

## Pollen Morphology and Sucrose Giving on Stigma Relation with Incompatibility of Sweetpotato [*Ipomoea batatas* (L.) L.]

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### ABSTRACT

This research aimed to increase the success rate of self-pollination in sweetpotato through sucrose treatment on stigma and pollen nuclei check. Various concentration of sucrose was applied on the stigma. Prior to the crosses, the stigma was excised and lubricated with sucrose. Pedicel was lubricated with 100 ppm of 2,4 D in lanolin. The result showed that the stigma of both varieties was longer than the anther. Papua Solosa showed 94,7% anther dehiscence and 70, 25% pollen viability; whereas MSU 03028-10 showed 87, 3% anther dehiscence and 87, 84% pollen viability. Both of these varieties showed receptive stigma. Sucrose level in compatible pollination was lower than in incompatible pollination. Sugar in 50 ppm concentration can increase the formation of fruit in self-pollination of Papua Solosa varieties from 0% to 70%; 0% to 60% in self-pollination of MSU 03028-10; and 10% to 90% of Papua Solosa > MSU 03028-10. The pollen nuclei of both of varieties were trinucleate. Sweetpotato incompatibility system was sporophytic self-incompatibility type that can be fixed by sucrose treatment using 50 ppm concentration, stigma excision, and 100 ppm of 2,4D in lanolin.

**Keywords:** *pollen morphology, sucrose giving, incompatibility of sweet potato*

### INTRODUCTION

The limited varieties of sweet potato (*Ipomoea batatas* L.) have been a problem that needs to be solved by the farmer. The efforts in breeding program for producing new varieties still continue. However incompatibility system of sweet potato may resist any plant breeding effort because it isn't followed by sexual embryo formation to produce new variety [9, 11]. Incompatibility system is controlled by single S gene locus with multiple alleles [8]. Incompatibility mechanism related to the type of pollen has been resulted in plants [2]. According to the pollen nuclei, Convolvulaceae family has binucleate and trinucleate type of pollen. Gemetophytic self incompatibility was observed in binucleate

species using site of inhibition in pollen tube growth. Sporophytic self-incompatibility was occurred in trinucleate species using site of inhibition of early pollen tube growth [2].

One of efforts which is aimed to increase the possibility to conduct plant crossbreeding to obtain new varieties is addition of an essential substance that may unavailable in sweet potato incompatibility [3, 5, 10]. Sucrose is an essential substance that seemed to be the key of successful germination and growth of in vivo pollen. Treatment of sucrose on sweet potato's stigma is expected to initiate the germination and penetration of pollen tube when incompatibility occurs; thus, fertilization can be preceded. The result of this research was assumed to increase the success rate of cross-pollination.

### MATERIALS AND METHODS

Quantitative descriptive researches on flower biology observe the number of pollen nuclei, distance between anther and stigma, pollen

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viability, and measurement of pistil's sucrose concentration. The crossbreeding of sweet potato was conducted using straw technique. Flower emasculated at evening and crossed in the morning. Before crosses conducted, stigma were excised and lubricated with various sucrose concentration (0 ppm, 50 ppm, 100 ppm, and 150 ppm) while the pedicel was lubricated with 2,4D in lanolin. Parameters measured were percentage of (%) fruit set from each treatment. Each treatment performed to 10 flowers that replicated in 3 times. Papua solosa varieties and MSU 03028-10 are selected to be the parental of this re-search. The type of cross were Papua solosa, MSU 03028-10, and Papua solosa  $\times$  MSU 03028-10.

Preliminary study was conducted to know the incompatibility degree of sweet potato. Acetolysis method [4] was used to observe the pollen nuclei; fluorescence method staining using aniline blue was used to determine the viability of pollen; and pistil receptivity was observed by Olympus microscope FSX 100. Acetocarmine was chosen as a comparison method to determine the viability of pollen. Pistil sucrose levels were measured with spectrophotometer UV-Vis

Varian/Cary 50 (each measurement used 30 pistils). Pollen were germinated in vitro in Bhojwani and Bhatnagar solid and liquid (100 g sucrose, 10 mg boric acid, 30 mg calcium nitrate, 20 mg magnesium sulphat, and 10 mg potassium nitrate in 100 ml distilled water, solid medium were added by 1% agar); modified Bhojwani and Bhatnagar (25 g sucrose, 0,005 mg boric acid, 500 ml distilled water); and Murashige and Skoog to know the germination degree of viable pollen.

## RESULTS AND DISCUSSION

Both varieties showed self-incompatibility and cross compatibility because there was no fruit generated (Figure 1b) and/or only 10 % fruit emerged (Figure 1a). Pollen viability of both varieties that had range 70, 25% - 91, 52% could support fertilization. Based on the flower morphology, the distance between anther and stigma of both varieties was slightly exerted type where stigma was higher than highest anther with relative distance 5,12mm in MSU 03028-10 and 4, 90 mm in Papua solosa (Figure 1d).

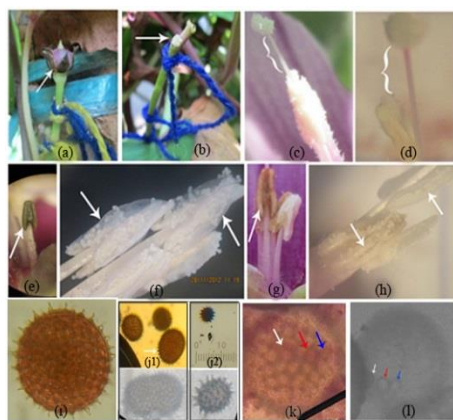


Figure 1. Sweetpotato's flower and pollen morphology. a) compatible crosses fruits, b) flower that fallen in incompatible crosses, c) and d) anther-stigma distance, e) and g) indehiscent anther were not developed, f) and h) opened anther, the MSU 03028-10 and Papua Solosa, i) circular pollen of sweetpotato, j1) and j2) very large and very small pollen, k) and l) trinucleate pollen of Papua Solosa and MSU 03028-10.

Based on pistil receptivity and anther dehiscence, both varieties showed 100% pistil receptivity and 80-100% anther dehiscence. Papua solosa showed 94, 7% anther dehiscence (Figure 1h) and MSU 03028-10 showed (Figure 1f) 87, 3% anther dehiscence. Therefore, anther dehiscence of MSU 03028-10 was lower than Papua solosa. One of anthers was not developed (Figure 1e). Papua solosa also showed few indehiscent anthers (Figure 1g). Anthers that were open-

ned during three hours of anthesis would be wilted [3, 7], thus anther dehiscence was one of important factors that determine the success rate of cross.

Pollen nuclei of both varieties were trinucleated type (3 nuclei) (Figure 1k and 1l) as Williams and Cope (1967) discovered that mature pollen in several species of genus *Ipomoea* were trinucleated. Trinucleate pollen related to the sporophytic self-incompatibility with inhibition

site of pollen germination in early tube growth. Circular pollen with normal size (119-153  $\mu\text{m}$ ) (Figure 1i) was compatible pollen and viable pollen to be applied in compatible crosses. Abnormal pollen can be very small (68  $\mu\text{m}$ ) (Figure 1j2) or very big (187  $\mu\text{m}$ ) (Figure 1j1). Formation of abnormal pollen may inhibit fertilization in the nature. Abnormal pollen suspected as the result of abnormal cleavage. Nondisjunction and other abnormalities can occur in high percentage as a result of genetic imbalance [3]. Hexaploid chromosomes of sweet potato were suspected to cause genetic imbalance in segregation, thus leakage produced bigger and/or smaller pollen than normal pollen. Papua solosa varieties showed lower pollen viability although percentage of dehiscent anther was higher than MSU 03028-10. The lower pollen viability showed that opened anther did not determine pollen viability. The lower pollen viability affected by normal but unviable pollen (Figure 2c, 2d, 2f, and 2g) and abnormal pollen. Investigation of *in vitro* pollen germination of viable pollen had been failed. Several medium of pollen germination showed that cell substance were lysed out and didn't produce pollen tube (Figure 2i1-2i4). The success growth of pollen was only found on Bhojwani and Bhatnagar solid medium (Figure 2h).

The failure of *in vitro* pollen germination caused by unmatched medium or none of essential substance in the medium can support *in vitro* germination of trinucleate pollen of sweet potato. Brewbaker (1957) stated that trinucleate pollen was rarely germinated. Heslop-Harrison and Shivanna (1977) said that trinucleate pollen was not ready to germinate *in vitro*. Jones (1980) assumed that pollen viability and storage of sweet potato was not much known because none of them can be germinated *in vitro*. *In vitro* studies showed that 80-85% pollen may viable (Jones 1980). Investigation on flower biology showed that one barrier that might exist in sweet potato was the incompatibility system since all of flowers component supported fertilization.

Treatment of various concentration of sucrose on stigma affected the increase of successful crosses (Table 1). Treatment in 50 ppm concentration increased the fruit formed of Papua Solosa, MSU 03028-10, and Papua Solosa < MSU 03028-10 crosses from 0% (preliminary test) to 70%, 0% to 60%, and 10% to 90%.

Control treatment also increased the fruit formed of Papua Solosa, MSU 03028-10, and Papua Solosa < MSU 03028-10 crosses from 0% (preliminary test) to 50%, 0% to 40%, and 10% to 70%.

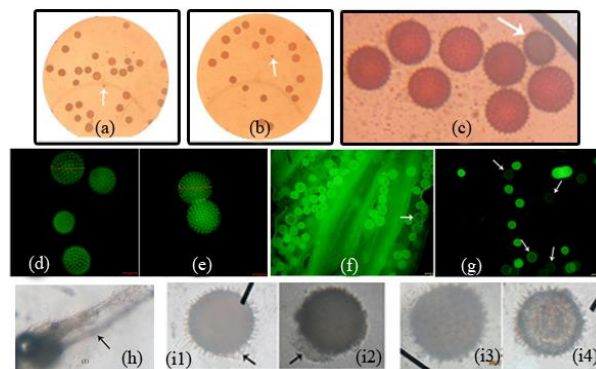


Figure 2. Pollen viability of sweetpotato. a) and d) pollen varieties Papua Solosa, b) and e) pollen MSU 03028-20, c), f) and g) viable and not viable pollen (designated arrows), h) pollen germination, i1 and i2 ) pollen on the medium Bhojwani and Bhatnagar solid and liquid, i3) pollen on the modified medium Bhojwani and Bhatnagar, i4) pollen on MS medium. a, b, c: acetocarmine staining method, d, e, f, g: fluorescence method, h, i1-4: methods of germination *in vitro*.

The increase of successful crosses assumed as the effect of stigma excision and giving 100 ppm of 2,4D in lanolin. Concentration 100 ppm and 150 ppm showed decreased successful crosses since there was no fruit produced in control treatment. Decreased succeed crosses might be caused by osmotic pressure on stigma. Measurement of sucrose concentration in pistil was conducted to discover the function of sucrose as an essential element that needed in pollination related with incompatibility of sweet potato. It is known that sucrose concentration in compatible crosses (80926,2 ppm) was lower than incompatible crosses (180055 ppm).

Regression analysis was used to obtain which sucrose concentration give maximum results to increase succeed crosses. Concentration of 55, 5893 ppm with R value = 0,647 and regression line equation  $y = 51,556 + 0,22x - 0,003x^2$ . was expected to produce maximum successful crosses in the next breeding. Trinucleate pollen and sucrose treatment affected the incompatibility of sweet potato.

Incompatible crosses before sucrose treatment showed abnormal germinated pollen

on stigma (Figure 3f) as the result of incompatibility mechanism. Compatible crosses showed normal pollen tube (Figure 3g and 3h). Incompatibility reaction happened on the surface of stigma of early development of pollen tube after fertilization. This arrested pollen tube growth; thus no seed formed. In short, sucrose is an essential element to obtain successful pollen germination and in vivo growth [2].

Physiological action from alleles which are related to the incompatibility system maybe mediated by simple mechanism of pollen sucrose uptake [2]. Trinucleate pollen is lack of sucrose

or relative small amount of certain metabolite that occur in maturity of second mitosis cleavage in late microsporogenesis. The limited sucrose inhibits pollen germination. Sporophytic self-incompatibility influenced by trinucleate pollen and site of inhibition of the pollen that occurs in early pollen growth. Sucrose helps trinucleate pollen to germinate on stigma. Sucrose in medium functioned to control osmotic pressure and served as substrate for respiration since respiration was an absolute need for pollen tube growth [1].

Table 1. Average Percentage Result of Fertilization on Sweet Potato Crosses

Treatment	P.Solosa><P.Solosa	P.Solosa><MSU 03028-10	MSU 03028-10><MSU 03028-10
S3	13,3a	40a	16,7a
S2	30ab	46,7a	23,3a
S0	40b	66,7ab	36,6ab
S1	60b	86,7b	53,3b

Note: number followed by the same letter in treatment and observation of the same parameters showed insignificant differences at 5% BNJ.

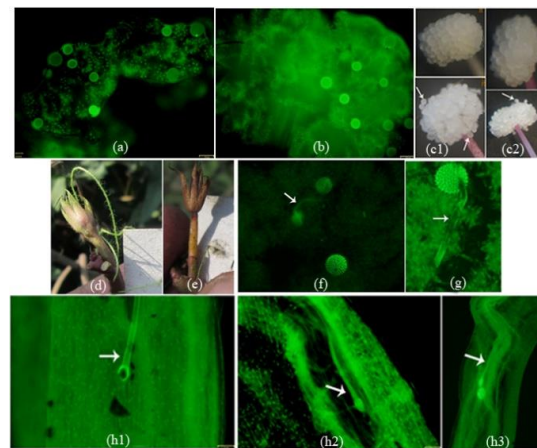


Figure 3. Pistil receptivity and pollen tubes of sweetpotato. a) and b) the receptive pistils (fluorescence), c1) and c2) stigma was receptive when anthesis occurred, MSU 03028-10 and Papua Solosa, occurred, d) and e) fruit formed and not formed after 2 weeks of compatible and incompatible crosses, f) abnormal pollen tube, g) pollen tube germinated on the stigma, h1), h2), and h3) pollen tube on the stylus.

In this research despite of sucrose treatment, excised stigma, and 100 ppm of 2,4D in lanolin also had important effect. Williams and Cope (1967) research on excised stigma proved that fruit were increased but seeds were not completely formed because of the fallen flower. Excised stigma made it possible to eliminate the site of inhibition, thus pollen can be germinated. Regulation of inhibition system was shown by the fallen of flower in incompatible crosses. Treatment of 100 ppm of 2,4D in lanolin at pedi-

cel was used to maintain the flower after pollination in compatible and incompatible crosses. The flower did not fall after 2 weeks although no fruit formed (Figure 3e). Combination of this treatment, i.e. sucrose treatment on stigma, excised stigma, and 100 ppm of 2, 4D in lanolin on pedicel can increase successful fertilization.

Sporophytic self-incompatibility occurs in trinucleate species and site of inhibition occurs in early pollen tube growth, whereas gametophytic self-incompatibility occurs in binucleate species



and site of inhibition occurs in pollen tube growth [2]. Inhibition of germination is caused by differences of available metabolite of each pollen type in anthesis stage and incompatibility mechanism which limits the metabolism and sucrose uptake of male gametes [2, 3, 5, 10]. Both varieties have trinucleate pollen and related to the sporophytic incompatibility. This research did not consider F1 offspring as a result of experimental crosses, but only focused on the increased successful crosses from lower rate to the higher rate as the effect of relationship of sweet potato's sporophytic self-incompatibility and its trinucleate pollen. Suppose that F1 offspring cannot germinate because of series of incompatibility mechanism, embryo culture can be conducted after fertilization occurred.

## CONCLUSIONS

Papua solosa varieties and MSU 03028-10 has trinucleate, viable, and compatible pollen, circular shaped, and 119-153  $\mu\text{m}$  in size. Incompatibility system in Papua solosa varieties and MSU 03028-10 are sporophytic self incompatibility where the inhibition occurs on stigma. Sucrose treatment can increase succeed crosses. 50 ppm concentration increased successful crosses up to 86,7% in Papua Solosa; 60% among Papua Solosa; and 53; 3% in MSU 03028-10 itself. Sucrose concentration for applying maximum result is 55, 5893 ppm.

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