Pollen Degeneration in Three Functional Male-Sterile Lines of Eggplant with the Wild Solanum Cytoplasms

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Abstract. Pollen developing process was studied to identify the stages of pollen degeneration and to elucidate the factors controlling low pollen fertility in three functional cytoplasmic male-sterile (CMS) lines of eggplant ‘Uttara’. The CMS lines of eggplant were developed by repeated backcrossing using the cytoplasms of wild Solanum species S. kurzii Brace & Prain, S. violaceum Ort., and S. virginianum L.. Anthers were squashed in 1% aceto-carmine to assess pollen staining ability and pollen degeneration at different stages of development. Unicellular microspores were released from tetrads after normal meiotic division. Pollen degeneration occurred at different stages of pollen development in CMS lines such as at unicellular microspore (29.3-36.3%), early bicellular pollen (5.5-12.2%), and late bicellular pollen (9.3-10.2%) stages. On the other hand, in eggplant, only 3.8% pollen was degenerated at unicellular microspore stage and there was negligible pollen degeneration at other stages. Among the stained pollen, abnormally stained (partly and faintly) pollen were found significantly higher in the CMS lines as compared to eggplant. Well stained pollen was varied from 23.2-31.9% in the CMS lines which was significantly lower than that of eggplant. Number of pollens per anther of CMS lines did not vary significantly from eggplant, except the CMS line with S. virginianum cytoplasm. In vitro pollen germination rate in the CMS lines was found to be significantly lower than that of eggplant. Starch accumulation and hydrolysis during pollen maturation were found incomplete in the CMS lines. Degeneration of pollen in different stages, abnormally stained pollen, incomplete starch accumulation and hydrolysis were most likely causes for low pollen fertility in these three CMS lines of eggplant.

Additional key words: male sterility, pollen characteristics, pollen development, pollen fertility, wild Solanum species

Introduction

Male sterility is the inability of a plant to produce functional anthers, pollen or male gametes, while female reproductive organs are functional. In eggplant (Solanum melongena), male sterility has been described in several reports. Genic male sterility caused by recessive nuclear genes was reported (Chauhan, 1984; Jasmin, 1954; Nuttall, 1963; Phatak and Jaworski, 1989; Phatak et al., 1991). Cytoplasmic male sterility (CMS) derived by substituting the cytoplasm of eggplant with that of wild Solanum species, S. gilo Raddi (Fang et al., 1985), S. kurzii Brace & Prain (Khan and Isshiki, 2009), S. violaceum Ort. (Isshiki and Kawajiri, 2002), S. virginianum L. (Khan and Isshiki, 2008), S. aethiopicum L. Aculeatum Group (Khan and Isshiki, 2010), S. anguivi Lam. (Khan and Isshiki, 2011) and S. grandifolium C.V. Morton (Hasnunnahar et al., 2012; Saito et al., 2009) was reported.

CMS lines with the cytoplasms of S. kurzii, S. violaceum and S. virginianum, showed anther indehiscent type of functional male sterility. Although these CMS lines contained pollen in their anthers but malfunctioning of the anthers prevented the release of pollen in these lines. These CMS lines showed stable expression of functional male sterility, low pollen fertility and high seed fertility. In commercial hybrid seed production, a large number of female parents (seed parent) with male sterility are required. For hybrid seed production, CMS lines are maintained by crossing it with the recurrent pollen parent. Propagation of CMS line by selfing is less tedious and more efficient method because no maintainer line is needed but the presence of viable pollen in CMS plant is an essential criterion for selfing. Although there was more than 50% stained pollen in aceto-carmine in these three CMS lines but in vitro pollen germination was less than 20%. Therefore, all of the stained pollen of the CMS lines did not germinate. There was no study to understand the reason of low pollen fertility and stages of degeneration.
pollen degeneration which was essential for the detail characterization of these CMS lines.

The development of fertile pollen involves a large number of genetical, physiological, biochemical, and morphological processes within the anther. Pollen development is initiated through the formation of tetrad of four unicellular microspores after two divisions of meiosis, which is enclosed within thick callosic wall (McCormick, 1993) and the unicellular microspores are released by the breakdown of callosic wall. The development of microspores are accompanied by progressive vacuolation with polarized nucleus, which undergo microspore mitosis (asymmetric) and bicellular pollen were produced. The first microspore mitosis plays a key role in the development of microspore to the maturation phase (Datta et al., 2002). At early bicellular stage, the central vacuole eventually disappeared and various subcellular organelles, e.g. the mitochondria, plastid, endoplasmic reticulum and lipid bodies, starch, etc., begin to develop and pollen become mature (García, 2007). In Solanaceae family, pollen is released from the anther at bicellular stage. The second pollen mitosis of the generative cell produce two sperm cells while the pollen tube grows through the female pistil (McCormick, 1993). Mutations that impair the development of the stamens, differentiation of the sporogenous cells, meiosis, development of the free microspores, microspore mitosis, pollen differentiation or anthesis may result male sterility in plants (Glover et al., 1998).

Developmental and cytological studies are necessary for characterizing the difference between normal and sterile pollen, which help to elucidate the sequence of events leading to male sterility. Investigating the characteristics of male sterility in eggplant may advance, not only our understanding of the mechanisms of male sterility, but also the application of this trait in heterosis breeding. In the present study, pollen developing process in three functional CMS lines of eggplant with the cytoplasms of S. kurzii, S. violaceum and S. virginianum was investigated to identify the stages of pollen degeneration and to elucidate the factors controlling low pollen fertility.

**Materials and Methods**

**Plant Materials**

Three functional CMS lines of eggplant, induced by the cytoplasms of wild *Solanum* species *S. kurzii* (Khan and Isshiki, 2009), *S. violaceum* (Isshiki and Kawajiri, 2002) and *S. virginianum* (Khan and Isshiki, 2008), namely CMS (Skur), CMS (Svio) and CMS (Svir), respectively, were developed previously. In the present study, eggplant ‘Uttara’ and the backcross progenies BC*₃* of CMS (Skur), CMS (Svio) and CMS (Svir) were investigated. Theoretically, the backcross progenies were contained more than 99% nuclear genes of eggplant ‘Uttara’ and were the isogenic lines of eggplant ‘Uttara’. All plant materials were grown in pots in a glasshouse during summer season from April to November. Minimum and maximum temperatures for that period were 15 and 38°C, respectively. To observe pollen characteristics and degeneration, at least three plants with seven flowers per plant were selected from each line and 500 pollens were counted for each flower.

**Pollen Degeneration and Staining with Aceto-carmine**

Pollen development and degeneration were studied at series of developmental stages from meiotic metaphase I of pollen mother cells (PMCs) to mature pollen at anthesis. Chromosome associations at meiotic metaphase I (MI) were observed in 20 PMCs of each plant by smear preparations of PMCs from fresh anthers in 1% (w/v) aceto-carmine. Four stages of pollen development were defined, namely unicellular microspore, early bicellular pollen (where the generative cell was attached to the intine), late bicellular pollen (where the generative cell was free within the vegetative cell), and mature pollen stage at day of anthesis (where the generative cell has flattened in the middle of the pollen). Anthers were squashed in 1% (w/v) aceto-carmine to assess their staining ability using the method described by Singh (2002). Percentage of degenerated pollen was counted from flowers at anthesis. Degenerated pollen were easily distinguished from normal pollen by staining properties, shape and size. Stained pollens were, plump and round while degenerated pollens were unstained and shriveled with different shapes and sizes (Fig. 1). In order to test the stainability of pollen, pollens from each flower were categorized by different size or staining properties and the number of unstained, partly stained, faintly stained and well stained pollens were counted.

**Number of Pollens Per Anther**

Number of pollens per anther was counted by using hemocytometer (Fuchs-Rosenthal). More than seven anthers from three flowers for each plant were examined for this counting. The anther was thoroughly crushed with a glass rod and then 5 ml distilled water with 1% aceto-carmine was added into each vial. After some stirring and shaking all pollens had loosened from the anther parts. Aliquot of this mixture was pipetted into a hemocytometer. The number of pollens per ml was counted by dividing the average number of pollens per square by the volume counted. The number of pollens per anther was counted by multiplying the number of pollens per ml by the original volume of mixture from which sample was removed.

**In Vitro Pollen Germination**

In vitro germination ability of pollen at flower anthesis was investigated according to Singh (2002) with a slight