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Pollen Viability and Pollen Tube Growth of IPB's Papaya

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ABSTRACT

The purpose of the research was to examine the pollen germination process and growth rate of pollen tubes of papaya. Pollen tube growth of nine genotypes of papaya (IPB 1, IPB 2, IPB 3, IPB 4, IPB 5, IPB 7, IPB 8, IPB 9, and IPB 10) was investigated in this experiment in order to study their pollen germination rate and pollen viability. The fresh pollen were excised from the flowers of papaya grown at Tajur Field Station of Research Center for Tropical Fruit, Bogor Agricultural University (IPB), Bogor. The extracted pollen was cultured aseptically on the Brewbaker and Kwack medium (pH 7.3) at ambient temperature of 26-28 °C. Pollen germination and pollen tube growth was observed under optical microscope with 100 and 400 magnification. Papaya pollen viability was not associated with size-based categories of papaya fruits. IPB 4 had the longest pollen tube at first 30 minutes after germination (115.5 µm), whereas IPB 10 had the shortest (99.5 µm). The distance from stigma to ovary in hermaphrodite flowers varies with genotypes, ranging from 7.38 to 13.44 mm. Average length of pollen tube within four hours of germination for small papaya fruit category (IPB 1, IPB 3, and IPB 4) was 1,030.67 ± 19.14 µm, while the distance between stigma and ovary was short (14.85 ± 2.19 mm) so that the expected of fertilization process occurred sooner. At the end of the experiment (four hours after germination), IPB 1 genotype had the longest pollen tube (1,052 µm) while IPB 9 genotype (913 µm) had the shortest pollen tube. Genotype with the highest percentage of germination at the end experiment was IPB 2 (65.65%), whereas the lowest was IPB 7 (42.56%).

Keywords: *Carica papaya*, germination rate, pollen germination

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the main fruit crops grown in Indonesia and the fruits have high economical values. Research Center for Tropical Fruit IPB conducted experiments and field trials on a number of papaya genotypes released by IPB, including IPB 1, IPB 2, IPB 3, IPB 4, IPB 5, IPB 7, IPB 8, IPB 9, and IPB 10. Each genotype has its unique physical and chemical properties. Physical characters of fruit include length, diameter, volume, weight, flesh weight, flesh colour and hardness, whereas chemical properties include pH, total soluble solids, total titrated acids, and vitamin C. These physical and chemical properties will determine fruit shapes, colours and flavours. The earliest state of fruit development is fertilization and formation of fruits (Gillaspy *et al*., 1993), therefore all plant parts involved in fertilization, i.e. ovary and pollen, will determine the success of fruit formation.

Pollen is an important vector of gene flow in plants; containing male sexual cells (gametophytes) which are the source of genes for plants improvement. Characters of pollen in the anther have important effects on fruit qualities, known as metaxenia. Fruit sizes and fruit ripening time are some of the characters affected, so that these phenomena has potentials to improve fruit quality (Sedgley and Griffin, 1989). Different pollen sources might result in differences in the stages of fruit maturity, fruit sizes and date-palm seed weight (Al Khalifah, 2006; Muhtaseb and Ghnaim, 2006). Similarly, pollen of *H. polyrhizus* were reported to affect fruit-ripening time, hence used to extend the marketing period of the cacti fruits (Mizrahi *et al*., 2004). Viability of pollen, i.e. the ability of pollen to germinate, or the growth of pollen tubes, affect fruit set. Pollen tube growth correlated well with fruit set. Knowledge on pollen viability will be useful to better estimate fruit production (Bolat and Pirlak, 1999).

Several methods to test pollen viabilities are available. One of the methods is to germinate the pollen *in vitro* (Galletta, 1983). Caricaceae family have binucleate pollen, so that they can be germinated *in vitro* and have long storage ability (Sedgley and Griffin, 1989). Media used to germinate pollen *in vitro* was developed by Brewbaker and Kwack in 1963 and have since been used to grow pollen from a number of species. The media composition consists of 10% of sucrose, 100 ppm of H_3BO_4, 300 ppm of $Ca(NO_3)_2.4H_2O$, 200 ppm of $MgSO_4.7H_2O$, and 100 ppm of KNO_3. Germination of durian (*Durio zibethinus*) pollen *in vitro* was reported to be optimal using 10% of sucrose in the media (Honsho *et al*., 2007). Studies on papaya pollen viability and their storage ability have been conducted since 1963. Cohen *et al*. (1989) have reported their results on

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Israeli papaya, whereas Perveen *et al*. (2007) reported on Pakistan papaya. Papaya pollen viability may vary with varieties and climates (Magdalita *et al.* 1998).

The objectives of the study were to evaluate pollen viability of several papaya genotypes, i.e. IPB 1, IPB 2, IPB 3, IPB 4, IPB 5, IPB 7, IPB 8, IPB 9 and IPB 10.

MATERIALS AND METHODS

The research was conducted at the Tissue Culture Laboratory and Plant Ecophysiology Laboratory in the Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, between February to June 2008. The pollen were taken from hermaphrodite flowers of IPB 1, IPB 2, IPB 3, IPB 4, IPB 5, IPB 7, IPB 8, IPB 9 and IPB 10 genotypes grown at the Fruit Teaching Farm, Research Center for Tropical Fruit, Tajur Field Station, University Farm, IPB, Bogor. Plants are grouped based on their fruit sizes, i.e. small (IPB 1, IPB 3, and IPB 4), medium (IPB 5, IPB 9, and IPB 10), and large (IPB 2, IPB 7, and IPB 8).

The media for germination contained 100 ppm H_3BO_4, 300 ppm $Ca(NO_3)_2.4H_2O$, 200 ppm $MgSO_4.7H_2O$, 100 ppm KNO_3, 5% sucrose and distilled water. Analytical scale, glass objects, spatula, micrometer, a camera mounted microscope "Olympus BX41" were used for the experiment.

Fruit samples were taken at one day before anthesis stage. Pollen grains were separated from the anther using tweezers and planted on the germination media. Isolated pollen of papaya IPB 1, IPB 2, IPB 3, IPB 4, IPB 5, IPB 7, IPB 8, IPB 9, and IPB 10 were then germinated in a glass object under room temperatures, as described in Burke *et al.* (2004). Burke *et al.* (2004) studied pollen germination of durian at room temperatures of 20-37 ^oC with relative humidity of 50-80%. One glass object is considered as one experimental unit and replicated ten times.

A preliminary study was conducted to determine the correlation between pollen tube length and fruit set by measuring the distance from stigma to the center of ovary, and distance from stigma to the base of ovary. Measurement was conducted on 10 hermaphrodite flowers for each genotype. Diameter and length of pollen tubes were measured under magnification of 100x and 400x. Germination was scored every 30 minutes for four hours using "Olympus BX41" microscope equipped with ocular micrometer and mounted camera.

Data were analysed using Anova at 95% confidence level. Means were analysed using contrast analysis at 5 and 1% using SAS (*Statistical Analysis System*) software version 6.12.

RESULTS AND DISCUSSION

There were significant differences in length of stigma to the center of ovary between small and large fruits, and between medium and large fruits. Length of stigma to the base of ovary (reflects the length of flowers) of the genotypes with small fruits, were significantly shorter than those of genotypes with large fruits (Table 1). The hermaphrodite flowers of small papaya fruits (genotypes IPB 1, IPB 3, and IPB 4) were clearly different from those from large fruits (genotypes IPB 2, IPB 7, and IPB 8), whereas the difference from the medium fruits were less pronounced (genotype IPB 5, IPB 9, and IPB 10). The size of anthers of these three groups (large, medium, and small fruits) were similar (Figure 1).

Table 1. Distance from stigma to ovary of several papaya genotypes with different fruit sizes

Fruit category	Genotype	Distance from stigma to the center of ovary (mm)	Distance from stigma to the base of ovary (mm)
Small papaya	IPB 1	7.38 ± 1.42	12.80 ± 2.31
< 1 kg	IPB 3	9.20 ± 2.43	14.59 ± 3.43
	IPB 4	11.18 ± 2.21	17.15 ± 2.74
	Means	9.25 ± 1.90	14.85 ± 2.19
Medium papaya	IPB 5	9.56 ± 2.42	14.96 ± 3.94
1-2 kg	IPB 9	12.19 ± 1.51	19.47 ± 2.91
	IPB 10	11.39 ± 2.89	20.47 ± 5.97
	Means	11.05 ± 1.35	18.30 ± 2.93
Large papaya	IPB 2	10.52 ± 2.10	17.01 ± 2.83
≥ 2 kg	IPB 7	11.83 ± 2.63	18.57 ± 4.22
	IPB 8	13.44 ± 2.70	21.09 ± 4.38
	Means	11.93 ± 1.46	18.89 ± 2.06
Contrast Test			
Small vs Large		**	*
Medium vs Large		*	ns

Note: * = significant at $P < 0.05$; ** = significant at $P < 0.01$; ns = not significant

There were no significant differences in pollen diameters, pollen length, and pollen germination between small and large fruits, as well as between medium and large fruits (Table 2, Figure 2). IPB 4 had the smallest pollen diameter (33.25 ±.0.64 µm) whereas IPB 10 had the largest (36.50 ± 1.75 µm) of the nine papaya genotypes studied. Previous study by Erdtman (1972) showed the similar sizes of pollen, i.e. 35 x 30 µm. Erdtman (1972) also reported the size of other pollen of Caricaceae family such as *C. platanifolia* from Peru (41 x 33 µm) and that the sizes were of irregular pattern, and *Jacaratia mexicana* from Mexico (33 x 26 µm).

Larger diameter of pollen (36.08 ± 0.14 µm and 35.75 ± 0.66 µm) of large and medium fruits did not result in longer pollen tubes (Table 2), so pollen diameter did not seem to affect pollen tube growth of papaya. Similar results were reported by Zebrowska (1997) on pollen of strawberries and by Aizen and Searcy (1998) on pollen of *Alstroemeria aurea*. Studies by Kelly *et al*. (2002) on *Mimulus guttatus* demonstrated that pollen size might be used as an indicator of pollen viabilities, being larger in viable pollen and smaller in less viable pollen.

Shapes and weight of pollen affected pollen viabilities, while the amount of pollen applied to the stigma affected fruit set. Studies by Janse and Verhaegh (2004) on apple and pear, and by Walters and Taylor (2006) on pumpkin demonstrated that the number of pollen applied to the stigma affected seed formation, number seeds per fruit and seed weight.

Plants with small, medium and large fruits have similar pollen tube length. The length of pollen tubes scored at 30 and 60 minutes after germination are on Figure 3. Scoring conducted up to four hours after germination showed a similar growth pollen tube growth between papaya genotypes (Figure 4). The length of pollen tubes of IPB 4, IPB 3, and IPB 10 were 115.5, 115.0 and 99.5 µm at 30 minutes after germination. IPB 8 and IPB 10 grew by 292.5 µm and 167.5 µm after 60 minutes. Pollen tube length from small (IPB 1 and IPB 3) and medium fruits (IPB 10 and IPB 9) were 1,052.0 µm, 1,025.0 µm, 937.0 µm, and 913.0 µm at four hours after germination. Studies by Buyyukkartal (2003) on *Trifolium pratense* L. reported that pollen tubes had length of 177.6 µm, 237.6 µm, 324 µm, and 376.8 µm at 1, 2, 3 and 4 hours after germination, respectively. Maximum germination rate occurred at two to three hours after germination, and the growth of pollen tubes slowing down after six hours. Miyajima *et al.* (2003) reported that the presence of pollen in the ovule determined seed formation in fruits. Shimizu-Yumoto and Ichimura (2006) demonstrated that pollen tube growth in styles of *Eustoma* flowers occurred earlier in flowers received more pollen than those received less.

It is suspected that the process of fruit set will be enhanced by longer pollen tubes and by the shortest length from stigma to ovary. Genotypes with small fruits such as IPB 1, IPB 3, and IPB 4 might set fruits earlier since they had a relatively long pollen tubes (1,030.67 ± 19.14 µm) and short length from stigma to ovary (14.85 ± 2.19 mm) which allows faster penetration to ovule. Therefore IPB 1 might set fruits earlier since this genotype had a longer pollen tubes (1,052 ± 120.4 0 µm) and a shorter length from stigma to the base of ovary (12.80 ± 2.31 mm) compared to

Table 2. Diameter of pollens, pollen tube length, pollen germination rate of several papaya genotypes with different fruit sizes during four hours after germination

Fruit category	Genotype	Pollen diameter (µm)	Length of pollen tubes (µm)	Germination rate (%)
Small Papaya	IPB 1	35.00 ± 1.67	1,052.00 ± 120.40	50.68 ± 10.13
< 1kg	IPB 3	35.50 ± 2.58	1,025.00 ± 182.90	62.66 ± 13.86
	IPB 4	33.25 ± 0.64	1,015.00 ± 189.40	55.46 ± 12.01
	Means	34.58 ± 1.81	1,030.67 ± 19.14	56.27 ± 6.03
Medium Papaya	IPB 5	35.25 ± 2.93	1,018.00 ± 175.00	65.09 ± 5.88
1-2 Kg	IPB 9	35.50 ± 2.29	913.00 ± 101.90	58.95 ± 17.19
	IPB 10	36.50 ± 1.75	937.00 ± 153.50	59.97 ± 18.31
	Means	35.75 ± 0.66	956.00 ± 55.02	61.34 ± 3.29
Large Papaya	IPB 2	36.00 ± 2.02	1,004.00 ± 214.40	65.65 ± 6.81
≥ 2 Kg	IPB 7	36.25 ± 1.67	981.50 ± 88.90	42.56 ± 8.55
	IPB 8	36.00 ± 1.64	1,002.00 ± 114.40	60.37 ± 8.39
	Means	36.08 ± 0.14	991.75 ± 12.45	56.19 ± 12.09
Contrast Test				
Small vs Large		ns	ns	ns
Medium vs Large		*	ns	ns

Note: * = significant at $P < 0.05$; ns = not significant

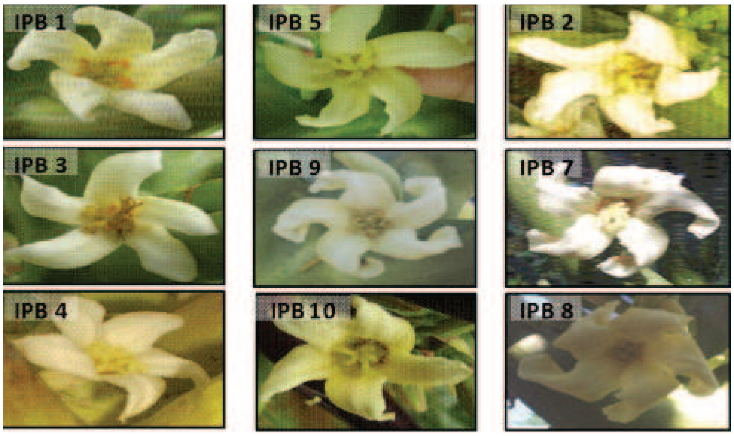

Figure 1. Hermaphrodite flowers of genotypes with small (IPB 1, IPB 3, and IPB 4), medium (IPB 5, IPB 9, and IPB 10), and large fruits (IPB 2, IPB 7, and IPB 8)

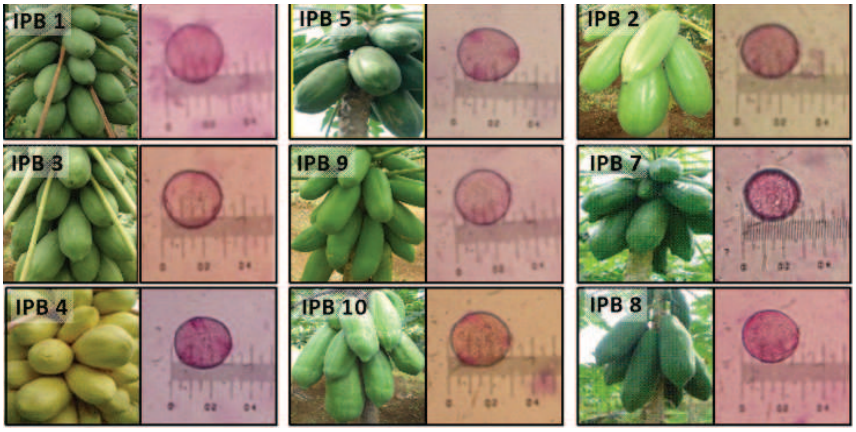

Figure 2. Fruits and pollen diameter of genotypes with small (IPB 1, IPB 3, and IPB 4), medium (IPB 5, IPB 9, and IPB 10), and large fruits (IPB 2, IPB 7, and IPB 8); (100X magnification, scale 0-2 = 30 μm)

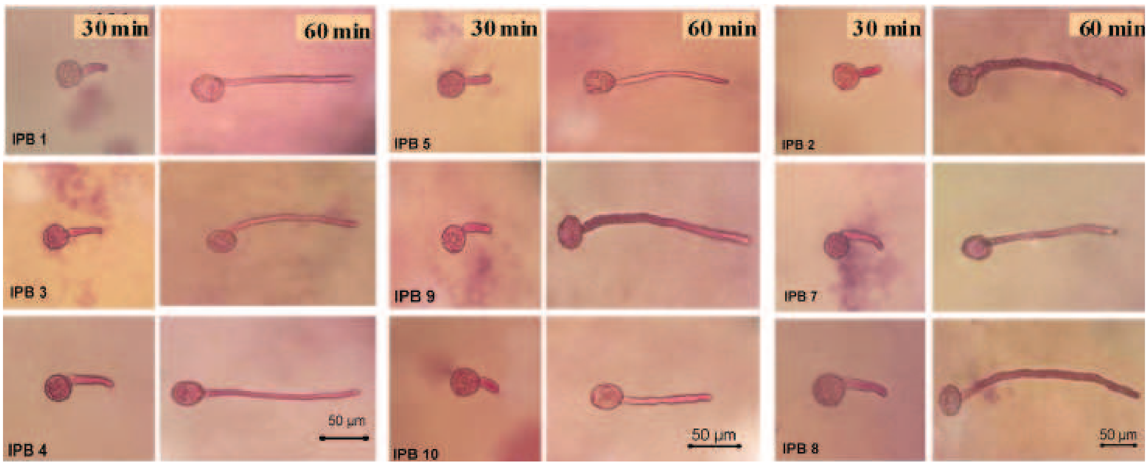

Figure 3. Comparison of pollen tubes of genotypes with small (IPB 1, IPB 3, IPB 4), medium (IPB 5, IPB 9, IPB 10), and large fruits (IPB 2, IPB 7, IPB 8); (100X magnification)

the other nine genotypes. Pollen tube growth commenced with the development of papilla tissue on the surface of the stigma, penetrate the styles through the pollen tube transmitting tissue, entered the fruits through micropils and then penetrated the embryo (Cheung, 1996). Therefore the shorter the distance from stigma to ovary would allow the pollen tubes reached the ovule earlier to start ferlitilization process.

Pollen germinability is an indicator of pollen viability. Germination rates varied with genotypes (Figure 5). Genotypes with small fruit i.e. IPB 3, IPB 1 and IPB 4 had germination rate of 62.66%, 50.68% and 55.46% after four hours, respectively. The genotypes with large fruits IPB 2, IPB 7 and IPB 8 had germination rate of 65.65%, 42.56% and 60.37%, whereas the genotypes with medium fruits IPB 5, IPB 9 and IPB 10 had 65.09%, 58.95% and 59.97%. These studies showed that there was no significant correlation between fruit sizes and pollen viability, indicated by pollen germinability and pollen tube length. Every genotypes demonstrated unique characteristics of pollen viability. Similar results were reported by Magdalita *et al.* (1998).

There have been limited reports describing the processes following pollen germination, penetration of pollen into styles in fruit trees, and fertilization, but this process involves interactions between parts of the flowers. Sexual reproduction in plants was affected by different factors, including complex biochemical and molecular systems involving different cells (Cheung, 1996). It is important to study the mechanism and pollen tube growth of different plant species, since these processes are linked to compatibilities between pollen and stigma, which are important for fruit set and fruit production.

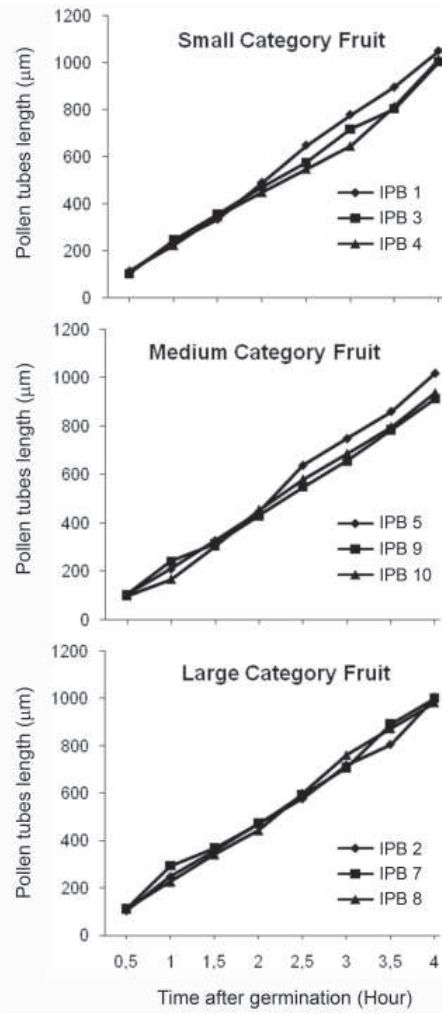

Figure 4. Pollen tubes length of IPB papaya genotypes

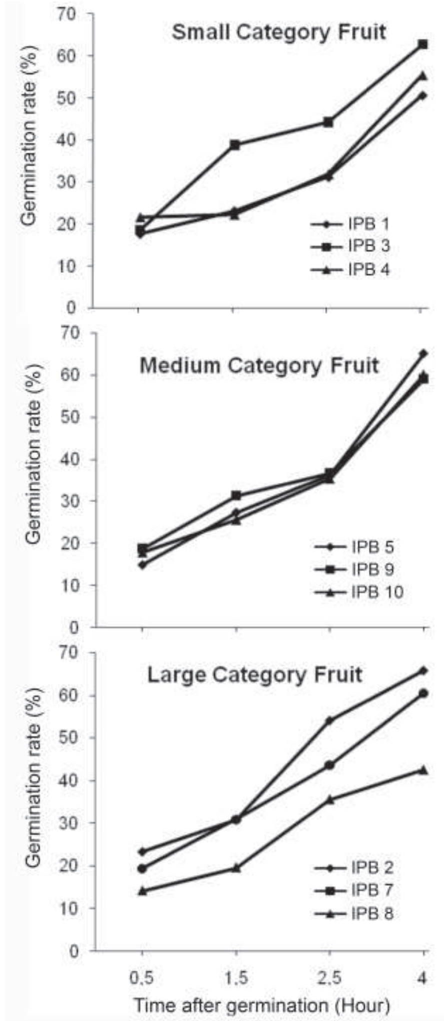

Figure 5. Pollen germination rate of IPB papaya genotypes

CONCLUSION

The success of papaya fruit set can be estimated using pollen viability as indicator, which are reflected by pollen germinabilities and speed of pollen tube growth. Fruit sizes did not show significant correlation with pollen viability.

Pollen tube growth of IPB 4, IPB 3, IPB 9 and IPB 10 scored every 30 minutes for the first two hours after germination were 115.5 µm, 115.0 µm, 104.5 µm and 99.5 µm, respectively. Small fruits (IPB 1, IPB 3 and IPB 4) had a relatively fast pollen tube growth of 1,030.67 ± 19.14 µm during the first four hours after germination, whereas the length from stigma to ovary were relatively short (14.85 ± 2.19 µm). It is expected that fruits will set earlier in plants with small fruits compared to plants with medium and large fruits.

IPB 1 had the longest pollen tube at four hours after germination (1,052 µm), whereas IPB 9 had the shortest (913 µm). IPB 2 had the highest germination rate of 65.65%, whereas IPB 7 had the lowest (42.56%).

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