INTRODUCTION

Eggplant (Solanum melongena L.) is an important and popular vegetable crop, especially in Asia (Yamaguchi, 1983). Most commercial cultivars of eggplant are F1 hybrids, as inter-breed hybrids of eggplant develop considerable heterosis, especially in yield (Kakizaki, 1931; Sambandum, 1962). Seed production in eggplant hybrid cultivars is cumbersome, as it requires manual emasculation, pollination, bagging, etc. If it is possible to establish a superior male sterility system in eggplant, it can reduce the labor required to produce F1 hybrid seeds.

In our laboratory, we have developed the cytoplasm substitution lines of eggplant by continuous backcrossing between Solanum kurzii Brace & Prain and eggplant using S. kurzii as cytoplasm donor and eggplant as nucleus one (Khan and Isshiki, 2009). The lines showed the anther indehiscent type (i.e., pollen non-release type) of functional male sterility in the BC1, BC 2, and BC 3 plants, however, the genetic segregation of anther dehiscent and indehiscent types occurred in each of the generation (Khan and Isshiki, 2009).

Cytoplasmic substitution lines of eggplant could be obtained in another method through the amphidiploid. This method restores the fertility of the F1 by chromosome doubling with colchicine treatment following Khan et al. (2013). Through repeated backcrosses to eggplant, using eggplant as the nucleus donor, five backcross generations, the BC1, BC 2, BC 3, BC 4 and BC 5 were produced by backcrossing plants of each generation to eggplant as nucleus donor. Three BC1, six BC 2, five BC 3, 16 BC4 and 12 BC5 plants were used in the present study.

MATERIALS AND METHODS

Plant materials

An interspecific F1 hybrid between S. kurzii and S. melongena ‘Uttara’ was made using S. kurzii as the cytoplasm donor and eggplant as nucleus one (Khan and Isshiki, 2009). The lines showed the anther indehiscent type (i.e., pollen non-release type) of functional male sterility in the BC1, BC 2, and BC 3 plants, however, the genetic segregation of anther dehiscent and indehiscent types occurred in each of the generation (Khan and Isshiki, 2009).

Cytoplasmic substitution lines of eggplant could be obtained in another method through the amphidiploid. This method restores the fertility of the F1 by chromosome doubling with colchicine consequently the backcrossing is often successful. It is a common practice in Brassica vegetables (Kanada and Kato, 1997). In the present study, therefore, we performed cytoplasmic substitution by the way of amphidiploid because this method might be able to accelerate genetic fixation of the anther indehiscent type. Each of the backcross generations was investigated for the fertility traits and the data were compared with the previous ones (Khan and Isshiki, 2009).

Keywords: amphidiploid, anther indehiscent, cytoplasmic male sterility, eggplant, Solanum kurzii

Development of the Functional Male Sterile Line of Eggplant Utilizing the Cytoplasm of Solanum kurzii by Way of the Amphidiploid

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To develop a male sterile eggplant (Solanum melongena L.), cytoplasm substitution lines of eggplant were produced by continuous backcrossing between S. kurzii Brace & Prain and eggplant using S. kurzii as cytoplasm donor and eggplant as nucleus one by way of the amphidiploid. Analyses of chloroplast DNA and mitochondrial DNA confirmed that all the backcross progenies (the BC1, BC 2, BC 3, BC 4, and BC 5) had the cytoplasm from S. kurzii. Anthers in all the backcross progenies were indehiscent type (i.e., pollen non-release type) without segregation. The pollen germination ability of the BC3, BC4 and BC5 plants were about less than 4%. The number of seeds per fruit increased dramatically in the BC1 plants and the average numbers of the BC1, BC 2, and BC 3 plants were about 230 and 360, respectively. The functional male sterility of the anther indehiscent type was shown to be attributed due to incompatibility between the cytoplasm of S. kurzii and nuclear genes of S. melongena. The present CMS plants is promising compared to the one that was backcrossed without going through the amphidiploid, because the CMS is fixed early and the pollen germination ability is extremely low.

Keywords: amphidiploid, anther indehiscent, cytoplasmic male sterility, eggplant, Solanum kurzii

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v(v) before hydrolyzing at 60°C for 10 minutes in 1 N HCl. The root tips were then stained with leucobasic fuschin for 20 minutes and placed separately on a glass slide with the addition of a drop of 45% acetic acid on them. The number of chromosomes in the root tip cells of the amphidiploid was observed and counted under a microscope.

Pollination release ability

Pollens were examined. An anther in which no pollen was released was assessed by tapping the pore end of anthers from open flowers onto a slide and observing the anthers using a stereomicroscope. Anthers from ten flowers of each plant were examined. A pollen released ability (anther dehiscent/indehiscent) was recorded as an anther indehiscent type.

Identification of cytoplasm

The cytoplasm from all the BC₃ plants was identified by analysis of its chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) to confirm maternal inheritance from S. kurzii. Total DNA was extracted from fresh leaves of each plant using the CTAB method, as described by Murray and Thompson (1980). For cpDNA, PCR-RFLP analysis of the region bounded by the conserved sequences in rbcL and ORF106 was conducted, following the method described by Ishiki et al. (1998). For mtDNA, PCR-RFLP analysis of the V7 region of mitochondrial small ribosomal subunit RNA (arRNA) gene was performed using the method described by Yamashita et al. (2000).

RESULTS

The chromosome number of the amphidiploid

The number of chromosomes in the root tip cells of the amphidiploid between S. kurzii and eggplant ‘Uttara’ was confirmed to be 48 (Fig. 1).

Pollination release ability

All the anthers of S. kurzii, S. melongena ‘Uttara’, their F₁ and the amphiploid plants released pollen. Anthers in all the backcross plants were pollen non-release type, i.e., anther indehiscent one, without segregation (Table 1, Fig. 2).

Pollination fertility

The pollen stainability of the backcross generations was low compared to S. kurzii and eggplant ‘Uttara’.

Table 1 Fertility traits in S. kurzii and S. melongena ‘Uttara’, F₁, the amphidiploid and their backcross generations.

| Plant material       | Anther release ability | Pollen stainability (%) | Pollen germination ability (%) | Fruit set (%) | Number of seeds/fruit |
|----------------------|------------------------|--------------------------|--------------------------------|---------------|-----------------------|
| S. kurzii            | +                      | 91.8                     | 78.2                           | 100           | 18.0                  |
| S. melongena ‘Uttara’| +                      | 97.0                     | 87.6                           | 100           | 351.0                 |
| F₁                   | +                      | 31.3                     | 2.2                            | 69.2          | 14.9                  |
| Amphidiploid         | +                      | 45.4                     | 9.9                            | 42.6          | 9.0                   |
| BC₁ (3 plants)       | −                      | 46.5±0.4a/^d^-/          | 4.4±0.1a                        | 25.2±4.2a     | 1.6±0.5a              |
| BC₁ (6 plants)       | −                      | 52.4±4.8a                | 10.7±3.1a                      | 23.7±2.2a     | 4.9±2.6a              |
| BC₂ (5 plants)       | −                      | 51.9±4.1a                | 1.1±0.8a                       | 31.4±5.0a     | 19.2±2.1a             |
| BC₂ (16 plants)      | −                      | 14.8±2.5b                | 0.8±0.96                       | 30.9±3.0a     | 232.3±7.86            |
| BC₃ (12 plants)      | −                      | 32.9±3.0ac               | 2.9±2.0ac                      | 54.1±5.1a     | 361.5±13.0c           |

^/ Values represent the means±SE, and those with the same letters within a column are not significantly different (P<0.05) by Scheffe’s multiple range tests.

Self pollination was performed.
Between the backcross generations, pollen stainability varied. The pollen germination ability of all the backcross generations was extremely low.

**Seed fertility**

Plants of all backcross generations set fruit after hand pollination with eggplant ‘Uttara’ pollen. All the backcross generations did not set any fruit without hand pollination. Fruit set percentage was 20±50% in most of them examined (Table 1). After hand pollination, all fruits of the backcross generations contained seeds. The number of seeds per fruit increased dramatically after the BC3 generation (Table 1). The average numbers of seeds per fruit in the BC4 and BC5 plants were about 230 and 360, respectively, almost the same as in the parental eggplant ‘Uttara’. The mean seed germination rate from four anther indehiscent type of the BC5 plants was 92%, and that of the parental eggplant was 91%.

**Identification of cytoplasm**

All backcross generations displayed the restriction patterns identical to those of *S. kurzii* in PCR-RFLP analyses of both cpDNA (Fig. 3) and mtDNA.

**The data of the previous study**

The data of the previous study (Khan and Isshiki, 2009) were adopted for comparison to those of the present ones. The fertility traits of the previous study were re-posted as Table 2.

**DISCUSSION**

Organelle inheritance in most plants is strictly maternal, although there are some exceptions (Reboud and Zeyl, 1994). CpDNA and mtDNA analyses in the present study showed that all backcross generations examined exhibited restriction patterns identical to that of female parent, *S. kurzii*. This indicates that both the chloroplasts and mitochondria of the backcross generations were derived from *S. kurzii* (Fig. 3). Therefore, the cytoplasm of *S. kurzii* was shown to be maternally inherited as shown in the previous study (Khan and Isshiki, 2009). This confirms that repeated backcross method by way of the amphidiploid is also suitable to develop cytoplasm substitution lines of eggplant.

In cases of true cytoplasmic male sterility (CMS), the degree of male sterility is known to increase with each subsequent backcross generation. This is supported by the data presented in Table 2, which shows a decrease in pollen fertility and male fertility traits across the backcross generations. The mean pollen germination rate decreased from 96.0% in *S. melongena* ‘Uttara’ to 30.0% in BC1, and further to 6.8% in BC5. This decrease in pollen fertility is consistent with the increase in male sterility observed in the backcross generations.

### Table 2 Fertility traits in *S. kurzii* and *S. melongena* ‘Uttara’, F1, their backcross generations not by way of the amphidiploid re-posted (Khan and Isshiki, 2009).

| Plant material | Anther dehiscent/indehiscent | Pollen stainability (%) | Pollen germination ability (%) | Fruit set (%) | Number of seeds/fruit |
|----------------|------------------------------|-------------------------|-------------------------------|---------------|----------------------|
| *S. kurzii*     | +                            | 85.0                    | 82.0                          | 100           | 18.0                 |
| *S. melongena* ‘Uttara’* | +                        | 96.0                    | 89.0                          | 100           | 351.0                |
| F1             | +                            | 30.0                    | 1.0                           | 50.0          | 43.0                 |
| BC1            | +/−                          | 63.3±9.7               | 7.5±3.0                       | 72.5±5.5      | 150.0±37.4           |
| BC2            | +/−                          | 68.3±3.5               | 14.0±5.2                      | 90.0±4.7      | 287.3±69.9           |
| BC3            | +/−                          | 66.0±6.8               | 23.5±10.0                     | 92.5±2.9      | 300.0±51.1           |

*Self pollination was performed.

*Segregation was observed.

*Mean±SE.

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cessive backcross generations (McVetty, 1997). All the present BC1, BC2, BC3, and BC4 plants were the anther indehiscent type (the pollen non-release type) without segregation. From the BC1 to the BC4 generations, the anthers were fixed to the indehiscent character. Therefore, the anther indehiscent character was proposed to be a form of CMS, induced by incompatibility between the cytoplasm of S. kuruvi and the nucleus of eggplant as in the previous study (Khan and Ishiki, 2009). No fertility restorer gene was able to detected in this CMS system. Although all the backcross generations produced pollen grains in their anthers, some malfunctions in the anthers prevented the release to pollen grains.

The segregation of the anther dehiscent and indehiscent types was recognized in all of the BC1, BC2 and BC3 generations reported in the previous study (Khan and Ishiki, 2009). However, in the present study, all of the backcross generations showed the anther indehiscent type without segregation. It is considered that anther indehiscent character was fixed in the BC1 plants. It is suggested that autosynthetic pairing occurred at a high frequency in meiosis of the amphidiploid, and the amphidiploid egg losing accidentally the S. kuruvi chromosome associated to the genetic factors for anther dehiscent fertilized eggplant ‘Uttara’ pollen. So, the BC1 plants did not possess the genetic factors and it is thought that the BC1 plants became the anther indehiscent type as a result. The occurrence of autosynthetic pairing at a high frequency in meiosis of the amphidiploid between eggplant and S. aethiopicum (S. integrifolium) was also reported (Isshiki et al., 2000).

The pollen stainability and germination ability of the backcross generations were no particular trend (Table 1). On the other hand, the number of seeds per fruit increased dramatically in the BC2 generation, whereas those of the BC1, BC2 and BC3 plants were less than 20 (Table 1). This was attributed to go through the amphidiploid in the cytoplasmic substitution. The BC1 plants should be allotriploid and the BC2 and BC3 ones might be aneuploid. So, the fertility of these generations was very low. However, after the BC1 generation, the BC2 and BC3 plants showed the anther indehiscence type as a result. The occurrence of autotetraploid pairing at a high frequency in meiosis of the amphidiploid between eggplant and S. aethiopicum (S. integrifolium) was also reported (Isshiki et al., 2000).

On the other hand, the number of seeds per fruit in the BC1 plants are about 360 and the mean seed germination rate from four individuals in the BC1 plants was 92%. On the other hand, the BC1, BC2 and BC3 plants not by way of the amphidiploid (Khan and Ishiki, 2009) considered to have been 24 of the normal chromosome number and therefore the number of seeds also increased dramatically. The mean number of seeds per fruit in the BC1 plants are about 360 and the mean seed germination rate from four individuals in the BC1 plants was 92%. On the other hand, the BC1, BC2 and BC3 plants not by way of the amphidiploid (Khan and Ishiki, 2009) considered to have been 24 of the normal chromosome number and therefore the number of seeds also increased dramatically. The mean number of seeds per fruit in the BC1 plants are about 360 and the mean seed germination rate from four individuals in the BC1 plants was 92%. On the other hand, the BC1, BC2 and BC3 plants not by way of the amphidiploid (Khan and Ishiki, 2009) considered to have been 24 of the normal chromosome number and therefore the number of seeds also increased dramatically.

The present cytoplasmic substitution by way of the amphidiploid was fixed with anther indehiscent type at the BC4 generation whereas the number of seeds per fruit recovered from the BC4 plants. Therefore, it should be recognized as the male sterile plants from the BC4 generation. On the other hand, the BC4 plants had the same number of seeds but their male sterility is not fixed in the previous study (Khan and Ishiki, 2009). From the point of view of fixing male sterility, the present cytoplasmic substitution should be better than previous study. This study is the first report for cytoplasmic substitution by way of the amphidiploid in eggplant; therefore, fertility traits using more amphidiploids should be collected for more data accumulation.

The pollen germination ability was extremely low and no recovery trend in the succeeding generations was observed in the present study. On the other hand, the pollen germination ability of the previous study (Khan and Ishiki, 2009) was higher than that in the present study. The reason of this difference is unknown. However, the extremely low pollen germination ability is advantageous for the male sterility. Since even if the anthers are releasing pollen grains accidentally, the male sterility can be maintained if there are few pollen grains which shows normal germination. The cytoplasmic substitution lines by way of the amphidiploid of the present study had the stable male sterility of the anther indehiscent type without segregation and extra low pollen germination ability. These two points are the great advantage against those in the previous study (Khan and Ishiki, 2009).

Recently, good parthenocarpy lines of eggplant have been developed by conventional cross-breeding (Kikuchi et al., 2008) and practically a Japanese seed company has started to selling the commercial cultivar with strong parthenocarpy. This parthenocarpy eggplant is not a genetically modified organism (GMO). If the parthenocarpy could be introduced into our CMS line, our lines might be used for not only hybrid seed production but also seedless eggplant production without requiring plant hormone treatment.

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