ROLE OF ASSISTED POLLINATION IN FRUIT SHAPE OF PURPLE PASSION FRUIT *(PASSIFLORA EDULIS SIMS.)*

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ABSTRACT

Purple passion fruit (Passiflora edulis sims.) or commercially recognized as Markisa among Malaysian is a vine species crop that can thrive well under hot and temperate climate region. In fruit industries, passion fruit is considered as one of tropical fruit that is traded globally among ASEAN, China, America and European countries. In Malaysia, the total passion fruit production in 2012-2017 was 579.6 MT. The flower of passion fruit possesses unique hermaphrodite feature with big and sticky pollens that causes low rate of pollination and fruit set. As part of an effort to increase the fruit production, it is fundamentally important to study the effect of assisted pollination. The present study examined the effect of assisted pollination (AP) and natural pollination (NP) in improving the percentage (%) of fruit formation as well as its growth and development characteristic. Length and diameter of fruit were measured to reflect the fruit size and shape. Final fruit size was significantly ($p>0.05$) influenced by pollination treatments. Bigger fruit was obtained by application of AP since it has resulted in length and diameter increment of 73.3 and 45.9%, respectively whereas NP resulted in only 29.2 and 37.7% in length and diameter, respectively. The final fruit shape obtained by AP was oblate spheroid while NP was spheroid. Overall, the study concluded that AP was not merely important to improve the fruit formation but vital for improving the size and shape of fruits.

Keywords: Assisted pollination, natural pollination, Passiflora edulis Sims., fruit quality, and fruit formation.

INTRODUCTION

Passion fruit plants has hermaphrodite, solitary flower that locate in its leaf axils (Juan et al., 2013). The flower consists of five stamens with three styles at its base. The flowers are protandrous as the anther dehiscence before stigma become receptive. Stigma will get receptive once anthesis until closing after 24-36 h. Due to this, passion fruit flower is one of self-incompatible and allogamous flower for fruit production (de Siqueira et al. 2009; Souza et al. 2006). Apparently, parthenorcarpic development does not occur in passion fruit. Various parthenocarpy-inducing chemical including naphthalene acetic acid, 2,4-dichloro phenoxyacetic acid and indole acetic acid were ineffective in fruit setting. Thus, pollination is an important criterion for fruit set in passion fruit (Das et al. 2013; Yamamoto et al. 2012; Suassuna et al. 2003). Pollination is act by transferring pollen grain from anthers of a flower to the stigma of the same or different flower to make the fertilization of flower happen (Rogers et al. 2014; Catalina et al. 2011).

Natural pollination (NP) process is initiated by wind, insect and climatic condition. NP is an uncertain process since it is depending on uncontrollable natural factors (Toledo et al. 2017; Silveira et al. 2012). The pollens of passion flower are very sticky in nature making wind to be ineffective for pollination since they cannot be dispersed easily and get through the stigma to make fertilization happen (Das et al. 2013; Amorim et al. 2011; Banu et al. 2009). Climatic condition affect pollination in different ways. For example, blossoming periods of fruit trees are not static and can vary from year to year depending on environmental conditions. This consequently result to miss-overlap between blooming period and pollinizer (Scaven & Rafferty, 2013). Adverse environmental conditions can affect negatively the production and the transport of pollen, either carried out by biotic or by abiotic vectors (Harsant et al. 2013; Arista et al. 2013).

Pollen transport by insects can also be affected by factors such as diseases or treatments with pesticides. These many factors, even in orchards with well-established pollination designs, can result in occasional pollination deficits that reduce production considerably. Dependability of passion fruit on insect's pollination has paramount importance in fruit production. For example, research conducted by Androcioli et al. (2017) bumblebee species such as *Xylocopa frontalis, X. grisescens* and *X. Suspecta* are the most favourable species in foraging the flower of passion fruit as bigger and sweeter fruits are obtained from the natural pollinated fruit (Junqueira & Augusto 2017). Pollination relying on entomophilous species does not always guarantee adequate pollination which consequently lead to small fruit size and inconstant size of fruit (Jacobs et al. 2009). In fact, relying on insect pollination greatly dependent on the insect habitat. If the insects are not inhabiting the farming site, then pollination will not happen (Klatt et al. 2014; Chautá-mellizo et al. 2012).

There is an alternative that guarantees an effective pollination in crops and lessens the uncertainty arise from the natural pollination, especially relying on insect pollination. Such an alternative is most commonly known as assisted pollination (AP), which can be defined as the biological or mechanical application of supplementary compatible pollen that has been previously collected apply manually by human on stigma of flower (Das et al. 2013; Klatt et al. 2014). This is not a recent technique. It has been found to increase fruit setting in various number of crops such as palm dates, dragon fruit, strawberry and apples (Awad 2018; Klatt et al. 2013; Suassuna et al. 2003). Fruit setting behaviours of crops that ecological dependent can be controlled by AP (Klatt et al. 2013).

Fruit size and shape are among the most important quality criteria for evaluation by consumer preference. Consumers prefer fruits of equal size and uniform shape (Bhat et al. 2015). During marketing and distribution process, fruit will be classified into different grades based on size and shape. Classification of fruit can increase uniformity, reduce packaging and transportation costs and also may provide an optimum packaging configuration (Keshavarzpour & Rashidi, 2010). Additionally, misshaped fruits are generally rejected according to sorting standards of fruit. The common method in classifying the fruit shape is by image processing and hedonic rating (Sadrnia et al. 2007). In grading, the United States Department of Agriculture (USDA) grade standard based in visual comparison that relative to reference drawing. The drawing serve as reference in classifying the shape of fruit. However, this method is subjective and biased as it is dependent on person who executes the rating. Measuring fruit shape by calculating the geometrical attributes of fruit is more reliable as this has been widely applied in numerous fruit such as watermelon, pear, tomato and kiwifruit. Geometrical measurement is simple as it can be performed by calliper and no complicated equipment is needed (Keshavarzpour and Achakzai, 2013)

There is close relationship between pollination and production of bigger and more symmetrical fruits (Junqueira & Augusto 2017). Shape of fruit was significantly improved by assisted pollination in few type of fruits such as tomato (Bashir et al. 2018), jackfruit (Lina & Protacio, 2015) and dates (Awad et al. 2018). Effect of pollination on passion fruit attributes has not been widely studies. There are reports on different pollination treatment on passion fruit studying the efficiency of pollinations on fruit formation (Das et al. 2013; Banu et al. 2009) but these workers do not consider the size and shape of fruit that represent the most important criteria in grading the appearance of fruit. Considering quality grading is essential in fruit industry, it is important to understand the effects of pollination on fruits shape and size. Therefore, the objective of this study was to investigate percentage fruit set, size and shape in purple passion fruit subjected to natural and assisted pollination treatments.

MATERIALS AND METHODS

STUDY AREA

Twenty purple passion fruit trees were cultivated at Ladang 10B, Universiti Putra Malaysia, Serdang using 3-month-old seedlings. The annual day temperature was about 28-35ºC, with heavy rainfall occur during November to March. Trees were planted in 25 L pot supplied with commercial planting soil. Plants were trellised in rows. Watering was supplied twice a day and fertilizer were given according to commercial practices (Joy, 2010).

POLLINATION TREATMENTS

For NP, flowers were tagged during anthesis and allowed for natural pollinators such as wind and insect. For assisted pollination, flowers were bagged before anthesis. On the day of anthesis, stamen from individual flower were rubbed gently on the stigma of flower and then re-bagged. About 120 flowers were used for each treatment.

PERCENTAGE OF FRUIT SETTING

Fruit set was scored as the number of fruit that remained on the vine after 14 days of pollination treatments. Aborted embryo that occur within 14 days after pollination was denoted as failure of fruit setting (Das et al. 2013).

FRUIT LENGTH AND DIAMETR

Fruit growth was recorded by measuring the length and widest diameter of each fruit at weekly intervals from the day of pollination until week 7 (Shiomi et al. 1996).

FRUIT SHAPE ANALYSIS

Fruit shape is analysed by calculating the outer dimension of fruit. By assuming the general shape of passion fruit as a sphere shape, the outer dimensions of each fruit i.e. length (L) and diameter (D) was measured to 0.1 cm accuracy by a digital calliper. Shape of fruit was detected based on aspect ratio (A.R). Shape was characterized as oblate spheroid when A.R $\leq$0.95, spheroid when A.R 0.95 – 1.05 and oblong spheroid when A.R $\geq$ 1.05. Calculation of A.R was based on equation proposed by Keshavarzpour & Rashidi (2010).

$$A.R. = L/D, \quad (A.R. = 1.0) \text{ where}$$

A.R. = Aspect ratio, non-dimensional
L = Length, cm
D = Widest diameter, cm

STATISTICAL ANALYSIS

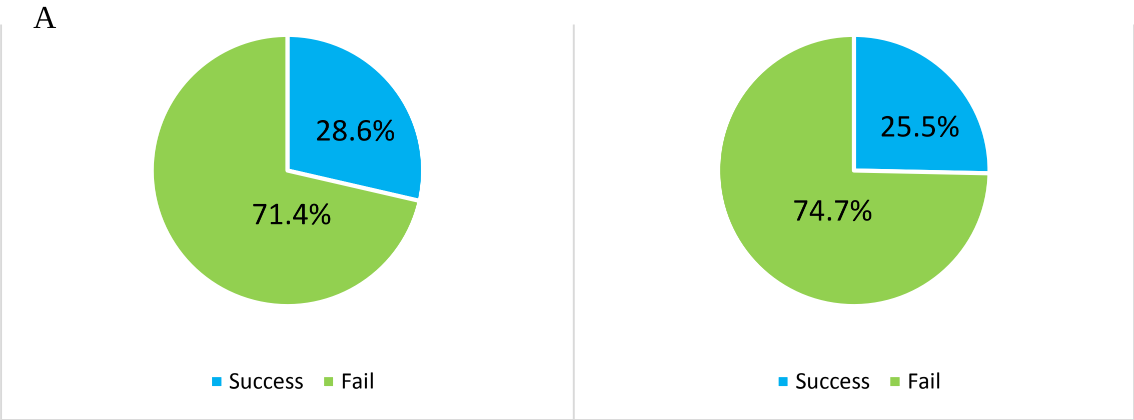
Student-t test was conducted to determine the effects between AP and NP in influencing the fruit setting behaviour. Fruit length, diameter and shape analysis were analysed by ANOVA to investigate the effect of pollination treatments and time after pollination. Mean of A.R were separated using least significant difference at 5% confidence interval.

RESULTS

Percentage of Fruit Setting

There was a non-significant difference in the % of fruit setting for AP (M=28.6, SD=5.40) and NP (M=25.33, SD=5.23) conditions; $t(4)= 0.43$, $p=0.05$. Fruit set was not strongly influenced by application of assisted pollination treatments. On average, the success rate of AP to form flower was just 28.6% (Figure 1A) as compared to NP with 25.5% (Figure 1B). AP resulted to non-significant ($P>0.05$) improvement in fruit setting as compared to NP.

Figure 1 Percentage of fruit setting A) Assisted pollination (B) Natural pollination

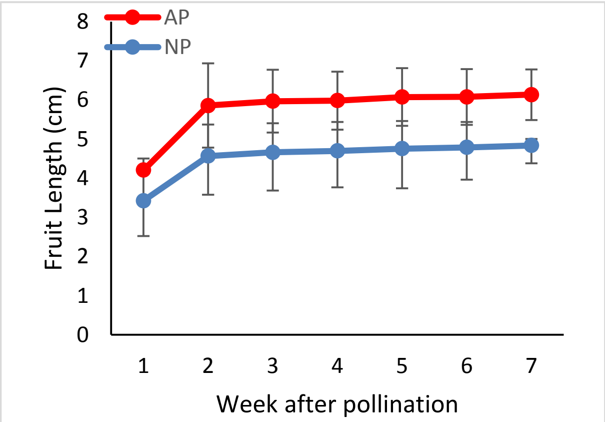


Fruit Length and Diameter

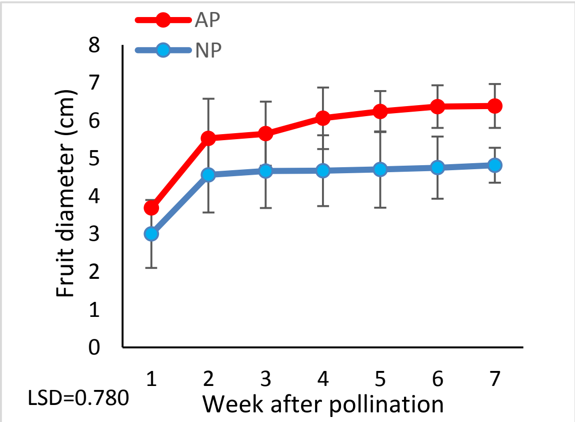
Growth curve of passion fruit from both treatments showed a sigmoid pattern (Figure 2). However, distinct development trend was observed between the pollination treatments in which length and diameter development of AP were significantly ($P<0.05$) higher than NP. From the results, growth rate was rapid during first week of development. Then, a bit slow during second week and eventually became plateau at 3 weeks after pollination (WAP). Pollination treatment can significantly ($p<0.05\%$) affect the fruit size. By 7 WAP, final fruit length obtained from AP and NP has increased by 45.86 and 29.18%, respectively as compared to initial fruit size. For fruit diameter, by 7 WAP the diameter of fruit obtained from AP and NP has increased by 73.33 and 37.72%, respectively as compared to initial fruit size. This result indicates that size of fruit obtained by AP was significantly ($P<0.05$) bigger than NP.

Figure 2 Growth and development of passion fruits (A) Changes in fruit length as a function of weak after pollination (B) Changes in fruit diameter as a function of weak after pollination

A



B



The initial shape of fertilized ovary was oblong spheroid at 1 WAP (Table 1). Then, the shape of fruits changed dynamically during fruit growth and development. During 7 WAP, NP produced spheroid shape while AP produced oblate spheroid shape fruit.

Table 1: Effects of natural and assisted pollination on shape and aspect ratio range of passion fruit during growth and development

Week after pollination	Natural Pollination		Assisted Pollination	
	Aspect Ratio	Shape	Aspect Ratio	Shape
1	1.35 a[z]	Oblong spheroid	1.15 abc	Oblong spheroid
2	1.07 a	Spheroid	1.07 bc	Spheroid
3	1.06 a	Spheroid	1.06 cd	Spheroid
4	1.07 a	Spheroid	0.99 cd	Oblate spheroid
5	1.09 a	Spheroid	0.99 cd	Oblate spheroid
6	1.07 a	Spheroid	0.96 d	Oblate spheroid
7	1.05 a	Spheroid	0.96 d	Oblate spheroid

[z]For each means (n=15) within same column followed by the different letters are significantly different by LSD at $P \leq 0.05$

DISCUSSION

Pollination is an important process in fruit setting of passion fruit. This study showed that, AP has limited effect in improving the fruit setting as compared to NP. This study shows deviation of result as compared to study conducted by Das et al. (2013) where they found out that AP was greatly enhancing the fruit setting of passion fruit. Low success rate of passion flower to become fruit even after introduction of assisted pollination can be explained by the self-incompatibility (SI) of passion fruit. SI is a genetic mechanism for the recognition and rejection of self-pollen or self-pollen tubes. Since the AP treatment in this experiment utilize the same pollen to pollinate the flower, the effect of SI evolved. In angiosperm, the SI stand as obstacle as it prevents self and cross pollination (Spinardi & Bassi 2012; Frrer et al. 2009). The pollen grains that are incompatible got rejected for fertilization. Study by Hérika et al. (2014) observed the pollen tube of self-pollinated passion fruit got arrested in the stigma in an incompatible combination. Under this circumstances, the pollen tube had to rearrange and undergone rapid deposition of callose in the stigma during the SI response. The damaged pollen tube caused failed fertilization.

Even though the act of AP does not affect the fruit setting success rate, it greatly beneficial in improving the fruit size of fruit. The standard of size grading for passion fruit can be separated into 3 categories (small, medium, large) based on fruit diameter (Joy, 2010). Marketable fruits size has dimeter ranged from 4 to 9 cm and length 4 to 12 cm. For export purpose, the average diameter of fruit is classified as small for dimeter of 5 cm, medium 6.5 and large 8 cm (Codex Allimentarius 2014). From this result, it showed that NP produced size of fruit below the export standard level since the highest diameter that it can obtained only 4.8 cm. However, the fruit is still marketable in local market. In contrast, applying AP capable of producing fruit that can meet the export standard since its diameter around 6 meet the medium grade quality of exported fruit.

Two distinct growth pattern was observed between the pollination treatments in which bigger fruits were obtained by assisted pollination. This study is in agreement with other assisted pollinated fruit such as kiwi, dates and olives (Gianni & Vania 2018; Awad 2018). AP allow complete pollination which consequently result in larger fruit, round and regular shape. Fruit that is obtained by AP usually contains more seeds than the natural pollinated fruit. The high number of seed is due to high number of fertilized ovule (Tuan & Chung-Ruey 2013). Seeds play important role in fruit setting process. Well pollinated flower will direct to rapid development of ovary, and the fecundated seed produce plant growth hormones leading to good development process and thus bigger fruit size are generated (Klatt et al. 2013).

Shape of fruit is dynamically changed during fruit growth and development suggesting that active cellular development of fruit changed the fruit morphology. During cellular development, there is active mitotic process happen after the fertilization of ovule in the ovary. Initially, the ovary will get swollen as it getting bigger in size (Rapoport & Rallo 1991). Further cell development causes the morphology of fruit to change (Adrienne et al. 2006). It seems that natural pollination produces more round shape than the assisted pollination. The oblate spheroid shows small deviation than spheroid. However, the fruit can still be considered to have complete round shape during the final week of development (Table 1). Round shape is normally preferred to oval-shaped fruit particularly in export market (Codex Allimentarius 2014; Joy 2010). This result indicated that both treatments are capable in producing symmetrical fruit shape (Codex Allimentarius 2014; Joy 2010).

To apply AP in farm practice is a laborious, expensive, require time and professionals. Operational efficiency of AP has been studied by Bonine et al. (2015) on eucalyptus flower. They found out that by applying conventional practice of AP, a worker can only pollinate 54 eucalyptus flower in an hour. To pollinate 1000 flower per hour, around 21 workers were needed. Man-power is needed to conduct the assisted pollination. Therefore, adapting this technique will consider of addition of labour-cost in producing the passion fruit. In contrast, considering the natural pollination is cost-free as it is only depending on environmental factor such as wind and insect for pollination to take place (Toledo et al. 2017).

CONCLUSION

It can be concluded that natural and artificial pollination produced non-significant effect in improving the percentage of fruit formation. However, AP was significantly importance in getting bigger size and round shape fruit as compared to NP. Fruit produced by NP is incapable to meet the export quality standard while AP can meet the export size criteria. Thus, it depends on growers to decide whether to adapt the AP in their agricultural practice by considering the market needs and labour cost. This study provides information about the influence of NP and AP on fruit formation, size and shape of fruit that will be beneficial to farmers, passion fruit producers and researchers. Fruit physicochemical properties subjected to different pollination treatments is important to be verified. Thus, future study can be conducted at this niche to understand the biochemical process that happen during fruit growth and development.

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