

## Pollen fertility differences in the progenies obtained from a cross between eggplant (*Solanum melongena* L.) as a seed parent and eggplant cytoplasmic substitution lines as pollen parents

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### Abstract

To the best of our knowledge, there is no report about pollen fertility of the progenies developed using eggplant (*Solanum melongena* L.) as a seed parent and eggplant cytoplasmic substitution lines as pollen parents. Pollen fertility of these progenies is very important to use as restorer line in the eggplant's hybrid breeding program. In this study, pollen fertility was investigated for the progenies which were produced using *S. melongena* 'Uttara' as a seed parent and the eggplant cytoplasmic substitution lines as pollen parents. To assess pollen fertility, pollen stainability and *in vitro* germination ability were investigated. Although the nuclear and the cytoplasmic genome of the progenies were almost identical to eggplant 'Uttara', a clear difference was observed in the pollen fertility due to the difference in the pollen parents having different wild *Solanum* cytoplasm. The progenies produced using the functional cytoplasmic male sterile (CMS) lines as a pollen parent, whose cytoplasm donor were *S. kurzii*, *S. violaceum* and *S. virginianum*, showed pollen release type and high pollen fertility almost equal to eggplant 'Uttara'. It is considered that the characteristics of these progenies were almost the same as eggplant. On the other hand, the progenies that produced using the fertility restored lines of the pollen non-formation type CMS lines as a pollen parent, whose cytoplasm donors were *S. aethiopicum*, *S. anguivi* and *S. grandifolium*, showed pollen release type and low pollen fertility, i.e., pollen staining ability was about 54% and pollen germination ability were about 35%. It is considered that the cause of this low pollen fertility was the incompatibility between the eggplant cytoplasm and the eggplant nuclear genome, which seems to be modified in the process of continuous backcrossing under the wild *Solanum* cytoplasm. It is suggested that complete nuclear substitution is difficult by continuous backcrossing with eggplant in the alloplasmic lines with *S. aethiopicum*, *S. anguivi* and *S. grandifolium* cytoplasm donors. Incompatibility between the normal eggplant cytoplasm and the modified eggplant nuclear genomes of the alloplasmic lines with *S. aethiopicum*, *S. anguivi* and *S. grandifolium* cytoplasm might be a cause for the low pollen fertility of the investigated progenies.

**Keywords:** cytoplasmic substitution line; eggplant; male sterility; pollen fertility difference; wild *Solanum* species.

### Introduction

In our laboratory, we have successfully developed six CMS systems of eggplant by developing cytoplasmic substitution lines utilizing the cytoplasm of six wild *Solanum* species closely related to eggplant through repeated backcrossing (Figs. 1 and 2). These CMS systems categorized into anther indehiscent and pollen non-formation types of sterility. CMS systems of eggplant developed utilizing the cytoplasm of *S. kurzii* Brace & Prain, *S. violaceum* Ort. and *S. virginianum* L. expressed anther indehiscent type functional male-sterility (Isshiki and Kawajiri, 2002, Khan and Isshiki, 2008, 2009). On the other hand, CMS systems of eggplant developed utilizing the cytoplasm of *S. aethiopicum* L. Aculeatum Group, *S. anguivi* Lam. and *S. grandifolium* C.V. Morton expressed pollen non-formation type sterility (Khan and Isshiki, 2010, 2011, Hasnunnahar, et al., 2012). Pollen non-formation type CMS systems has male fertile line (fertility restorer line), which carries the fertility restorer (*Rf*) genes against pollen non-formation type sterility. At present, these CMS lines are being put to practical use for the production of F<sub>1</sub> seeds and/or

seedless fruits of eggplant.

The traits of the wild *Solanum* species almost disappeared in the generation after the third generation of backcross, and the characteristics of the male sterility were stably expressed (Isshiki and Kawajiri, 2002, Khan and Isshiki, 2008, 2009, 2010, 2011, Hasnunnahar, et al., 2012). In this backcross generation, we attempted a reverse mating of the backcrossing, that is, an eggplant cultivar as a seed parent and the cytoplasmic substitution lines as a pollen parent for producing eggplant nuclear genome and cytoplasm with a nuclear *Rf* (restore fertility) gene/genes (Khan et al., 2013). These plants are predicted not to express male sterility because their cytoplasm is eggplant one and their pollen staining abilities are expected to be high almost equal to eggplant.

However, surprisingly, some of these plants showed low pollen staining ability when we examined the pollen fertility in the preliminary trial. This interesting phenomenon seems particularly noticeable in the progenies when fertility restored lines of the pollen non-formation type CMS lines used as a pollen parent. Therefore, we investigated the pollen fertility of

each of the six progenies produced using eggplant as a seed parent and the six cytoplasmic substitution lines of wild *Solanum* species as a pollen parent in this study. Furthermore, the progenies of the backcrossing and the selfing of (eggplant × the BC<sub>8</sub> of *S. anguivi*), were examined for elucidating the cause of the low pollen fertility.

## Results and discussion

### Pollen release ability

Pollen release ability was investigated for each of the progenies produced using eggplant 'Uttara' as a seed parent and the six cytoplasmic substitution lines as a pollen parent. Each of the progenies was male fertile, i.e., pollen release type like eggplant 'Uttara'.

### Pollen fertility

Although nuclear genome of the progenies was almost identical to eggplant 'Uttara', a clear difference was observed for pollen fertility when pollen parents from the two CMS systems differ. The progenies of the eggplant 'Uttara' × the functional CMS lines, whose cytoplasm donors were *S. kurzii*, *S. violaceum* and *S. virginianum*, showed high pollen fertility almost equal to eggplant 'Uttara' (Table 1, Figs. 3 and 4). It is considered that these progenies are almost the same to eggplant 'Uttara' for the characteristics of pollen release ability and pollen fertility. This may be since the alloplasmic cytoplasm of *S. kurzii*, *S. violaceum* and *S. virginianum* had no effect on the eggplant nuclear genome. These cytoplasm seem to be high affinity with the eggplant nuclear genome. On the other hand, the progenies of the eggplant 'Uttara' × the fertility restorer lines of the pollen non-formation type CMS systems, whose cytoplasm donors were *S. aethiopicum*, *S. anguivi* and *S. grandifolium*, showed low pollen fertility. Pollen staining ability was about 53 ~ 54 % and pollen germination ability was about 34 ~ 38 % (Table 2, Figs.3 and 4). This may be due to the fact that each of the alloplasmic cytoplasm of *S. aethiopicum*, *S. anguivi* and *S. grandifolium* had a strong effect on eggplant nuclear genome. This strong effect means certain parts of the eggplant nuclear genome may have been modified. It is believed that the incompatibility between the normal eggplant cytoplasm and the modified eggplant nuclear genomes caused low pollen fertility. These cytoplasm seem not to have a high affinity with the eggplant nuclear genome. In order to elucidate this further, we examined the progenies of backcrossing and selfing of the 'Uttara' × BC<sub>8</sub> of *S. anguivi* (Table 2). The pollen staining ability and germination ability of the backcrossed one were about 57 % and 33 %. These values almost the same as the parental plant 'Uttara' × BC<sub>8</sub> of *S. anguivi*. This indicates that the fertility of the backcrossed one has not been recovered at all even if eggplant crossed once more. Therefore, it is suggested that depending on the cytoplasmic parents, complete nuclear substitution is difficult by continuous backcrossing with eggplant in the alloplasmic lines.

On the other hand, the pollen staining ability and germination ability of the selfed progenies were found in a recovery trend, which were about 90 % and 42 %, respectively (Table 2). The recovery trend may be since the frequency of homozygosity of the modified eggplant nuclear genes increased through selfing. Of the six wild species surveyed this time, the five wild species,

excluding *S. grandifolium*, have clarified their genetic distances based on ISSR variations (Isshiki et al., 2008). The respective genetic distance between eggplant 'Uttara' and the five wild species is similar in the dendrogram, however, there were differences in cross compatibility. This indicates that the cross incompatibility is not always related to the genetic distance between eggplant and wild *Solanum* species. There was a little cross incompatibility between eggplant and the three wild species, *S. kurzii*, *S. violaceum* and *S. virginianum* (Isshiki and Kawajiri, 2002, Khan and Isshiki, 2008, 2009). On the other hand, there was a considerable cross incompatibility between eggplant and the three wild species, *S. aethiopicum*, *S. anguivi* and *S. grandifolium* (Khan and Isshiki, 2010, 2011, Hasnunnahar, et al., 2012). When F<sub>1</sub> hybrids were made in the first cross to perform cytoplasmic substitution, they showed high hybrid sterility. It is thought that the difference in the presence or absence of the hybrid sterility might be one of a cause of the occurrence of this interesting phenomenon in the present study.

Prevalence of low pollen fertility found in the progenies between eggplant and the cytoplasmic substitution lines of eggplant having *S. aethiopicum*, *S. anguivi* and *S. grandifolium* cytoplasm is the first evidence in *Solanum* to our knowledge. Moreover, similar reports are also scarce in the case of cytoplasmic substitution lines of other vegetables. These findings would be valuable for elucidating speciation in *Solanum*, however, further studies should be needed to reveal the detailed mechanism.

## Materials and Methods

### Plant materials

*Solanum melongena* 'Uttara' was crossed with each of the six cytoplasmic substitution lines using 'Uttara' as a seed parent (cytoplasm parent) and cytoplasmic substitution lines of eggplant as pollen parents (Figs. 1 and 2). Forty seven seedlings of 'Uttara' × the BC<sub>5</sub> of *S. kurzii*, 50 of 'Uttara' × the BC<sub>10</sub> of *S. violaceum*, 69 of 'Uttara' × the BC<sub>5</sub> of *S. virginianum*, 84 of 'Uttara' × the BC<sub>5</sub> of *S. aethiopicum*, 82 of 'Uttara' × the BC<sub>8</sub> of *S. anguivi*, 46 of 'Uttara' × the BC<sub>5</sub> of *S. grandifolium* and their parental lines were used as the experimental materials. Further, 74 crossed seedlings of ('Uttara' × the BC<sub>8</sub> of *S. anguivi*) × 'Uttara' and 69 selfed seedlings of ('Uttara' × the BC<sub>8</sub> of *S. anguivi*) were also used for investigation of the present study.

### Pollen release ability

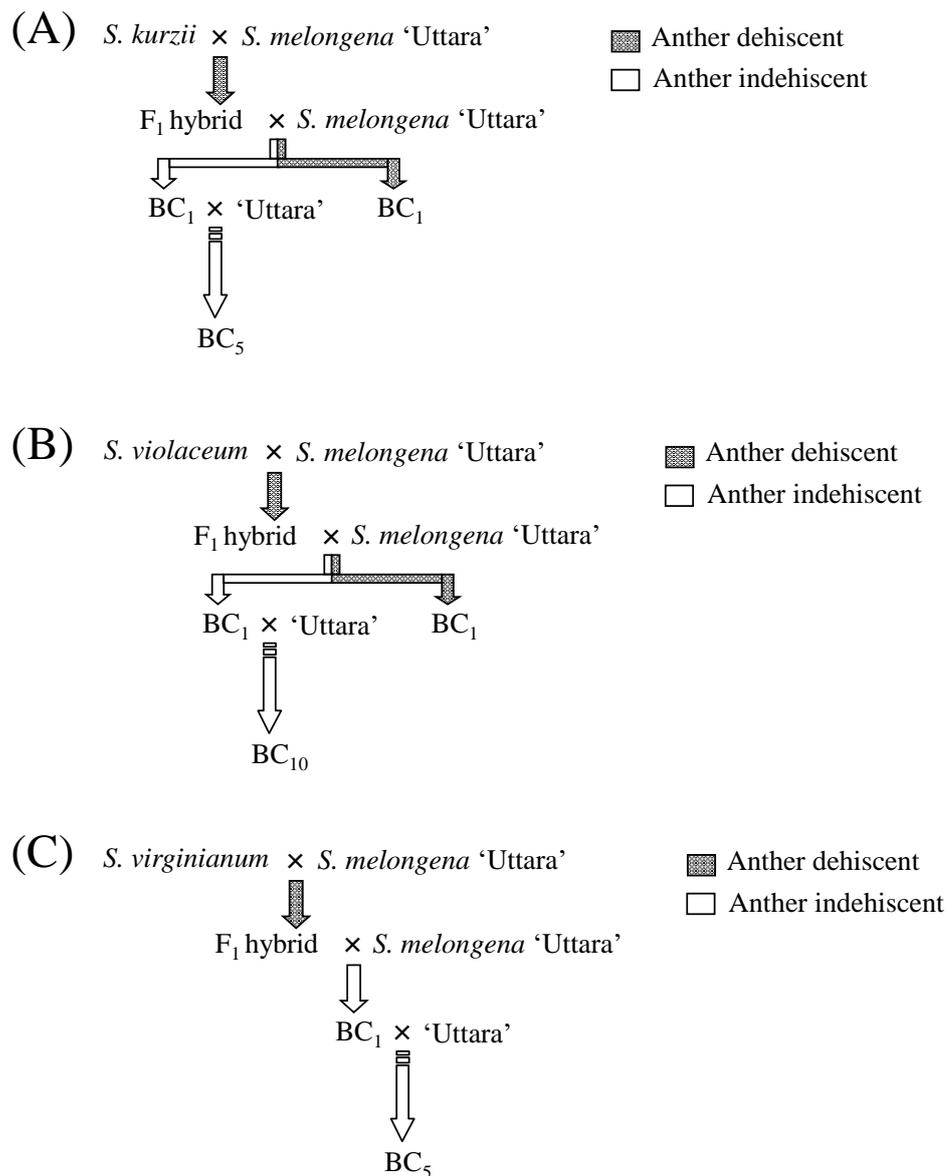
Anther tips were observed under stereomicroscope for assessing pollen release ability. Anthers from 15 flowers were examined for each plant.

### Pollen fertility

The pollen stainability and *in vitro* germination ability of pollen were investigated to assess pollen fertility. Pollen grains from freshly opened flowers were extracted from the anthers by dissection, then smeared in 1% (w/v) acetic carmine to assess their staining ability using the method described by Singh (2002). The *in vitro* germination ability of pollen was investigated according to Singh (2002) with a slight modification using germination medium consisting of 1% (w/v)

**Table 1.** Pollen release ability, pollen stainability and pollen germination ability in the progenies that produced using *Solanum melongena* 'Uttara' as a seed parent and the functional type of CMS lines as a pollen parent.

Plant materials	Pollen release ability	Pollen stainability <sup>1</sup>	Pollen germination ability <sup>1</sup>
<i>Solanum melongena</i> 'Uttara'	+	98.6	72.1
BC <sub>5</sub> of <i>S. kurzii</i>	-	58.2	4.6
BC <sub>10</sub> of <i>S. violaceum</i>	-	41.8	2.8
BC <sub>5</sub> of <i>S. virginianum</i>	-	58.0	4.5
<i>S. melongena</i> 'Uttara' ♀ × BC <sub>5</sub> of <i>S. kurzii</i> ♂	+	92.6±0.9	68.2±0.9
<i>S. melongena</i> 'Uttara' ♀ × BC <sub>10</sub> of <i>S. violaceum</i> ♂	+	95.3±0.3	66.5±0.5
<i>S. melongena</i> 'Uttara' ♀ × BC <sub>5</sub> of <i>S. virginianum</i> ♂	+	93.3±0.4	67.2±0.7



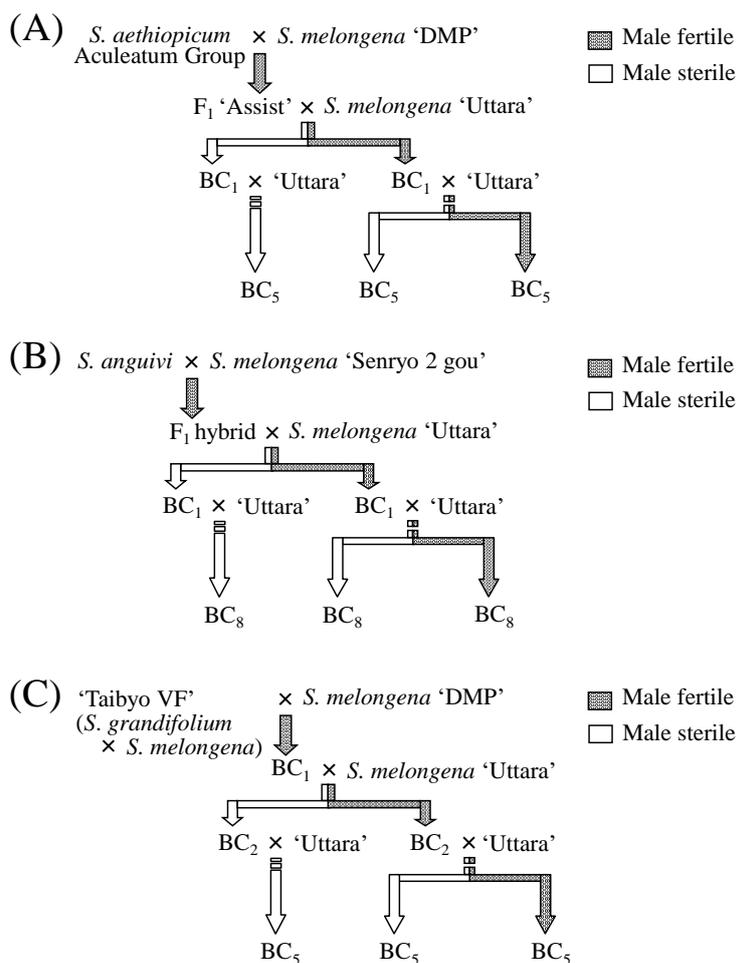
**Fig 1.** Procedure for substituting the cytoplasm of *S. kurzii* (A), *S. violaceum* (B) and *S. virginianum* (C) for that of *S. melongena* 'Uttara' by continuous backcrossing.

<sup>1</sup> Values represent the mean ± SE.

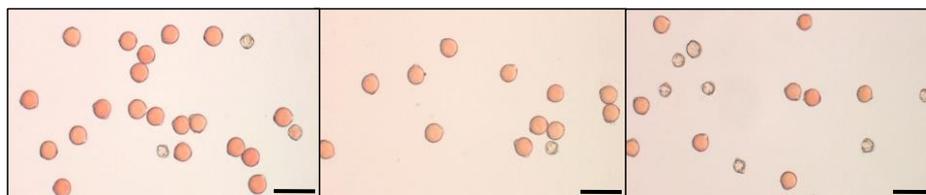
**Table 2.** Pollen release ability, pollen stainability and pollen germination ability in the progenies that produced using *Solanum melongena* ‘Uttara’ as a seed parent and the fertility restored lines of the pollen non-formation type CMS lines as a pollen parent and their further progenies.

Plant materials	Pollen release ability	Pollen stainability <sup>1</sup>	Pollen germination ability <sup>1</sup>
<i>Solanum melongena</i> ‘Uttara’		98.6	72.1
BC <sub>5</sub> of <i>S. aethiopicum</i> (CMS with <i>Rf</i> )	+	57.1	6.0
BC <sub>8</sub> of <i>S. anguivi</i> (CMS with <i>Rf</i> )	+	53.5	25.4
BC <sub>5</sub> of <i>S. grandifolium</i> (CMS with <i>Rf</i> )	+	53.0	1.5
<i>S. melongena</i> ‘Uttara’ ♀ × BC <sub>5</sub> of <i>S. aethiopicum</i> (CMS with <i>Rf</i> ) ♂	+	54.1±0.8	38.2±0.94
<i>S. melongena</i> ‘Uttara’ ♀ × BC <sub>8</sub> of <i>S. anguivi</i> (CMS with <i>Rf</i> ) ♂	+	54.6±0.8	34.4±0.95
<i>S. melongena</i> ‘Uttara’ ♀ × BC <sub>5</sub> of <i>S. grandifolium</i> (CMS with <i>Rf</i> ) ♂	+	53.2±0.1	35.1±0.16
{‘Uttara’ × BC <sub>8</sub> of <i>S. anguivi</i> (CMS with <i>Rf</i> )} ♀ × ‘Uttara’ ♂	+	56.5±1.7	32.9±1.57
Selfing of {‘Uttara’ × BC <sub>8</sub> of <i>S. anguivi</i> (CMS with <i>Rf</i> )}	+	89.6±0.9	41.7±2.14

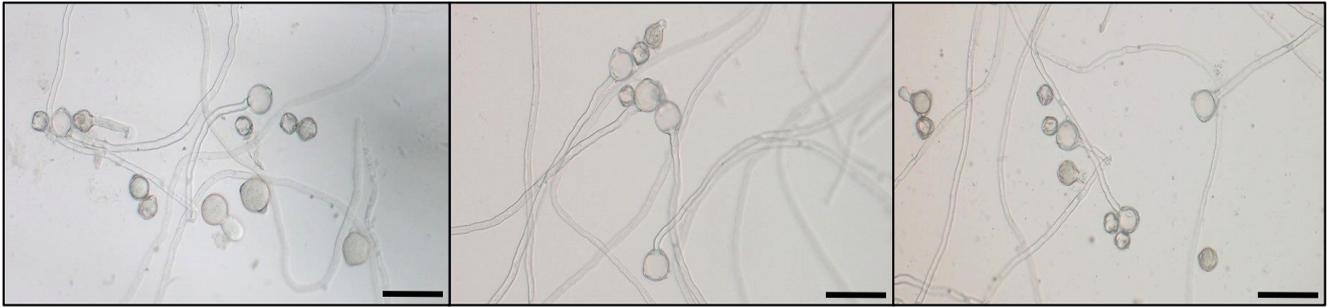
<sup>1</sup>Values represent the mean ± SE.



**Fig 2.** Procedure for substituting the cytoplasm of *S. aethiopicum* Aculeatum Group (A), *S. anguivi* (B) and *S. grandifolium* (C) for that of *S. melongena* ‘Uttara’ by continuous backcrossing.



**Fig 3.** Pollen stainability of *S. melongena* ‘Uttara’ (A), a progeny of ‘Uttara’ × BC<sub>5</sub> of *S. kurzii* (B) and a progeny of ‘Uttara’ × BC<sub>8</sub> of *S. anguivi* (CMS with *Rf*) (C).



**Fig 4.** Pollen germination ability of *S. melongena* 'Uttara' (A), a progeny of 'Uttara' × BC<sub>5</sub> of *S. kurzii* (B) and a progeny of 'Uttara' × BC<sub>8</sub> of *S. anguivi* (CMS with *Rf*) (C).

agar, 5% sucrose and 50 mg l<sup>-1</sup> boric acid.

### Conclusions

Although it is presumed that the progenies produced using *Solanum melongena* eggplant 'Uttara' as a seed parent and the eggplant cytoplasmic substitution lines as pollen parents possess nuclear and cytoplasmic genome almost identical to eggplant 'Uttara', pollen fertility differences in the progenies compared to 'Uttara' might be due to the effect of wild *Solanum* cytoplasm of the pollen parents. In the cytoplasmic substitution lines, cytoplasm of *S. kurzii*, *S. violaceum* and *S. virginianum* had almost no effect on the eggplant nuclear genome. In contrast, cytoplasm of *S. aethiopicum*, *S. anguivi* and *S. grandifolium* had a strong effect which leads some modification in the eggplant nuclear genome. Incompatibility between the normal eggplant cytoplasm and the modified eggplant nuclear genomes of the alloplasmic lines might cause for the low pollen fertility of the investigated progenies.

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