to pollen activity, the stigma pollen acceptance capacity also reaches a peak and then goes down, the capacity being determined by the fruit setting percentage. The pollen used in this experiment was freshly collected or stored at 4°C for less than 24 h. The germination rate (37.6%–65.1%) for each time point is shown as a line chart and the corresponding fruit setting percentage is shown as a boxplot in Figure 4b. At –2 h, it can be observed that the stigma already has the capacity to accept the pollen, with a fruit setting percentage of 93.3%, while pollinate at -4 h or earlier, the highest fruit setting percentage is only 71.7%; between 0 h and 8 h, the fruit setting percentage is almost 100%; at 10 h, that is, at 6:00 am the next morning, the fruit setting percentage is 92.5%, after which it drops to less than 89.2% and finally to 2.5% at +12 h (Figure 5b, Table S11). The fruits were weighed after maturation (Figure 5c, Table

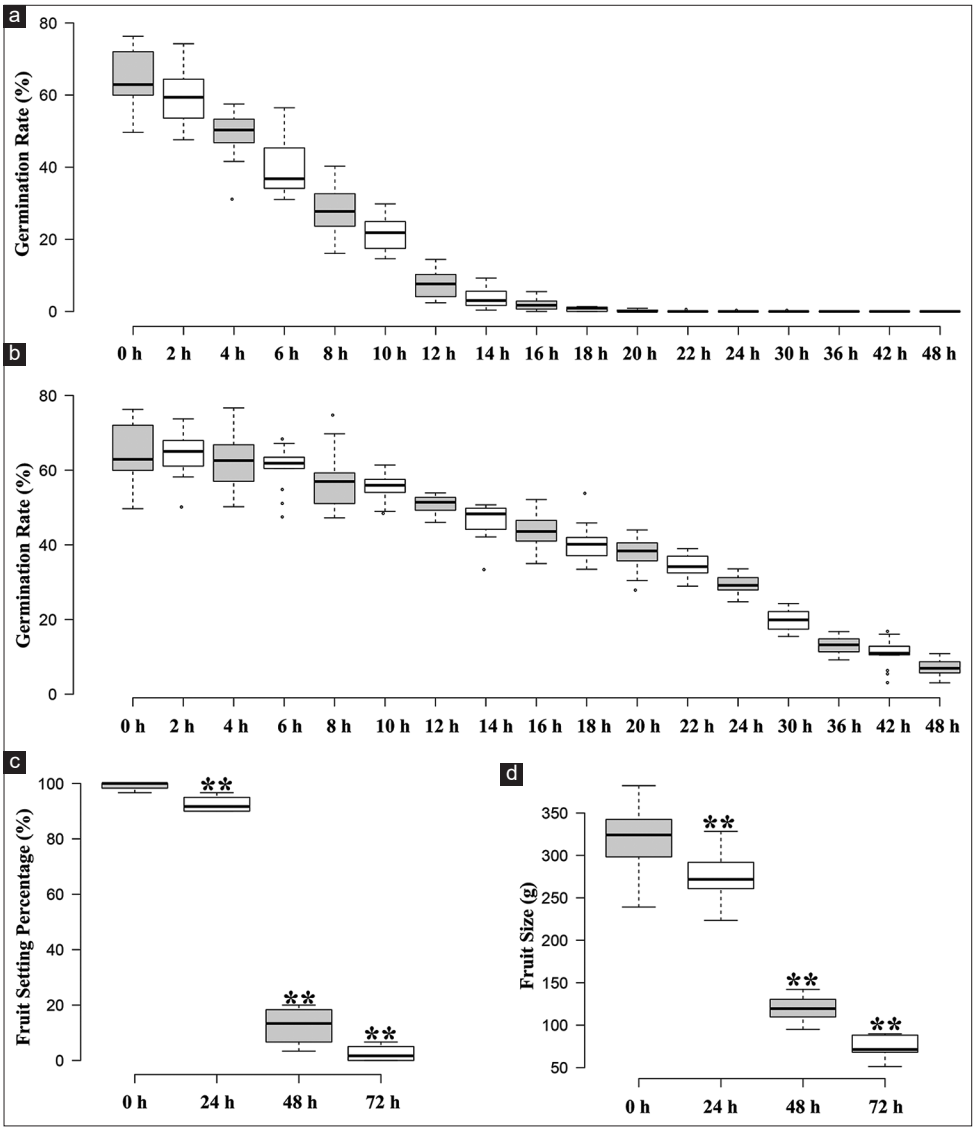

Fig 3. (a) Time-varying activity of pollen stored at room temperature. (b) Time-varying activity of pollen stored at 4°C. (c) Fruit setting percentage of pollinations with pollen stored for different time periods. (d) Fruit size of pollinations with pollen stored for different time periods.

S12); the fruit size at –2, 0, 2, 4, and 6 h was the same level and that of fruits at –4, 8, 10, 12, and +2h was significantly than the fruits of 0 h (p-value<0.01), size of other time points were didn't measured due to inadequate numbers of fruit.

The data mean that pollination taking place in 8 h after blooming will result in about 100% of fruit setting and significantly bigger fruits than those occurring at other time points. Pollination at –2 h will result in a reduced fruit setting percentage but will not affect the fruit size. Pollination at 8 h, when the flowers are still open, the fruit setting percentage is as high as 99.2%, but the size of fruits is already became significantly smaller (10%) than those of 0 h, the size reduction became more and more serious for fruit pollination at 8 h or later. Pollination in 6 h after blooming is the best choice; however, considering the fact that it is much easier carry out the process during daytime, pollination before dark or the next morning is usually carried out. Our data show that pollination at –2 h is much better than that carried out the next morning with respect to both fruit setting percentage and fruit size.

DISCUSSION

Our results developed a relatively optimal medium (20% sucrose + 300 mg/L H_3BO_3 + 100 mg/L $Ca(NO_3)_2.4H_2O$ + 300 mg/L KNO_3 + 300 mg/L $MgSO_4.7H_2O$ under 28°C, pH 7.0) for the pollination of pitaya pollen. However, there is no best medium because in natural, pollen germinate and grow in the pistil, which is a complex, time-changing environment involving different phases of pollen growth and different requirements (Holdaway Clarke and Weddle et al., 2003). The highest pollen germination rate with

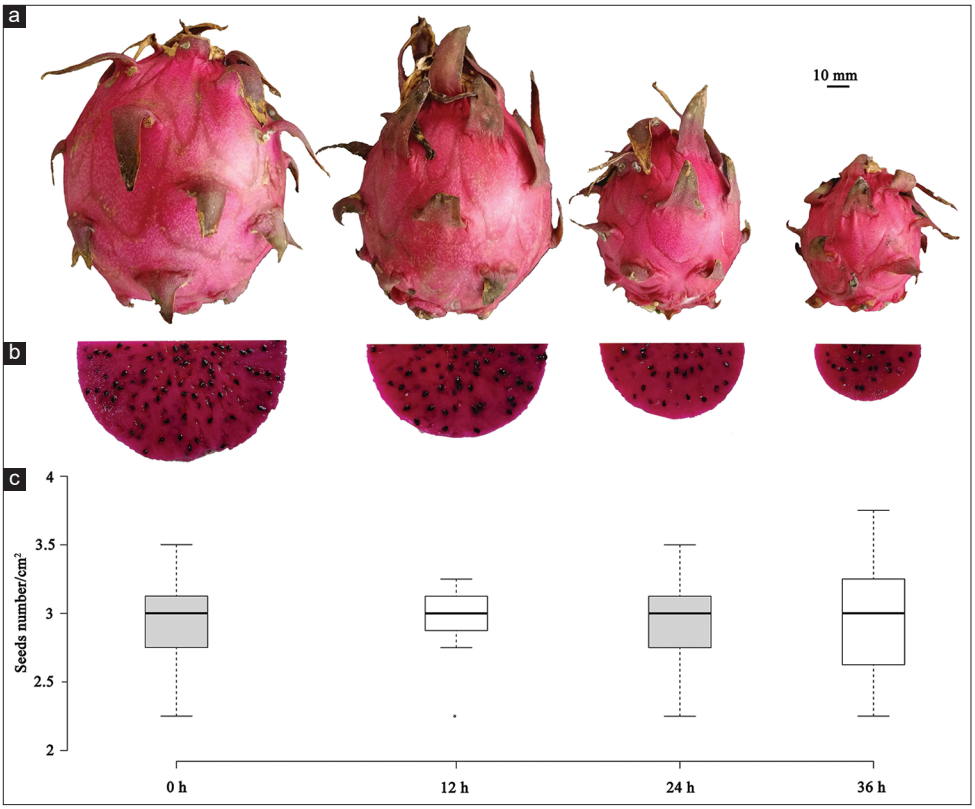

Fig 4. Fruit size (a), fruit equatorial longitudinal section (b) and seeds intensity (c) for pitaya pollinated with fresh pollen (0 h) or pollen stored for 24 h, 48 h and 72 h.

this optimal medium is 65.1%, which is reached in 10 h, increasing the time does not result in a higher germination rate or a longer pollen tube, that may be because 65.1% is very close to that obtained by natural pollen activity or the bound defect of the medium.

Pollen storage is an established technique in many species, it could be cryopreservation at -20°C, -80°C or -196°C, organic solvent preservation, sealed dry preservation and so on(Harrington, 1970; Iwanami and Nakamura, 1972; Pinney and Polito, 1989; Shivanna et al., 1991; Martínez-Gómez et al., 2002; Towil, 2010). In our study, we also refer to those methods, but no activity was detected after one week of storage (data not show). At present, there is no effective method for long-term pollen storage. Further study need be carrying out on pitaya pollen storage.

With the optimal medium for germination, our results suggest that pollen collected at 22:00 pm (about 2 h after blooming) have the highest activity, which means that pollen collected at 22:00 pm are the most suitable to be stored for hand pollination later. However, a higher pollen activity does not mean a higher fruit setting percentage or fruit size since there seems to be a saturation value for pollen activity. The germination rate changes from 56.3% to 65.1% (from 8:00 pm to 2:00 am the next day) result the same fruit setting percentage (about 100%) and same level of fruit size (from 306 to 313 g with no significant difference). Even pollination using pollen with a germination rate of 37.6% (pollination at 6:00 pm with pollen collected at 10:00 pm the day before) does not affect too much of the yielding. Although pollination during daytime is much easier, it is better to carry out the pollination not earlier than 2 h before blooming to minimize production losses. Pollination the next morning will lead to more production losses. The best choice for hand pollination is to pollinate within 6 h after blooming (from 8:00 pm to 2:00 am next morning). For pollination at 4:00 am, the fruit setting percentage can reach up to 99.2% but the fruit size is significantly 10.5% smaller. In pollinations carried out after 4:00 am, both the fruit setting percentage and fruit size drop rapidly.

No matter pollination at earlier than 2 h before or later than 8 h after blooming will lead to production losses, but the pattern is different. The production loss of pollination at morning is caused by the decrease in the fruit size, although the fruit setting percentage is still at a relatively high level. We speculate that it is because in the morning, the temperature becomes higher and higher, with water loss from the stigma inhibiting pollen germination, thus reducing the fruit size. The production loss of pollination before blooming is mainly caused by low fruit setting percentage, while the fruit size has not been affected too

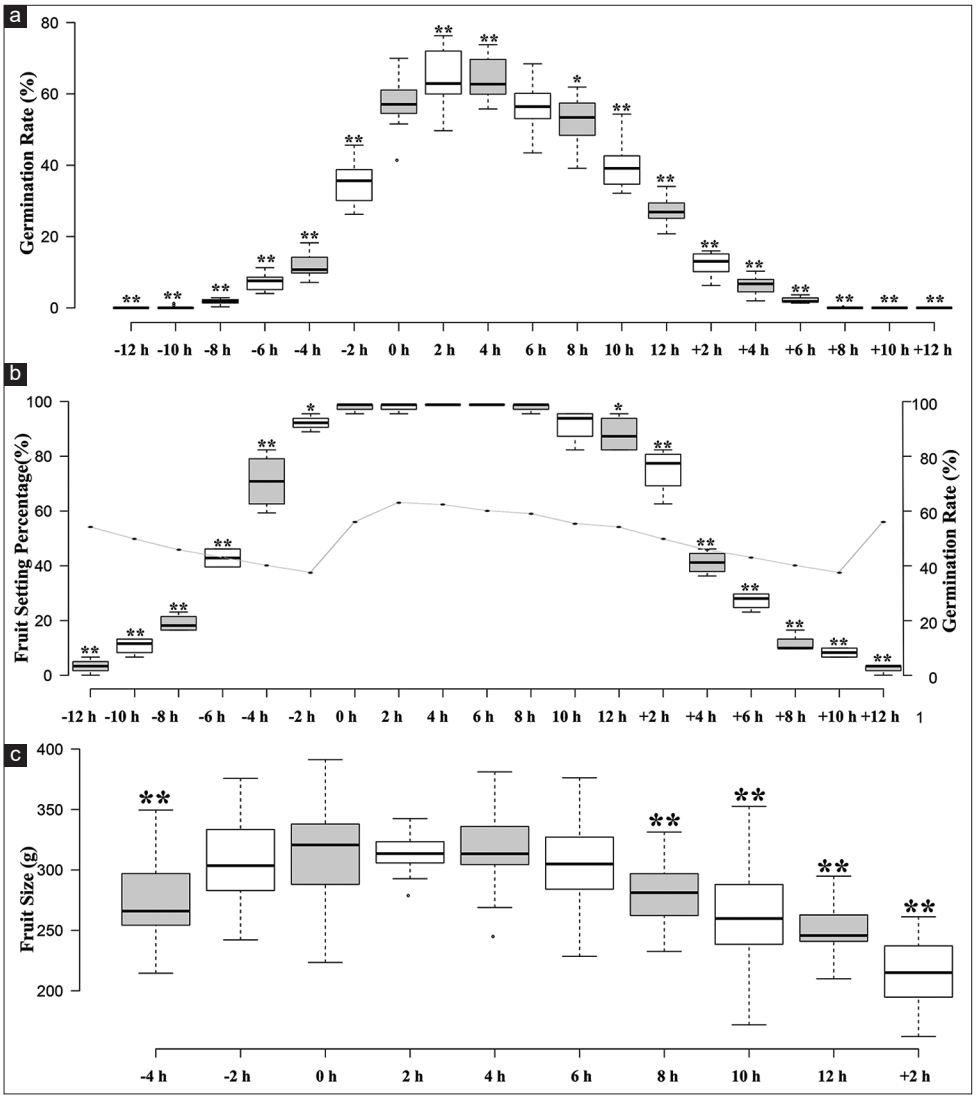

Fig 5. (a) Activity of pollen collected from 12 h before blooming to 12 h after withering; (b) Fruit setting percentages for pollination from 12 h before blooming to 12 h after withering are shown as boxplots, and the corresponding pollen activities are shown as line charts; (c) Fruit size of fruits from pollination at different time points. All the comparisons, fruit size, germination rate, fruit setting rate are compare with the data at 0 h, ** and * means extremely significant different and significant different.

much. It seems is because that the pistil is not totally ready yet before blooming, but the pollen on the stigma can wait for the mature of pistil with a tiny reduction of activity thus lower the fruit setting percentage. Pollen stored at 4°C for 24 h will show a reduction in their activity, thus decreasing the fruit setting percentage and fruit size. However, it can still be considered as an alternative option with an 18% reduction in production.

In pitaya production, the process of hand pollination is usually by virtue of experience. But experience won't always work. In some complex scenario, like a short-term rainstorm, the work of pollination will be ruined. So, will hand pollination be successful before or after rainstorm, i.e. before blooming or several hours after blooming? Our study confirmed that the pollen had a high germination rate (>35.5%) and the stigma had a high receptivity (>92.5%) from 2 h before blooming to 10 h after blooming (>=92.5%), during which the pollen collection and pollination can ensure normal fruit setting and yield. Our research on pollen activity and stigma receptivity can be a technical guidance to hand pollination, and thus, avoid losses or improve production and breeding efficiency.

CONLUSION

In this study, the pollen germination medium and environment factors of pitaya were optimized. Based on this, it was found that the highest pollen activity of pitaya occurs between 2 and 4 hours after flowering, and the optimal pollination time is within 6 hours after flowering. The fruit size was more affected by pollen activity than

stigma receptivity, and with regard to current pollen storage technology, the use of non-fresh pollen for pollination will leads to significantly lower fruit set rate and fruit size.

FUNDING

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Authors' contribution

Qingming Sun designed experiments; Juncheng Li, Yulin Wang carried out experiments; Juncheng Li, Hongfen Dai, Junsheng Zhao analyzed experimental results; Juncheng Li wrote the manuscript.

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Table S1: Germination rate under different temprature from 1 to 24 h, 4 replications multiply 3 visual fields for one treatment were measured

		Time	1 h	2 h	3 h	4 h	6 h	10 h	12 h	24 h
Temperature and Germination Rate	25 ℃	Replication1-visual field1	2.8	2.3	7.8	18.5	20.8	20.3	36.3	36.5
		Replication1-visual field2	1.9	8.7	14.4	26.9	29.5	20.7	27.6	36.1
		Replication1-visual field3	1.8	8.9	10.2	11.2	19.2	44.8	32.4	36.1
		Replication2-visual field1	6.2	6.0	4.9	18.0	30.4	39.2	29.2	35.0
		Replication2-visual field2	8.1	5.5	8.5	13.8	32.0	35.8	34.6	33.6
		Replication2-visual field3	8.7	11.3	9.7	20.2	27.0	35.3	28.4	32.8
		Replication3-visual field1	8.0	6.0	13.9	11.1	13.8	24.7	35.7	31.8
		Replication3-visual field2	5.9	5.6	12.8	14.6	15.7	35.4	26.7	30.6
		Replication3-visual field3	2.4	5.4	7.7	14.5	30.2	20.6	31.4	29.2
		Replication4-visual field1	5.6	8.5	10.8	15.3	28.7	30.6	36.8	28.3
		Replication4-visual field2	2.5	11.5	10.0	19.1	24.8	26.7	30.9	27.3
		Replication4-visual field3	8.4	5.6	11.0	22.9	19.4	32.4	33.1	25.8
		Average	5.2	7.1	10.1	17.2	24.3	30.5	31.9	31.9
		SE	0.8	0.8	0.8	1.4	1.8	2.3	1.0	1.1
	28 ℃	Replication1-visual field1	3.0	3.2	9.7	19.4	22.6	22.6	46.9	37.2
		Replication1-visual field2	2.8	9.4	14.7	28.1	31.3	21.9	28.8	33.7
		Replication1-visual field3	2.8	9.4	12.1	11.4	21.2	45.2	33.3	31.5
		Replication2-visual field1	6.3	6.5	6.5	19.4	32.4	40.0	29.4	28.1
		Replication2-visual field2	8.6	5.7	9.4	15.2	32.3	38.2	35.1	36.4
		Replication2-visual field3	9.7	11.4	11.4	21.2	28.1	35.5	28.6	29.0
		Replication3-visual field1	8.8	6.1	15.2	12.5	15.6	27.3	36.4	34.5
		Replication3-visual field2	5.9	6.3	13.9	25.5	16.1	36.4	28.6	29.0
		Replication3-visual field3	3.1	6.3	8.6	5.7	30.3	21.9	31.4	28.8
		Replication4-visual field1	5.7	9.1	12.5	16.7	29.0	31.3	27.3	30.8
		Replication4-visual field2	2.9	11.8	10.5	9.4	27.3	28.1	31.4	34.2
		Replication4-visual field3	8.6	6.5	11.4	25.0	20.0	42.9	33.2	37.0
		Average	5.7	7.6	11.3	17.5	25.5	32.6	32.5	32.5
		SE	0.8	0.7	0.7	2.0	1.8	2.4	1.5	1.0
	30 ℃	Replication1-visual field1	2.2	3.5	6.6	18.3	14.8	17.2	29.6	20.0
		Replication1-visual field2	3.8	8.8	11.7	14.3	20.0	31.3	21.0	29.0
		Replication1-visual field3	3.1	8.5	10.6	7.1	20.3	31.0	28.6	24.9
		Replication2-visual field1	5.7	5.5	3.3	17.8	29.3	32.7	24.9	24.6
		Replication2-visual field2	4.6	6.0	7.2	12.5	20.2	32.4	34.1	27.8
		Replication2-visual field3	8.0	6.3	9.1	21.0	20.9	25.5	26.9	24.7
		Replication3-visual field1	5.3	7.3	12.0	10.5	19.5	17.4	24.9	30.0
		Replication3-visual field2	6.7	5.7	13.4	24.7	9.4	28.5	26.1	26.9
		Replication3-visual field3	3.5	6.7	8.2	8.4	19.5	25.3	30.2	29.5
		Replication4-visual field1	5.1	8.8	9.5	13.8	19.0	27.3	18.6	28.3
		Replication4-visual field2	3.5	8.6	8.5	8.9	27.1	18.5	22.4	22.3
		Replication4-visual field3	7.6	6.2	10.1	21.0	12.0	34.2	31.0	30.7
		Average	4.9	6.8	9.2	14.9	19.3	26.8	26.5	26.6
		SE	0.5	0.5	0.8	1.6	1.6	1.8	1.3	1.0
	32 ℃	Replication1-visual field1	1.5	1.8	6.5	17.6	19.9	27.7	21.9	18.3
		Replication1-visual field2	1.8	6.1	13.8	17.9	19.0	14.8	12.2	28.4
		Replication1-visual field3	1.1	4.9	7.4	4.4	15.9	23.0	23.2	22.5
		Replication2-visual field1	5.4	2.2	4.8	14.7	20.0	16.6	19.5	23.0
		Replication2-visual field2	5.7	2.1	7.2	14.9	19.4	29.1	27.6	12.5
		Replication2-visual field3	7.1	5.6	9.5	15.0	18.9	19.5	22.2	25.2
		Replication3-visual field1	6.4	2.8	10.1	9.0	12.6	32.7	15.4	28.2
		Replication3-visual field2	5.3	1.9	13.0	13.2	18.2	12.2	32.6	27.5
		Replication3-visual field3	0.6	3.4	6.6	2.3	11.9	23.5	19.3	22.1
		Replication4-visual field1	4.3	7.6	9.6	9.3	13.4	17.2	21.0	21.9
		Replication4-visual field2	0.8	6.5	5.4	2.9	22.2	20.0	28.0	21.0
		Replication4-visual field3	7.6	4.1	6.4	12.9	29.5	29.2	22.3	19.5
		Average	4.0	4.1	8.4	11.2	18.4	22.1	22.1	22.5
		SE	0.8	0.6	0.8	1.6	1.4	1.9	1.6	1.3

Table S2: Average tube length measured after germination for 12 h under different temprature

Temperature	25 ℃	28 ℃	30 ℃	32 ℃
Tube Length	307.8	642.3	357.7	246.3
	291.1	646.3	467.6	395.1
	284.1	541.6	453.3	314.4
	443.8	525.8	487.1	395.5
	346.4	354.1	368.8	469.9
	566	456.4	655.1	303.2
	315.9	376.4	360.1	410
	314.4	450.2	396.3	345
	334.7	661.5	398.9	266.1
	341.1	680.3	300.4	422.9
	296.2	734.4	244.2	372.1
	291.5	379.9	275.2	275.2
Average	344.4166667	537.4333333	397.0583333	351.3083333
SE	23.636505	38.54715792	31.87424499	20.36282434

Table S3: Germinate rate of the one factor expriment, the 7 level means 7 different concentration of surose or boic, calcium, potassium, magnesium and different PH values. For PH, 1 to 7 represent PH 5.5, 6, 6.5, 7, 7.5, 8, 8.5; for sucrose, 1 to 7 represent the concentration of 0%, 10%, 15%, 20%, 25%, 30%, 35%; for boic, calcium, potassium and magnesium, 1 to 7 represent the concentration of 0, 50, 100, 200, 300, 500, 800 ppm

	Level	Germination Rate						
		Replication1-visual field1	Replication1-visual field2	Replication1-visual field3	Replication2-visual field1	Replication2-visual field2	Replication2-visual field3	Replication3-visual field1
PH	1	20.43221	21.95727	26.02118	27.98227	19.87178	23.24973	15.26234
	2	33.06451	22.90941	31.14362	21.18388	31.85419	22.45877	29.28628
	3	30.45865	23.02812	30.31479	31.08299	38.20811	27.11076	38.1076
	4	35.65192	33.10063	32.85591	30.82556	31.52689	34.57011	28.60858
	5	42.08658	31.41082	24.86602	25.3049	30.29492	27.33138	34.15684
	6	26.31063	23.82272	26.91572	28.65474	28.49176	32.06684	28.24136
	7	25.21557	26.32672	20.97861	12.62166	23.77392	28.7102	15.12685
Sucrose	1	14.94056	15.30323	16.52266	23.70522	16.64818	19.42684	17.69401
	2	35.65192	33.10063	32.85591	30.82556	31.52689	34.57011	28.60858
	3	32.06643	35.34193	29.83608	26.00485	32.57926	35.35168	37.98747
	4	29.37649	27.99442	37.00657	36.77636	41.05875	36.53185	39.27002
	5	35.36332	33.43186	38.45989	38.9447	36.71206	28.56589	34.74464
	6	30.1373	22.53793	26.69799	20.1313	24.22283	22.18084	27.13178
	7	12.171588	9.952111	12.581638	12.579993	11.267362	15.457963	11.76439
Boric	1	19.63257	19.45242	20.87013	23.37835	19.71525	24.39982	23.03706
	2	26.48614	27.14387	23.50385	22.97171	25.85114	26.83265	27.3369
	3	35.65192	33.10063	32.85591	30.82556	31.52689	34.57011	28.60858
	4	35.83443	33.36191	42.71121	33.83048	30.95986	29.27024	19.79425
	5	29.84874	21.69989	26.22268	28.48982	28.99682	25.16161	30.39189
	6	26.38972	25.8116	23.98717	28.39909	29.14297	27.19661	22.17512
	7	24.9718	27.41011	23.72758	22.80846	28.97413	25.91545	21.5849
Calcium	1	26.69978	27.76733	26.07363	25.8863	25.79811	30.03087	24.94813
	2	28.41047	30.55183	34.6615	27.65911	31.40369	30.67284	20.19212
	3	30.03316	33.99053	37.94655	34.95194	36.43867	33.28078	35.63253
	4	31.80044	30.34895	31.14809	29.09635	33.65692	36.6999	28.3117
	5	35.65192	33.10063	32.85591	30.82556	31.52689	34.57011	28.60858
	6	30.79366	35.22381	33.00801	27.65102	23.71549	45.9699	36.93069
	7	31.117	29.17025	27.11482	35.20034	32.63607	19.96331	31.53992

(*Contd...*)

Table S3: (*Continued*)

	Level	Germination Rate						
		Replication1-visual field1	Replication1-visual field2	Replication1-visual field3	Replication2-visual field1	Replication2-visual field2	Replication2-visual field3	Replication3-visual field1
Potassium	1	28.53649	21.8807	27.97306	27.778	29.60897	27.51038	26.84764
	2	34.67276	29.40016	35.14634	31.19681	29.90082	30.40872	32.59089
	3	35.65192	33.10063	32.85591	30.82556	31.52689	34.57011	28.60858
	4	30.81943	36.51502	30.11475	31.696	30.39626	25.86288	29.94777
	5	35.71998	33.96405	31.52687	29.85446	27.51247	37.70517	35.79503
	6	29.32527	33.31955	32.60896	28.56434	29.07381	32.2971	26.93575
	7	28.54754	28.47815	29.39177	29.49259	26.67135	31.07318	32.31395
Magnesium	1	31.86064	25.15374	28.45537	30.15632	28.55185	28.80327	27.00081
	2	32.87635	39.79537	34.47471	33.09311	27.37388	32.29419	34.18573
	3	37.53526	33.26153	35.66163	19.01634	32.07332	38.6063	30.78356
	4	35.65192	33.10063	32.85591	30.82556	31.52689	34.57011	28.60858
	5	33.34966	43.09145	35.78891	36.17198	34.32379	34.05618	28.94807
	6	33.2416	33.27633	29.85398	26.3437	27.27071	35.39324	33.19877
	7	31.60111	39.75189	38.55672	30.76673	31.01651	27.5265	29.79229

	Level	Germination Rate						
		Replication3-visual field2	Replication3-visual field3	Replication4-visual field1	Replication4-visual field2	Replication4-visual field3	Average	SE
PH	1	28.045	25.74913	26.4884	22.12918	20.39817	23.13222167	1.114122883
	2	28.25482	31.01257	23.61353	26.53794	23.94613	27.10547083	1.207522683
	3	24.23229	36.0532	29.30818	31.65694	31.02681	30.88237	1.393672326
	4	39.14848	28.83695	32.83268	33.02108	29.2935	32.52269083	0.878528001
	5	34.85538	36.15677	31.86806	43.22806	27.91949	32.45660167	1.725097593
	6	27.42936	33.3959	26.04671	28.2233	26.80574	28.03373167	0.746032087
	7	29.09086	23.19109	23.63	20.68338	25.64037	22.91576917	1.437455499
Sucrose	1	22.68313	22.61719	20.2951	14.00802	18.2291	18.50610333	0.940478876
	2	39.14848	28.83695	32.83268	33.02108	29.2935	32.52269083	0.878528001
	3	35.0468	35.24625	31.84198	31.06696	30.51994	32.7408025	0.935568864
	4	36.62397	39.45095	33.1495	43.39217	40.77111	36.78351333	1.338836151
	5	34.05411	30.78631	39.88646	32.12078	40.86041	35.32753583	1.095414098
	6	30.82374	36.16014	27.13654	21.67509	30.22473	26.58835083	1.357485413
	7	11.052771	11.864158	12.262751	13.740998	10.546465	12.10351567	0.421467054
Boric	1	19.79626	21.28696	21.23525	15.86089	18.84232	20.62560667	0.664397223
	2	21.36497	26.50134	26.73598	22.93867	25.98459	25.3043175	0.585750253
	3	39.14848	28.83695	32.83268	33.02108	29.2935	32.52269083	0.878528001
	4	36.57514	26.4347	34.79474	30.51481	31.25392	32.1113075	1.638737821
	5	27.68045	26.47599	29.83534	28.78427	24.81975	27.36727083	0.743646666
	6	28.52768	25.99633	29.1614	26.48885	28.00168	26.773185	0.611690918
	7	28.40806	21.81523	22.56942	28.72442	19.41295	24.6935425	0.921752207
Calcium	1	26.0118	26.43316	28.6603	28.02582	28.53523	27.07253833	0.435358077
	2	39.5185	38.2077	44.15506	32.8018	37.42639	32.97175083	1.834058383
	3	27.04568	27.04293	36.14434	29.50537	29.91455	32.66058583	1.094312844
	4	30.46329	30.46185	31.54242	33.70605	36.05564	31.94096667	0.751838609
	5	39.14848	28.83695	32.83268	33.02108	29.2935	32.52269083	0.878528001
	6	29.63274	31.12805	37.88246	34.81286	41.07591	33.98538333	1.749094455
	7	25.74497	34.79536	23.89161	32.66894	35.27461	29.92643333	1.405218444
Potassium	1	31.28632	35.17087	24.7933	25.03628	31.36719	28.1491	1.008671288
	2	26.05207	24.81833	31.47427	34.99442	35.19128	31.3205725	1.00218424
	3	39.14848	28.83695	32.83268	33.02108	29.2935	32.52269083	0.878528001
	4	31.69664	32.20408	31.38223	32.7991	30.57214	31.16719167	0.700343155
	5	34.64632	32.44826	32.65576	33.2853	34.3631	33.28973083	0.800196086
	6	30.13166	32.93176	35.21662	31.768	35.21742	31.44918667	0.762958039
	7	27.80077	28.04022	33.68887	32.28422	25.18851	29.41426	0.723916012

Table S4: Pollen germination rate under combination of different concentrations of sourose (10%, 15%, 20%, 25%), boric acid (100, 200, 300, 500 ppm) and PH (6.5, 7, 7.5)

	100-6.5	100-7	100-7.5	200-6.5	200-7	200-7.5
10	34.14916325	37.2082175	38.16810482	41.25384	42.88515962	40.35641
15	34.14539882	42.22548036	36.2011096	30.5213441	31.66039774	30.52134075
20	46.82192123	49.99591933	47.78082001	47.2657136	48.72216551	42.39818976
25	40.56384521	33.63235843	27.22437	36.02248496	34.08342138	25.80413703
	300-6.5	**300-7**	**300-7.5**	**500-6.5**	**500-7**	**500-7.5**
10	42.73909222	35.77708343	33.64432397	27.63813098	35.23010927	35.87714916
15	31.33489797	26.74787051	25.31680506	22.79000985	24.61114278	28.19430829
20	53.27445778	59.16511901	52.27444959	49.89774387	53.64502341	50.96687993
25	45.58418212	46.13200266	39.95380239	55.24701516	50.40961124	46.55191192

Table S5: Germination rate of pollen stored under room temprature fro 0 to 48 h

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h
Replication1 - visual field1	57.09387	60.40149	53.6702	56.46966	30.55242	19.550084	6.661383	8.1192529	1.4211205
Replication1 - visual field2	62.54804	58.50519	47.08894	32.06214	29.03101	22.15055	7.082225	0.7779326	2.6340557
Replication1 - visual field3	72.27734	65.90714	55.06167	36.53183	35.37272	25.113972	13.904228	2.4116447	0
Replication2 - visual field1	76.29391	63.0975	31.12372	34.69285	28.50149	23.452999	6.651461	0.3777298	2.8121274
Replication2 - visual field2	66.24519	59.50745	57.51796	36.00057	26.97808	19.895231	2.772938	5.3364232	1.8886087
Replication2 - visual field3	63.281	54.99342	50.7191	35.99974	20.37384	29.821847	3.31347	3.018196	2.9663349
Replication3 - visual field1	49.68078	59.16008	50.48188	33.58757	25.25525	14.723454	9.802242	1.8699033	0
Replication3 - visual field2	59.91553	48.24596	50.1562	31.05413	40.29603	29.811493	3.428615	1.4130015	5.4980162
Replication3 - visual field3	59.84413	52.21151	54.15383	55.70268	25.07916	21.775093	10.263873	4.0517868	3.0256577
Replication4 - visual field1	74.80611	47.60845	52.97115	49.80516	22.45258	19.095501	4.803798	1.0670223	1.53247
Replication4 - visual field2	74.15314	74.22291	47.36556	31.372	24.8214	24.736466	2.41682	3.2302255	0.9546226
Replication4 - visual field3	61.8366	64.337	48.8158	41.8448	16.06653	14.610256	8.45233	7.7462885	0.9184954
Average	64.83130333	59.01650833	49.9271675	39.59359417	27.0650425	22.06141217	6.629448583	3.284950592	1.970959092
SE	2.352070708	2.192895876	1.93753496	2.677314546	1.869697751	1.424753226	1.018467525	0.749091563	0.444681629
	18 h	**20 h**	**22 h**	**24 h**	**30 h**	**36 h**	**42 h**	**48 h**	
Replication1 - visual field1	0.8706903	0	0	0	0	0	0	0	
Replication1 - visual field2	0	0	0	0	0	0	0	0	
Replication1 - visual field3	1.3568746	0	0	0	0	0	0	0	
Replication2 - visual field1	0.5221509	0.642476377	0	0	0	0	0	0	
Replication2 - visual field2	0	0	0.555555	0	0.31598	0	0	0	
Replication2 - visual field3	1.3396715	0	0	0	0	0	0	0	
Replication3 - visual field1	1.1973593	0.887193845	0	0	0	0	0	0	
Replication3 - visual field2	1.1700268	0.463843602	0	0.333333	0	0	0	0	

(*Contd...*)

Table S5: (*Continued*)

	18 h	20 h	22 h	24 h	30 h	36 h	42 h	48 h
Replication3 - visual field3	0	0	0	0	0	0	0	0
Replication4 - visual field1	1.3553631	0.280333933	0	0	0	0	0	0
Replication4 - visual field2	0	0.659510986	0	0	0	0	0	0
Replication4 - visual field3	0	0	0	0	0	0	0	0
Average	0.651011375	0.244446562	0.04629625	0.02777775	0.026331667	0	0	0
SE	0.178809679	0.095803306	0.04629625	0.02777775	0.026331667	0	0	0

Table S6: Germination rate of pollen stored under 4□ fror 0 to 48 h

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h
Replication1 - visual field1	57.09387	60.84872	62.8668	62.79249	59.42674	55.39805	46.86833	49.90993	47.17858
Replication1 - visual field2	60.95052	61.339	59.27033	61.78696	51.6608	51.07339	45.99857	49.7027	41.18545
Replication1 - visual field3	71.48422	58.18097	62.63223	68.34254	59.0786	56.53031	49.30285	44.43216	41.7363
Replication2 - visual field1	76.29391	71.63309	56.80244	64.62597	50.52778	50.95811	53.92815	48.24358	45.93805
Replication2 - visual field2	69.77592	64.66608	67.0842	47.45754	69.75144	61.3795	53.07601	43.81506	44.64973
Replication2 - visual field3	63.281	66.22215	66.02287	62.40937	47.24936	48.45624	48.46627	50.52993	34.98249
Replication3 - visual field1	49.68078	73.73716	76.67573	60.51078	71.768	60.22662	49.24168	48.80492	44.25038
Replication3 - visual field2	60.91553	64.84728	50.20421	60.8241	58.11524	57.08326	53.01815	48.80492	41.72764
Replication3 - visual field3	60.84413	64.99726	63.5925	67.1737	51.43524	54.03435	52.32222	43.70403	40.85511
Replication4 - visual field1	72.80611	60.8359	51.43449	51.07745	58.41427	59.95656	50.84482	50.71069	48.31067
Replication4 - visual field2	73.15314	69.81361	62.43705	61.47973	51.44481	54.04658	52.51112	33.36782	39.29348
Replication4 - visual field3	63.8366	52.09262	62.51732	63.16547	53.70704	57.51214	52.89546	48.945	52.1291
Average	65.00964417	64.10115333	61.79501417	60.97050833	56.88161	55.5545925	50.70613583	46.74756167	43.51974833
SE	2.255735359	1.733177746	2.034340655	1.736480759	2.188330554	1.162744687	0.768932399	1.420946089	1.313702523

	18 h	20 h	22 h	24 h	30 h	36 h	42 h	48 h
Replication1 - visual field1	53.79309	30.40852	32.44124	30.80107	17.64149	12.985769	11.309178	3.468714
Replication1 - visual field2	40.83353	37.66	37.66011	28.47483	20.11499	12.564445	16.810865	8.496271
Replication1 - visual field3	34.25646	35.82402	34.99905	32.07645	21.36	16.774046	10.797513	10.869731
Replication2 - visual field1	39.95431	41.18668	28.90712	30.76355	17.96722	13.811106	3.0737	4.731705
Replication2 - visual field2	43.09163	40.09535	32.04489	26.35269	15.44962	16.723959	16.072174	6.602743
Replication2 - visual field3	40.28005	40.96773	36.94643	33.56873	21.99437	9.535037	10.822932	9.43457
Replication3 - visual field1	37.62534	27.81582	32.64606	30.91906	18.50108	9.203198	10.646512	4.941091
Replication3 - visual field2	39.11057	35.73452	38.5093	31.50698	18.32576	9.944628	8.981039	5.951448
Replication3 - visual field3	43.83691	39.38906	32.89349	26.36435	19.69065	13.056377	10.63211	5.964899

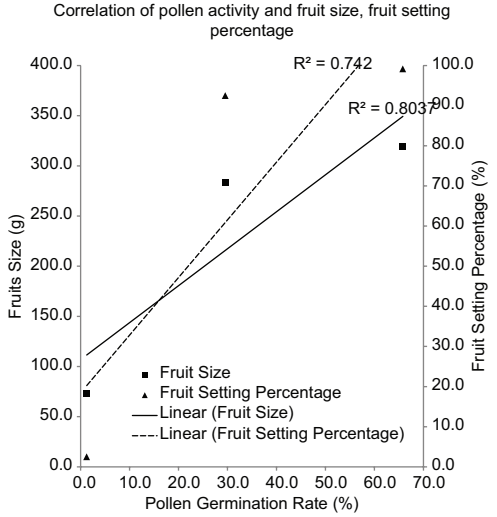
(*Contd...*)

Table S6: (*Continued*)

	18 h	20 h	22 h	24 h	30 h	36 h	42 h	48 h
Replication4 - visual field1	40.68465	37.95495	36.94528	27.23955	20.66796	14.42836	11.101862	7.250931
Replication4 - visual field2	33.46114	41.69239	32.33953	28.96528	19.14464	12.326329	5.458311	8.423837
Replication4 - visual field3	40.05357	39.51393	36.51849	27.76896	22.70068	16.026703	11.530205	8.224001
Average	40.58177083	37.35358083	34.40424917	29.56679167	19.463205	13.11499642	10.60303342	7.029995083
SE	1.492125389	1.253863043	0.845982005	0.683413117	0.592888997	0.759862098	1.086886709	0.620980371

Table S7: Fruit Setting percentage of pitaya pollinate with pollen stored for 0 to 72 hours under 4℃. There are 120 flowers randomly assigned to 4 replications were used for each treatment in this study, a 2 sample T test is conduct compare with fruit setting percentage of "0 H" treatment for other three treatments

	0 H	24H	48 H	72 H
Replication-1	100	93.33333333	3.333333333	3.333333333
Replication-2	96.66666667	90	10	0
Replication-3	100	96.66666667	20	6.666666667
Replication-4	100	90	16.66666667	0
Average	99.16666667	92.5	12.5	2.5
T test		0.003198202	6.38943E-06	8.47647E-08



Pollen Germination Rate	Fruit Setting Percentage	Fruit Size
65.8	99.2	319.5
29.5	92.5	283.6
1.3	2.5	73.3

Table S8: Fruit size of pitaya pollinate with pollen stored for 0 to 72 hours under 4℃. Fruit size is represent by fruit weight.There are 30 randomly selected fruits for group "0 H" and "24H", all fruits for froup "48 H" and "72 H" were used for size determination. A 2 sample T test is conduct compare with fruit size of "0 H" treatment for other three treatments

Time	0 H	24H	48 H	72 H
Fruit weight	284.57	241.92	133.20	68.12
	321.88	312.00	108.30	74.12
	342.44	272.88	121.88	51.28
	379.26	292.27	117.29	88.23
	372.25	256.53	142.16	89.81
	258.41	306.86	113.72	68.49
	298.31	286.70	94.97	
	303.27	270.67	119.87	
	239.19	223.42	138.05	
	313.09	329.10	137.17	
	333.03	315.63	108.89	
	328.24	295.25	108.76	
	330.05	269.44	127.50	
	340.64	284.66	119.47	
	344.59	260.94	110.51	
	382.18	291.41		
	369.79	270.06		
	346.47	285.52		
	305.23	324.25		
	306.53	328.31		
	326.35	258.30		
	288.34	287.40		
	299.91	265.76		
	260.59	254.62		
	280.66	260.12		
	330.57	260.78		
	304.25	276.50		
	379.20	339.56		
	284.85	281.64		
	332.25	304.84		
Average weight	319.55	283.58	120.12	73.34
T test		2.5727E-05	2.79499E-11	2.79243E-05

Table S9: Seed density (seeds number in an unit area of fruit flesh) of fruits from different treatment. There were no difference between different treatment

Time	0 H	24H	48 H	72 H
Seeds number	12	13	12	12
	11	12	9	15
	14	12	12	10
	12	11	11	11
	9	12	9	13
	10	13	13	12
	13	12	12	11
	12	13	14	10
	12	12	13	9
	11	9	11	14
	12	12	12	13
	13	11	12	12
Average	11.75	11.83333333	11.66666667	11.83333333
SE	0.391674726	0.3217691	0.43228311	0.505025252

Table S10: Germination Rate of pollen collected at different time. The title line -12 to 12 means 12 h before the flower bloom to 12 h after bloom

Time	-12 h	-10 h	-8 h	-6 h	-4 h	-2 h	0 h	2 h	4 h	6 h
Pollen germination Rate	0	0	1.3716525	6.199803	7.288392	45.62095	58.0599	57.09387	70.98944	51.68925
	0	0	2.4963105	5.030946	10.871906	36.51993	58.46501	62.54804	59.75349	68.42814
	0	0	2.2714767	8.995363	17.51165	27.80494	61.81923	72.27734	63.28791	59.53424
	0	0.1	0.2651472	7.971588	8.571732	38.54348	60.35156	76.29391	61.49603	60.83872
	0	0	1.6299272	4.025463	9.851943	30.16072	52.33849	66.24519	62.79563	56.63015
	0	0	1.8545802	7.619218	18.227365	38.98623	56.09095	63.281	65.12066	56.44562
	0	0.7	1.0174207	4.978677	14.509519	43.85995	69.97848	49.68078	68.34113	55.09337
	0	0	1.8251017	4.016994	15.58049	33.94306	59.36183	59.91553	57.78381	62.1267
	0	0	1.8167489	8.198534	10.525868	34.7359	54.17155	59.84413	73.79353	56.41175
	0	0	0.7230084	9.84804	9.981529	26.23575	41.40792	74.80611	71.46552	51.70799
	0	0	2.3356668	7.423581	10.192606	34.64147	55.74486	74.15314	73.10287	54.3985
	0	0	1.5121117	11.26065	13.181782	37.52272	51.54662	61.8366	60.07596	60.71446
	0	1.2358	2.8113324	7.493145	7.102719	29.99049	66.26462	71.77592	55.777	54.53164
	0	0	1.3558928	5.193241	13.841527	37.83717	55.78113	59.99882	61.43012	57.58616
	0	0	2.6906154	10.543966	9.667119	43.37469	54.92472	60.95052	62.67783	51.56787
	0	0	1.8631494	7.855245	13.470278	27.83467	63.93452	71.48422	58.64311	43.44391
Arvage	0	0.1272375	1.740008906	7.290903375	11.89852656	35.4757575	57.51508688	65.13657	64.1583775	56.32177938
SE	0	0.099111933	0.202498185	0.645425811	0.982738316	1.720367332	1.90350375	2.185389114	1.646591602	1.618634024

	8 h	10 h	12 h	+2 h	+4 h	+6 h	+8 h	+10 h	+12 h
Pollen germination Rate	52.56414	32.1508	27.19994	10.951915	8.12716	2.894981	0	0	0
	54.23682	40.10402	20.75744	13.257779	3.332699	1.310464	0	0	0
	50.56471	36.52632	26.52451	13.304859	7.27074	3.201496	0	0	0
	57.50446	38.3636	25.83725	15.337193	9.493479	1.324737	0	0	0
	48.35809	43.91872	34.03564	15.695012	6.924928	1.796597	0	0	0
	56.6096	39.15046	26.30563	15.984883	2.918674	1.315774	0	0	0
	48.43982	44.27456	25.15467	8.228072	7.822042	3.065503	0	0	0
	50.32718	33.5272	28.42832	11.269473	5.189512	1.715491	0.2	0	0
	47.14546	48.27534	27.39358	6.239853	9.579863	2.237218	0	0	0
	57.34791	40.28652	30.35809	12.806474	5.383977	3.637476	0	0	0
	56.97036	35.82748	27.69059	15.471724	7.013005	2.640674	0	0	0
	47.0203	54.33082	23.0462	8.340704	4.167446	2.501646	0	0	0
	61.9198	33.45637	25.0876	14.929945	6.499452	1.848356	0	0	0
	58.59823	41.32217	23.50625	11.749024	10.276858	1.637333	0	0	0
	59.51155	39.10239	30.819	9.31926	1.962531	1.558683	0	0	0
	39.14908	33.22822	32.89286	13.256076	4.778434	1.730697	0	0	0
Arvage	52.89171938	39.61531188	27.18984813	12.25889038	6.2963	2.151070375	0.0125	0	0
SE	1.732243983	1.720497268	1.022398865	0.867390091	0.716062743	0.215486111	0.014433757	0	0

Table S11: Fruit setting percentage polinate at different time points. The title line -12 to 12 means 12 h before the flower bloom to 12 h after bloom. A 2 sample T test is conduct compare with fruit setting percentage of "0 H" treatment for other treatments

Time	-12 h	-10 h	-8 h	-6 h	-4 h	-2 h	0 h	2 h	4 h	6 h
Fruit setting percentage	3.333333333	13.33333333	16.66666667	40	66.66666667	96.66666667	100	100	100	100
	3.333333333	13.33333333	20	46.66666667	60	90	100	96.66666667	100	100
	6.666666667	6.666666667	16.66666667	46.66666667	83.33333333	93.33333333	96.66666667	100	100	100
	0	10	23.33333333	40	76.66666667	93.33333333	100	100	100	100
Average	3.333333333	10.83333333	19.16666667	43.33333333	71.66666667	93.33333333	99.16666667	99.16666667	100	100
SE	1.360827635	1.595711846	1.595711846	1.924500897	5.181877252	1.360827635	0.833333333	0.833333333	0	0
T.test	1.43231E-09	4.80454E-09	8.69437E-09	1.85413E-07	0.001939071	0.01063468		1	0.355917684	0.355917684
	8 h	**10 h**	**12 h**	**+2 h**	**+4 h**	**+6 h**	**+8 h**	**+10 h**	**+12 h**	
Fruit setting percentage	100	96.66666667	96.66666667	83.33333333	43.33333333	30	10	10	3.333333333	
	100	96.66666667	83.33333333	63.33333333	36.66666667	26.66666667	16.66666667	6.666666667	0	
	96.66666667	93.33333333	93.33333333	80	46.66666667	23.33333333	10	6.666666667	3.333333333	
	100	83.33333333	83.33333333	76.66666667	40	30	10	10	3.333333333	
Average	99.16666667	92.5	89.16666667	75.83333333	41.66666667	27.5	11.66666667	8.333333333	2.5	
SE	0.833333333	3.154949081	3.435921355	4.383259399	2.151657415	1.595711846	1.666666667	0.962250449	0.833333333	
T.test	1	0.087080023	0.030019745	0.001957921	2.74831E-07	1.6789E-08	6.25144E-09	5.09721E-10	2.21121E-10	

Table S12: Fruit size of fruits harvest from different time point pollination

Time	-4 h	-2 h	0 h	2 h	4 h
	279.2	320.4	342.6	342	309.5
	290.2	338.1	327.6	313.8	311.8
	277.1	249.7	300.5	299.5	336
	274.7	255.2	237.9	342.3	354.4
	251.4	375.8	240.1	296.3	341.2
	304.4	298.8	312.5	292.7	340.5
	347.8	269	318.8	306	335.9
	214.6	349.3	322.6	278.7	347.6
	256.2	321.1	283.1	321.5	268.9
	254.3	275.8	328.9	304.7	313.5
	258.6	289.6	223.5	326.3	304.4
	289.5	242.2	250.8	310.9	381.1
	264.5	288.3	287.8	305.8	332.2
	238.4	283	319.8	294.2	314.6
	301.8	314.3	331.2	340.6	283.3
	340	364.5	288	311.3	308.2
	292.9	270.8	307.3	319.2	314.2
	240.6	301.4	391.2	323.4	330.4
	297	294.5	262.4	310.7	309.2
	349.5	305.7	341.2	309.3	287.6
	255.7	348.2	337.9	327.3	300.2
	299.4	341.1	321.5	328	336.6
	265.7	349.1	342.7	325.1	310.3
	300.2	325.3	333.2	304.6	330
	262.2	296	370.5	312.6	316.4
	231.4	329.2	313.3	322.8	272.7
	264.5	326.6	364.6	323.3	303.9
	227.3	282.2	305.1	319.9	305.3
	227.6	333.4	334.1	313.1	313.1
	266.2	287.7	360.4	316.8	244.8
Average	**274.0966667**	**307.5433333**	**313.37**	**314.7566667**	**315.26**
SE	6.27475783	6.274146337	7.365215153	2.685643754	4.996473699
T. test	0.00014942	0.549374121	1	0.860218767	0.832572291
	6 h	**8 h**	**10 h**	**12 h**	**+2 h**
	284.6	288.8	265.6	246.6	216.1
	300.5	298.5	352.6	257.7	238.4
	328.7	260.9	272.7	266.8	202.9
	283.2	273.7	246	272.5	179.9
	371.1	286.3	268.5	291.9	236.6
	327.2	267.5	253.3	242.3	239.6
	294.4	331.3	310.9	240.4	252.5
	323.5	273.2	238.2	224.5	162.2
	305.4	286.7	308.9	244.5	212.2
	228.5	296.8	256.5	245.2	207.2
	284.2	262.9	275	246.3	219.3
	376.2	256	272	231.9	244.6
	319.1	298.6	289.6	210	207.6
	288.3	283.8	288.1	242.3	261.3
	284.1	262.3	226.1	267.7	194.8
	307.2	280.6	246.8	241.7	185.3
	268.8	292.1	263.1	262.7	169.9
	320.9	321.4	272.6	241.7	179.4
	331	290.8	251.1	241	215.6
	281.5	246	298.3	255.4	214.5

(*Contd...*)

Table S12: (*Continued*)

	6 h	8 h	10 h	12 h	+2 h
	338.2	268.4	253.8	236.1	214
	362.1	281.8	287.9	273.2	190.4
	301.7	255.4	211	254.9	193.8
	326.4	324.5	242.3	249.8	222.7
	334.1	266	226.3	270.3	259.7
	262.3	303.5	171.9	248.7	227.5
	310.2	325.3	319.3	235.4	204.9
	304.4	234.5	238.5	294.8	237.2
	272.7	232.6	200.2	226	225
	262.5	249.9	211.9	243.9	245.4
Average	306.1	280.0033333	260.6333333	250.2066667	215.35
SE	6.083478663	4.727910484	6.940431822	3.423122636	4.774387486
T. test	0.449718065	0.000335562	2.60293E-06	1.44954E-10	4.42771E-16