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Effect of Various Pollen Sources to Ability Fruit Set and Quality in 'Long Red B' Wax Apple

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***Abstract*—**By hand pollination was conducted to evaluated different pollen sources and their affects on fruit set and quality of wax apple. The following parameters were recorded: fruit set, seed set, fruit characteristics. Results showed that fruit set percentage with seed were significantly high in 'Long Red B' when 'Black', 'Thyto' were used as pollen parents. Pollen of 'Black', 'Thyto' resulted in high fruit weight, fruit diameter, fruit length, bigger flesh thickness, better total soluble solids as compared with other pollens. The observation of pollen-growth *in vitro* revealed that pollen germination at 15% sucrose concentration are required for optimum pollen germination with the high pollen germination were found in 'Black', 'Thyto'. From the result, we concluded that 'Black', 'Thyto' were proved to be good pollinizers in 'Long Red B'. Therefore, artificial cross-pollination using 'Black', 'Thyto' as pollinizers were strongly recommended for 'Long Red B' cultivar in wax apple orchard.

***Keywords*—**Wax apple, pollination, pollen source, *in vitro*, fruit quality.

I. Introduction

THE wax apple or jambu air madu, is a non-climacteric tropical fruit from the *Myrtaceae* family and botanically identified as *Syzygium samarangense* [15]. It has become economically important fruit crop in Southeast Asia such as Thailand, Taiwan, Malaysia and Indonesia [22]-[27]. Wax apple fruit is pear-shaped, about 3.4-6cm long and 4-5cm wide, with 1-4 seeds or without seed. The skin is very thin and waxy glossy appearance. Normally, the fruit is harvested when blossom-end is fully expanded and skin shows desired market color. The skin color can be green to deep-red depending upon cultivars [17]. Moreover, it has become an increasingly popular fruit in the tropical region where it can fetch a price of up to 3USD per kilogram and has the potential to bring great benefits to local farmers and the country's economy [11].

Fruit set is an essential event among many events that must occur successfully for fruit production. In this process, adequate pollen needs to be transferred to the stigma and pollen tube growth has to occur successfully for the pollen tube to fertilize the embryo sac [7]. In addition, fruit sugar content produced under cross-pollination was higher than estimated sugar content for self-pollination fruits. Moreover, benefits of cross-pollination can be explained for higher and earlier fertilization that caused fruits with more seeds [6]. Therefore, fruit and seed set are especially depended on successful pollination when the species cannot be selfed. Thus, this is especially important for cultivated plants, where pollination is usually a critical step in crop production [1]. Based on it, we have been suggested that the artificial cross-pollination to achieve a high and stable fruit set. Reference [1] showed that insect pollination has been considered to improve fruit quality in pepper and thus to increase the incomes of the farmers. There were some scientists have indicated that pollination of greenhouse sweet pepper with different pollinators, such as the solitary bees, *Osmia cornifrons* (Radoszkowski); bumblebees, *M. rotundata;* or the honey bee, *Apis mellifera* and all these works have found that insect pollination increased fruit weight and the percentage of extra-large and large fruit compared with self-pollinated fruit [5]-[14]. On the other hand, the most successful way to improve the fruit set, size, and shape by hand pollination, which is in accordant with reported by [20]. Reference [19] indicated that studied the effect of pollination in pepper and compared four different pollination methods. She revealed that hand pollinating, vibration, and bee pollination (with *Megachile rotundata* F.) resulted in better fruit set, higher yield, and better quality.

Although some references are available in literatures and effort have been made to control fruit set, stimulate fruit growth and development lastly increased the yield and quality improvement but there is no precise recommendation for the control fruit set, enhanced growth and quality improvement in wax apple. Furthermore, pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed-producing plants. Pollen performance is clearly affected by the genotype of the pollen [23]. It is well established that the total number of pollen grains on a stigma often exceeds the number necessary to fertilize all the ovules, leading to competition among growing pollen tubes in the style [9]. However, an adequate germination medium, to test pollen viability in wax apple is so far lacking. In order to improve the crop through development of new varieties, basic knowledge on the fertilization system was needed. Furthermore, higher and more sustainable production of wax apple would be possible by interplanting a proper pollinizer if compatibility of self- and cross-pollination is elucidated.

Therefore, in this paper we study the effects of various pollen sources on seed set in wax apple cultivars. By hand pollinating part of the flowers on female plants at different pollen from males and comparing percentage of fruit set with seed, seed set, and fruit quality with self - pollination flowers,

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we also test for ability pollen germination of wax apple cultivars.

II. Materials and Methods

A. Plant Materials

Field pollination experiments were conducted in Tropical Fruit Orchard, Department of Plant Industry located at National Pingtung University of Science and Technology, Taiwan. Nine wax apple cultivars, 'Thub Thim Chang' (TTB), 'Thyto' (TT), 'Long Red B' (LRB), 'Big Pink apple' (BPA), 'Atu' (AT), 'Black' (BL), 'Mallay apple' (MA), 'Light pink apple' (LPA), and 'Twenty Century' (TC) were used in this experiment. The experimental design was in Randomized Complete block Design and all trials were submitted to the same conditions.

B. Cross - Pollination

Cross-pollination was carried out artificially by hand. Ten flowers cluster that were thinned to two to five flowers per cluster and the filament of flowers were emasculated in the afternoon. After emasculation, clusters were covered with paper bags to prevent invasion of other undesirable pollen, and the flowers were pollinated at anthesis around 6-8.00 a.m. the following day when pollen of paternal plants matured and was released from the anther. Pollination was performed manually by touching anthers containing pollen to stigma. After pollination, clusters were re-covered with paper bags. Additionally, open pollination was designed as a control, in which no flowers were emasculated and bagged [8].

C. Pollen Viability in vitro

The trial was applied the following of sucrose concentrations to culture: 10%; 15%; 20%. One drop of the culture medium with the respective levels of sucrose was applied on each slide which was followed by the addition of the pollen grains. Each plot consisted of a single slide and each treatment was done with three replicated. The slides were placed in moist chambers and incubate at 25°C under fluorescent for 4h. After the incubation period germinated and non germinated pollen grains were photographed with a digital camera in an optical microscope with 10x objective, in order to increase images. Three areas of each image were evaluated, where geminated and non germinated pollen grains were counted. The emission of the pollen tube was considered as the germination criteria [4].

D. Fruit Sampling

The fruit sampling procedure was exactly the same for all cultivars and plantings. Samples consisting of fifteen fruits each were randomly collected from each one of 9 trees (totally, 135 fruits) of each variety cultivated in orchard. The concentration of total soluble solids (TSS) of the juice was determined with a refractometer (PR-1 Digital Refractometer, Atago Co Ltd., Japan) and the result was expressed as °Brix. Finally, the number of seeds per fruit was counted and the mean fresh fruit weight, fruit length, fruit diameter, flesh thickness, percentage of fruit set with seed were measured.

E. Statistical Analysis

The data were analyzed using the SAS 9.1 for Windows statistical package. For comparison of the means and Duncan's multiple range test, both for $P \leq 0.05$, were employed.

III. Results

A. Pollen Germination

The results of pollen viability, which was evaluated by in vitro germination tests, revealed that the best germination was 23.9% in (TT) followed by 23.7% in (BL) and 21.9% in (LPA) at 15% sucrose concentration (Fig. 1). Moreover, there was significant decreased pollen germination as sucrose increased for all cultivars excepted (TC) and (MLA) at 20% sucrose concentration. Finally, pollen germination was decreased very quickly at low sucrose concentration, only 1.1% pollen germination in (BPA), followed as (TTC) with 2.7% at sucrose 10%.

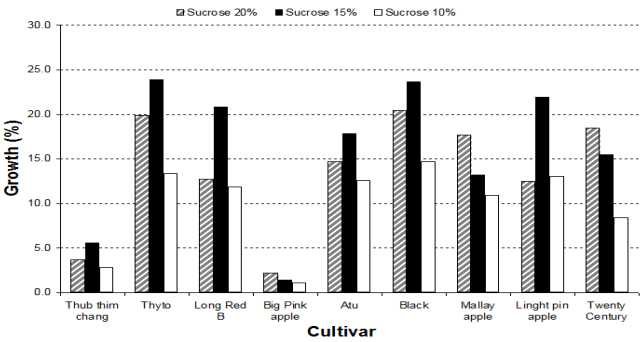

Fig. 1 The pollen germination percentage of wax apple cultivars

B. Fruit Set after Cross–Pollination by Hand

TABLE I
Fruit Set of Controlled Pollination in Wax Apple 1

Female	Male	No. of fruit set	No. of fruit set with seed	No. of seed/fruit	% of fruit set with seed
LRB	LRB	2.8ab	0.6cd	0.6de	21.4d
	TTC	2.5b	0.0e	0.0e	0.0e
	TT	3.3ab	1.5b	2.6b	45.5ab
	BPA	3.0ab	0.1de	0.1de	3.3e
	AT	2.5b	0.5cde	0.8d	20d
	BL	3.8a	2.1a	4.3a	55.3a
	MLA	1.1c	0.0e	0.0e	0.0e
	LPK	2.6ab	0.7cd	1.6c	26.9cd
	TC	2.1bc	0.8c	1.8c	38.1bc

1 Mean in each column followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

The result in Table I showed that fruit set after self-pollination (21.4%) was superior to that after cross–pollination with BPA (3.3%), TTC and MLA (0.0%). Moreover, collected TC, BL, TT as pollen parent, significantly higher fruit set were achieved, especially in BL (55.3%). Despite of there was no significant difference with self–pollination when LPK selected as pollen parent, the fruit set of LPK was also high at harvest (26.9%). Furthermore, the results summarized in Table I showed that highest seed number was 4.3 seeds/fruit in (BL)

cultivar and 2.6 seeds/fruit for (TT) cultivar, whereas the lowest seed number for (TTC), (MLA) and (BPA) cultivars.

C. Fruit Quality Variables

TABLE II
EFFECT OF POLLEN SOURCES ON FRUIT CHARACTERS IN WAX APPLE 1

Female	Male	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Flesh thickness (mm)	Soluble solid (o Brix)
	LRB	43.9b2	47.8b	58.5bc	10.1c	10.7bc
	TTC	27.7d	41.7d	51.7c	9.3c	9.5c
	TT	71.1a	52.0a	68.8a	13.0b	12.2ab
	BPA	31.9cd	45.0bc	55.3c	9.3c	6.5d
LRB	AT	41.0bc	45.1bc	57.4c	9.7c	10.7bc
	BL	81.8a	54.3a	69.3a	14.7a	12.8a
	MLA	28.5d	42.6cd	49.3c	9.1c	5.9d
	LPK	44.8b	46.3b	67.1ab	10.2c	11.8ab
	TC	50.6b	47.1b	67.7ab	11.8b	11.6ab

1 Mean values for the characteristics weight (WF), length (LF), diameter (DF) and number of seed (NS) of fruits resulting from wax apple fruit pollination by hand.

2 Means with different letters are significantly different by Duncan's multiple range tests at $P \leq 0.05$

The various fruit quality parameters of LRB in relation to self- and cross-pollination with other cultivars are presented in Table II. Based on the data the mean values of all parameters were significant differences among cultivars. It was found that fruit weight of TT (71.1g/fruit) and BL (81.8g/fruit) was significantly greater compared to remainder cultivars in self and cross-pollination. Moreover, cross-pollination of BL and TT with LRB resulted in a significant higher fruit diameter (54.3mm, 52.0mm, respectively), whereas cross-pollination of remainder cultivars with LRB did not affected in fruit diameter. However, low fruit diameter was found in TTC (41.7mm) and MLA (42.6mm) compared to self-pollination (47.8mm). Furthermore, fruit length of MLA (49.3mm) and BPA (55.3mm) in cross-pollination did not significant compared to self-pollination. However, cross-pollination caused a significant increase in the fruit length of BL (69.3mm) and TT (68.8mm). Moreover, significantly higher flesh thickness was found in BL, TT, and TC compared to cultivars remainder in the study. Finally, the concentrations of total soluble solids (TSS) in the juice of BL and TT fruit (12.8 ^{o}Brix; 12.2 ^{o}Brix, respectively) were significantly than TTC, BPA and MLA in cross-pollination with LRB whereas no significant differences total soluble solids among remainder cultivars compared to self-pollination excepted MLA (5.9 ^{o}Brix) and BPA (6.5 ^{o}Brix).

IV. DISCUSSION

A. In vitro Pollen Germination at Different Sucrose Concentration

In pollen germination assay in vitro, sucrose is generally used as an energy source in many plant species, because it usually stimulates pollen germination and subsequent tube growth. Pollen germination tests of *Primula obconica* and *Diospyros kaki* as in [16]-[18] showed that germination was promoted most effectively by sucrose. Therefore, experimental conditions, sucrose had a clear effect on pollen germination with the best results at 15% but sucrose fewer than 10% and over 25% reduced pollen germination. Similar findings have been reported that media containing 10% sucrose caused 20.4% germination, whereas media containing 15% sucrose caused 29.4% germination [16]. Therefore, an adequate germination sucrose concentration has been set up for wax apple pollen and the requirements for successful germination have been determined. Our results will also contribute to better understanding pollen germination and lead to improvements in controlling pollination and fertilization in fruit trees.

B. Fruit Set by Controlled Pollination

In an agriculture context, the yield of fruit is low and erratic, mainly due to inadequate fruit set [24]. Self-incompatibility may be one reason for the low fruit set in durian, but it is empirical and there are few studies which have clearly confirmed this [13]-[26]. Moreover, fruit set after open pollination was extremely low compared with that after artificial pollination in all cultivars tested, because this might be due to lack of pollination. If pollination was not successful, the pistil would shrivel and fall off within a few days after anthesis [25]. Therefore, the percentages of fruit set with seed of the cultivars used in this experiment were different. These differences are mainly due to the different averages of pollen source because the controlling pollination per cultivar was similar to [21]. Hence, used pollen of BK, TT and TC to pollination resulted in high fruit set in LRB as compared to pollen of remainder cultivars in this study. Therefore, this result suggested that BL, TT and TC are good pollen source in wax apple orchard.

C. Effect of Pollination on Seed Set

Seeds play an important role in fruit setting process, since bad developed fruits are the result of an unequal seed distribution inside the fruit [10]. Thus, in a well-pollinated flower occurs a rapid development of ovary, and the fecundated seeds produce plant growth hormones, leading to good fruit development. Moreover, cross-pollination caused a significant increase in the number of seeds per fruit of BL and TT cultivars. The results of this study are in agreement with those of [2] that found fruit obtained from hand pollination are always larger, heavier and with more seeds that in fruit obtained from natural pollination. The results also revealed that the pollen source of (BL) resulted in an increase in number of seeds/fruit, compared to pollen source of remainder varieties in study with the values of 1.8; 1.6; 0.8; 0.6; 0.1; 0.0 seeds/fruit, respectively.

D. Effect of Pollination on Fruit Character

Reference [3] reported that pollinated fruits were distinguished by superior quality and could be better stored than non pollinated ones. This assertion was reinforced by [28], who also reported workers of mandarins suggest that it may be possible to find a pollinizer that would increase fruit set and fruit size, even in very different growing environments and seasons. Therefore, previous studies on cross-pollination of wax apple cultivars have shown the existence of a wide variation in parameters with different pollinizers. The overall

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pomology characters of LRB fruit could be improved by using either BL or TT as pollinizer. However, cross-pollination of TTC, BPA and MLA with LRB did not affect the most of fruit quality parameters determined (Table II). Reference [8] reported that cross-pollination improved both fruit shape and size in Durian. Interplanting a suitable pollinizer may be valuable for improving not only productivity but also fruit quality. Thus, results of the present investigation demonstrated that pollen source is the most suitable provides high productivity and fruit quality for BL and TT as pollinizers whereas pollen source considered less effected by using TTC, BPA and MLA as pollinizers. Therefore, in the study interplanting BL, TT trees as a pollinizer with LRB in wax apple orchards are recommended

V. Conclusions

Based on the present results, we concluded that sucrose greatly stimulates wax apple pollen germination in 15% sucrose concentration, while 10% and 20% sucrose concentration strongly inhibits pollen germination. This also could be concluded since cross-pollination of LRB with either BL or TT significantly increased the percentage of fruit set with seed, number of seeds/fruit and fruit quality attributes.

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