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RESEARCH ARTICLE

PRE-FERTILIZATION BARRIER IN SOMEINTERSPECIFIC HYBRIDS OF *SOLANUM* SPECIES

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ABSTRACT

Interspesific F_1 hybrids between *S. aethiopicum* Acc.8971 and *S. melongena* var. DLP and Acc. 3305 were produced. Yet, recovered crosses were observed to have high percentage of flower abscission and fruit setting was never noted in them. Pollen tube study was conducted to unveil the pre-fertilization barriers causing hybrid sterility and breakdown. Fluorescence microscopy was used to determine pre-fertilization barriers. Pre-fertilization barrier in the form of inhibited pollen tube growth was noted.

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INTRODUCTION

Plant development programs aim to produce superior eggplant varieties resistant to pests and diseases. The hybrids of eggplant have many advantages compared with open-pollinated cultivars in terms of yield and disease resistance. Interspecific F_1 hybrids between *S. aethiopicumn* Acc. 8971and *S. melongena* var. DLP and Acc. 3305 were generated.However, recovered crosses were observed to have high percentage of flower abscission and fruit setting was never noted in them.Interspecific hybridization must produce viable hybrids to gain relevance in plant breeding. But in the attempt toincorporate beneficial genes across interspecific and intergeneric hybridization, deleterious genes are introduced as well (Raghavan, 1986). These deleterious genes which are difficult to eliminate also find their way into the hybrids causing hybrid sterility and breakdown. It is thought that wide hybridization triggers hybrid sterility and breakdown. The general objective of this study was to unveil the pre-fertlization barriers governing the hybrid sterility and breakdown in the interspecific crosses made. Exploitation of the existing parameters e.g. pollen tube growth assessment is very important. By knowing such barriers, one could explain the occurrence of hybrid breakdown in *Solanum's* interspecific crosses.

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MATERIALS AND METHODS

Pollen tube growth on the style was studied in the selfed F_1 hybrids at the National Plant Genetic Resources Laboratory (NPGRL) field, Institute of Plant Breeding, University of the Philippines Los Baños, Laguna. These hybrids were observed to have high percentage of flower abscission and no fruit setting. Germinating pollen stained with aniline blue was observed under fluorescence microscope at the International Rice Research Institute (IRRI). The status of pollen germination and pollen tube growth were monitored by harvesting flowers one hour after pollination and every hour thereafter up to 24 hrs. Ten to fifteen flowers were collected for dissection per harvesting time. The styles were dissected properly using a dissecting microscope. The dissected specimens were then stained for 3 to 4 hours with aniline blue. Stained styles were placed under a coverslip before microscopy. Fluorescence microscopy was used to observe pollen tube growth and pollen germination (Khan 1929; and Grubben 1977; Mendioro and Ramirez 1963).

RESULTS AND DISCUSSION

At 8:00 AM, pollens of selfed plants were observed clinging to the stigma. After an hour (9:00 AM), the same scenario was observed in the stigma until 11:00 AM where pollen germination occurred (Figure 1). Pollen tube growth was not able to occur one day after pollination because most of the

selfed flowers were already abscising on the following day. In eggplant, fertilization is completed within 3 days after pollination (Shrivastra and Raira, 2008), but since early flower abscission occurred, the potential to reach the complete fertilization was hampered. As observed, pollen germination occurred but did not show any pollen tube growth (Figure 2).

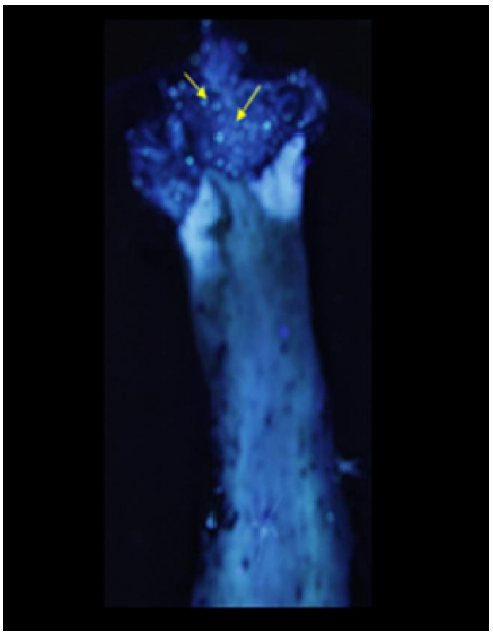

Figure 1. Pollen germination on the stigma of *Solanumaethiopicum* L. Acc. 8971 x *S.melongena* var. DLP at 11:00 AM

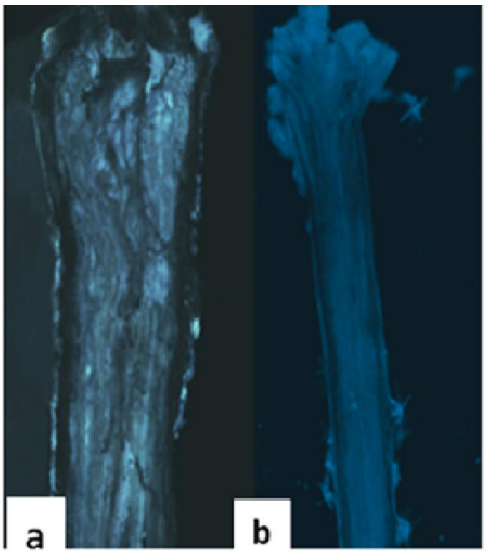

Figure 2. No occurrence of pollen tube growth in *Solanum*hy brids a) *Solanumaethiopicum* L. *x S. melongena* var. DLP and b) *S. aethiopicum x S. melongena* Acc. 3305 one day after pollination

Inhibited pollen tube growth could likely account for the high percentage of flower abscission leading to zero fruit setting. In addition, inhibited pollen tube growth is not only observed in interspecific hybridization in *Solanum* species. It was also observed in the cross between rye (*Secalecereale*) and barley (*Hordeumvulgare*) (Heslop-Harrison 1982). Pollen tube entered the stigma, but upon reaching the style, growth was retarded and ultimately the tips enlarged. According to Sari-Gorla *et al*. (1995), the ability of the pollen tube to be developed may be considered as indicator of the pollen quality, independent of the environment in which the flowers are pollinated, but the performance of pollen varies according to the genetic combination of the species combined. Furthermore, if pollen grains germinate, barriers operate in the course of micropyle penetration. Micropylar or short-range pollen tube guidance appears to be governed by species-specific chemotaxis (Higashiyama *et al*., 2001; Palanivelu and Preuss, 2006). Physiological malfunctions due to species-species chemotaxis signaling might cause its inhibited pollen tube growth leading to hybrid sterility and breakdown.

Conclusion

Pre-fertilization barriers were identified. Inhibited pollen tube growth was observed in the selfed F_1 hybrids. These phenomena including low pollen fertility would likely be the reason for high percentage of flower abscission and zero fruit setting. Furthermore, failure to obtain fruits in the F_1 hybrids can be due to physiological malfunctions during pollen germination and tube growth. The mechanism of hybrid sterility and breakdown has been elucidated by pollen tube growth study. Over the years, this protocol paved the way in ascertaining pre-fertilization barriers across different plants species and cross incompatibilities in many interspecific and intergeneric crosses. It is suggested that the potential of biochemical/physiology experiments e. g. extensive *in vitro* germination and pollen tube growth and molecular techniques e. g. assessment of sperm dynamics during pollen tube growth and delivery using germline-specific markers can be conducted to further elucidate the mechanism of hybrid breakdown in the interspecific hybrids. Total genomic DNA can be used to rapidly detect alien DNA in the hybrids.

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