Australian Journal of Crop Science AJCS
AJCS 6(3):475-479 (2012) ISSN:1835-2707

Inheritance analysis of fertility restoration genes (*Rf*) in a male sterile system of eggplant using cytoplasm of *Solanum grandifolium*

Mst. Hasnunnahar[1], Md. Mizanur Rahim Khan[2], S. Isshiki[2,*]

[1]The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065, Japan
[2]Faculty of Agriculture, Saga University, Saga 840-8502, Japan

[*]Corresponding author: isshiki@cc.saga-u.ac.jp

Abstract

Analysis of pollen and seed fertility of the test cross progenies was performed to assess the inheritance and relevancy of male fertility restoration ability of *Solanum grandifolium*, induced male-sterile eggplant. In this study, 'Taibyo VF' (*S. grandifolium* × *S. melongena*) was continuously backcrossed to recurrent pollen parent *S. melongena*, to develop a cytoplasmic substitution line of eggplant. All the examined BC_1 plants were male fertile while the BC_2 plants segregated into male fertile and sterile types. The BC_3, BC_4, BC_5 and BC_6 progenies, obtained from the male fertile seed parents, were also segregated into the above mentioned types, whereas backcross progenies obtained from male-sterile seed parents were all male-sterile without any segregation. Backcross and selfed progenies obtained from male fertile plants were fitted well to either 1:1 or 3:1 and 3:1 or 15:1 ratios, respectively. The progenies obtained from crossing between male fertile and sterile backcross progenies were fitted to 3:1 ratio. The segregation patterns indicated that two independent dominant fertility restorer (*Rf*) genes control the pollen formation of the *S. melongena* with the cytoplasm of *S. grandifolium*. It is suggested that progenies containing one or both of dominant *Rf* loci will allow pollen formation while recessive *Rf* loci will result the non pollen-forming plants. The pollen stainability was about 50% and 85%, when the male fertile plants pollinated with a pollen donor containing *S. melongena* and *S. grandifolium* cytoplasms, respectively. Knowledge of male fertility restoration inheritance of this Cytoplasmic male sterility (CMS) is useful to develop and utilize the restorer lines in the breeding of eggplant.

Keywords: Cytoplasmic male sterility; eggplant; fertility restoration (*Rf*); *Solanum grandifolium*.
Abbreviations: CMS-Cytoplasmic male sterility; cpDNA-chloroplast DNA; mf-male fertile; ms-male-sterile; mtDNA-mitochondrial DNA.

Introduction

Eggplant (*Solanum melongena* L.) is an economically important vegetable crop in tropical and temperate parts of the world (Kashyap et al., 2003). Most of the inter-varietal hybrids of eggplant reportedly have considerable vigor in economic characters, particularly the yield (Sambandam, 1962). Useful male-sterile (ms) lines could contribute to reduce the costs of labor and hybrid seed production and production of high quality seedless fruits in eggplant (Khan and Isshiki, 2010). There are some reports about male sterility of eggplant. Genic male sterility was reportedly derived by a spontaneous mutation in eggeplant (Jasmin, 1954; Nuttall, 1963; Chauhan, 1984; Phatak and Jaworski, 1989; Phatak et al., 1991). Cytoplasmic male sterility was derived by interspecific crosses, in which wild *Solanum* species was used as female parent followed by repeated backcrossing to the cultivated species to achieve cytoplasm substitution (Fang et al., 1985; Isshiki and Kawajiri, 2002; Khan and Isshiki, 2008; Khan and Isshiki, 2009; Saito et al., 2009; Khan and Isshiki, 2010; Khan and Isshiki, 2011). 'Taibyo VF', an interspecific hybrid between *S. grandifolium* and *S. melongena*, is a commercial rootstock cultivar, resistant to *Verticillium* wilt, released by Takii & Co. Ltd (Kyoto, Japan). Saito et al. (2009) developed the promising pollen non-formation MS lines of eggplant by substituting the cytoplasm of eggplant with 'Taibyo VF', which is indicated to contain the cytoplasm of *S. grandifolium*. They described that this male sterility is cytoplasmic (CMS) which is caused by the cytoplasm of *S. grandifolium*. On the other hand, the fertility restoration is controlled by a single dominant gene. However, they did not describe pollen fertility of the male fertile lines. In our laboratory, we newly developed this ms line of eggplant with *S. grandifolium* cytoplasm by continuous backcrossing method for characterizing this male sterility in detail. We studied pollen and seed fertility of the backcross progenies and distinguished two independent dominant fertility restorer genes, controlling fertility restoration, in contradiction with the results obtained by Saito et al. (2009). In the present study, genetic analysis and investigation of pollen and seed fertility of the test cross progenies were performed to find out the inheritance of male fertility restoration of *S. grandifolium* induced male-sterile eggplant.

Results

Segregation of fertility restoration

All the BC_1 progenies produced pollen grains while the BC_2 progenies were segregated into the male fertile (mf) and sterile (ms) types (Table 1). The ms plants did not produce any pollen grains. The BC_3, BC_4, BC_5, and BC_6 progenies

obtained from the mf seed parents were also segregated into the both types, respectively. The succeeding backcross progenies obtained from mf plants fitted well to either 1:1 or 3:1 ratio (Table 1). On the other hand, the succeeding backcross progenies obtained from ms plants were all ms without segregation. The progenies obtained from crossing between msBC_3No.10 and mfBC_3No.16 backcross progenies fitted to a 3:1 ratio. The selfed progeny of mfBC_3No.16 segregated into the mf and ms types and the segregation ratio fitted well to 15:1 ratio. The selfed progeny of mfBC_4No.18 also segregated and the ratios showed statistically significant deviations from 15:1 and instead fitted to 3:1 ratio.

Pollen stainability and germination ability

Pollen stainability was very high in *S. melongena* 'Uttara' although very low stainable pollen was observed in 'Taibyo VF' (Table 2 and Fig. 2). Pollen stainability differed among backcrossed and selfed progenies and the progenies obtained from crossing between ms and mf BC_3 (Fig. 3). Pollen stainability was found 50% in all the backcrossed progenies except BC_1. There was no tendency for the pollen stainability in the succeeding backcross generations. The selfed progenies showed about 95% pollen stainability (Fig. 3). The progenies obtained by msBC_3 × mfBC_3 showed about 85% pollen stainability. Pollen germination ability in the 'Taibyo VF', backcross and selfed progenies and those progenies obtained from msBC_3 × mfBC_3 were extremely low compared to *S. melongena* 'Uttara'.

Seed fertility

Fruit set percentage, number of seeds per fruit and seed germination rate were high in *S. melongena* 'Uttara' (Table 3). Fruit set percentage in BC_4 progeny was about half of that in *S. melongena* 'Uttara', but it was increased in BC_5 progenies. A high number of seeds per fruit were observed in all backcross progenies, irrespective of male sterility. The seed germination rate was also high in all backcross progenies.

Cytoplasm identification

In the analyses of chloroplast DNA (cpDNA), both of the mf and ms BC_4 and BC_5 plants showed the restriction patterns, identical to those of non-recurrent female parent 'Taibyo VF' (Fig. 4). In the analyses of mitochondrial DNA (mtDNA), also both of the mf and ms BC_4 and BC_5 plants had the restriction patterns identical to those of 'Taibyo VF'. These results confirmed that the chloroplasts and mitochondria of the BC_4 and BC_5 plants have been derived from 'Taibyo VF', which contain the cytoplasm of *S. grandifolium*.

Discussion

In this study, six backcross generations were successfully produced (by continuous backcrossing) to substitute the cytoplasm of *S. melongena* with *S. grandifolium*. In the analyses of both cpDNA and mtDNA, all the BC_4 and BC_5 progenies had the restriction patterns similar to 'Taibyo VF'. These results provide clear evidence for maternal inheritance of cpDNA and mtDNA in the backcross progenies, which confirm the successful substitution of the cytoplasm of *S. grandifolium* in eggplant by backcrossing procedure. Maternal inheritance observed in this study is in accordance with most plant species with strict organelle maternal inheritance (Reboud and Zeyl, 1994). The expected ratios of mf and ms should be 1:1 and 3:1 in backcrossed and selfed progenies, respectively, if the pollen formation is controlled by one dominant gene (Zhang et al., 2010). We found that the segregation patterns of mf and ms in the test cross progenies cannot be explained by one-locus model because 3:1 and 15:1 ratios exist in backcrossed and selfed progenies, respectively. Thus, one-locus model is not suitable and fitted in our case. On the other hand, if the pollen formation is controlled by two dominant genes, the expected ratios of mf and ms should be 3:1 and 15:1 in backcrossed and selfed progenies, respectively. In this study, 3:1 and 15:1 ratios exists in backcrossed and selfed progenies, respectively. Thus, we suggest a two-locus model for the present study. From the segregation patterns, we proposed that, two independent dominant fertility restorer (*Rf*) genes control the pollen formation of the *S. melongena* with the cytoplasm of *S. grandifolium*. It is suggested that the plant contains the *Rf* gene at one or both of these loci allows to form pollen while the recessiveness for both loci results in non pollen forming plants. The segregation patterns for mf and ms in the test crossed progenies indicated that the genotype of the randomly selected mf backcross progenies might be heterozygous at one restorer locus and recessive homozygous at the other locus or heterozygous for the *Rf* genes at both loci. In this study, the BC_2 and BC_3 progenies fitted to 1:1 ratio but BC_4 and BC_5 progenies fitted well to a 3:1 ratio, which is unusual. However, this might be due to a lower number of plants observed in the earlier backcross progenies or due to some unknown reasons. The backcrossed and selfed progenies obtained from mfBC_3No.16 fitted 3:1 and 15:1 ratios, respectively. The progenies obtained by pollination of ms plants with mfBC_3No.16 fitted a 3:1 ratio. It could be concluded that mfBC_3No.16 was heterozygous for the *Rf* genes at both loci. Similar to this, the seed parent of BC_2 and BC_3 also might be heterozygous for the *Rf* genes at both loci. On the other hand, after selfing mfBC_4No.18, the following progenies segregated to a 3:1 ratio, which indicates that mfBC_4No.18 might be heterozygous for the *Rf* gene at one loci and homozygous recessive at the other locus. The ms backcross progenies do not carry any restorer genes and the genotype might be homozygous. Saito et al. (2009) suggested that the fertility restoration of *S. grandifolium* induced CMS is controlled by a single dominant nuclear gene (*Rf*), whereas from our study it is revealed that two independent dominant fertility restorer genes controlled fertility restoration. The dissimilar findings of number of *Rf* gene(s) in the present study with results obtained by Saito et al. (2009) might be due to loss of *Rf* gene from one locus before their systematic genetic analysis. Pollen non-formation type CMS lines of eggplant induced by the cytoplasm of *S. anguivi* (Khan and Isshiki, 2011) and *S. aethiopicum* (Khan and Isshiki, 2010) were reported to be controlled by two independent dominant fertility restorer (*Rf*) genes for fertility restoration. Fertility restoration controlled by two dominant genes has also been reported in several plant species such as *tour* CMS in *Brassica* (Pahwa et al., 2004); kos CMS in radish (Koizuka et al., 2000); WA, Dissi and Gambiaca CMS in rice (Sattari et al., 2008). The pollen stainability of all the mfBC_4 and selfed progenies of mfBC_3No.16 was about 50% and 95%, respectively. In the backcrossing, the cytoplasm of pollen donor plant was origintaed from *S. melongena*.

Table 1. Segregation for male fertile (mf) and sterile (ms) plants in the test crossed progenies.

Plant materials	No. of plants		Expected ratio (mf : ms)	χ^2	*P*	Expected ratio (mf : ms)	χ^2	*P*
	mf	ms						
BC_1 ('Taibyo VF' × 'DMP')	2	0	-[1]	-	-	-	-	-
BC_2 ($mfBC_1$No.1 ×'Uttara')	6	5	1 : 1	0.09	0.76	3 : 1	2.45	0.12
BC_3 ($mfBC_2$No.9 ×'Uttara')	9	12	1 : 1	0.43	0.51	3 : 1	11.57	0.00
BC_4 ($mfBC_3$No.16 ×'Uttara')	41	17	1 : 1	9.93	0.00	3 : 1	0.57	0.45
BC_5 ($mfBC_4$No.3 ×'Uttara')	46	18	1 : 1	12.25	0.00	3 : 1	0.33	0.56
BC_6 ($mfBC_5$No.27 ×'Uttara')	18	20	1 : 1	0.11	0.75	3 : 1	15.47	0.00
BC_4 ($msBC_3$No.10 ×'Uttara')	0	11	0 : 1	-	-	-	-	-
BC_5 ($msBC_4$No.4 ×'Uttara')	0	11	0 : 1	-	-	-	-	-
$MsBC_3$No.10× $mfBC_3$No.16	20	6	1 : 1	7.54	0.01	3 : 1	0.05	0.82
Selfed progeny of $mfBC_3$No.16	19	2	3 : 1	2.68	0.10	15 : 1	0.38	0.54
Selfed progeny of $mfBC_4$No.18	40	9	3 : 1	1.14	0.28	15 : 1	12.28	0.00

[1]Not investigated.

Table 2. Pollen fertility in the *S. melongena* 'Uttara', 'Taibyo VF' (*S. grandifolium* × *S. melongena*), backcrossed progenies, selfed progenies and the progenies obtained from crossing between male-sterile (ms) and male fertile (mf) BC_3 progenies.

Plant material	Pollen stainability (%)	Pollen germination ability (%)
S. melongena 'Uttara'	95.44 ± 1.0[1]	85.46 ± 1.9
'Taibyo VF'	7.12 ± 1.32	4.01 ± 0.73
BC_1 ('Taibyo VF' × DMP)	26.11 ± 7.23	13.56 ± 3.01
BC_2 ($mfBC_1$No.1 ×'Uttara')	51.32 ± 0.94	4.8 ± 0.76
BC_3 ($mfBC_2$No.9 ×'Uttara')	55.05 ± 1.41	3.35 ± 0.37
BC_4 ($mfBC_3$No.16 ×'Uttara')	53.08 ± 1.16	3.09 ± 0.42
BC_5 ($mfBC_4$No.3 ×'Uttara')	51.38 ± 0.78	1.87 ± 0.22
$MsBC_3$No.10× $mfBC_3$No.16	85.78 ± 1.44	2.45 ± 0.64
Selfed progeny of $mfBC_3$No.16	95.29 ± 0.31	3.30 ± 0.47
Selfed progeny of $mfBC_4$No.18	94.00 ± 0.38	2.98 ± 0.23

[1]Values represent the mean ± SE.

Table 3. Seed fertility in the *S. melongena* 'Uttara', 'Taibyo VF' (*S. grandifolium* × *S. melongena*) and backcrossed progenies.

Plant material		Fruit set (%)	Number of seeds per fruit	Seed germination rate (%)
S. melongena 'Uttara'		100	532 ± 68.6[1]	91
'Taibyo VF'		17.7	2.7	-[2]
BC_2	1 (male fertile)	84.6 ± 10.9	137.2 ± 29.0	-
	2 (male-sterile)	84.6 ± 5.4	148.2 ± 85.6	-
BC_3	1 (male fertile)	45.5 ± 2.8	259.8 ± 50.3	92
	2 (male-sterile)	60.0 ± 17.0	207.2 ± 29.5	91
BC_4	1 (male fertile)	63.9 ± 9.0	239.6 ± 136.6	78
	2 (male-sterile)	50.0 ± 23.6	510.5 ± 12.0	90
BC_5	1 (male fertile)	72.2 ± 18.0	215.8 ± 32.3	96
	2 (male-sterile)	75.0 ± 35.4	343.5 ± 3.5	98

[1]Values represent the mean ± SE. [2]Not investigated.

However, in selfing, the cytoplasm of pollen donor plant was from *S. grandifolium*. Hence, the difference of pollen stainability in backcross and selfed progenies might be due to the difference in the cytoplasm of pollen donor plant instead of the genotype of *Rf* genes. Cytoplasm of pollen donor plant might be attributed to the difference in pollen stainability between backcross and selfed progenies. High fruit set, number of seeds per fruit and seed germination rate found in the BC_5 progenies indicate that the cytoplasm of *S. grandifolium* has no notable negative effect on seed fertility of eggplant. High seed fertility was also found in the *S. grandifolium* induced CMS line by Saito et al. (2009). Inheritance of male fertility restoration of *S. grandifolium* induced ms line in the present study may lead to a more detailed evaluation of this male sterility system in eggplant. In conclusion, the male sterility and fertility restoration in the present case would potentially be helpful to characterize the CMS-*Rf* system and may contribute to develop and utilize the restorer lines in the breeding programs of eggplant. During the last few decades, the use of molecular markers, revealing polymorphism at the DNA level, is playing important role in plant biotechnology and genetics studies (Kumar et al., 2009). Molecular elucidation of this male sterility and fertility restoration system is needed for further study.

Materials and methods

Production of cytoplasmic substitution line

To develop a cytoplasmic substitution line of eggplant, 'Taibyo VF' (*S. grandifolium* × *S. melongena*) was continuously backcrossed to recurrent pollen parent *S. melongena*. Six backcross generations, BC_1, BC_2, BC_3, BC_4, BC_5 and BC_6 were produced (Fig. 1). In backcrossing, progenies were selected in the two directions of male fertile

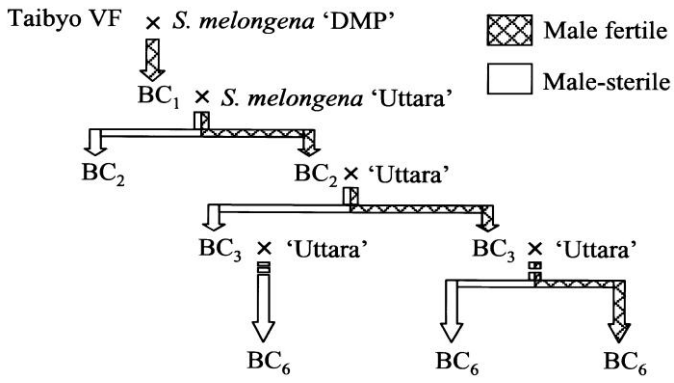

Fig 1. A schematic procedure for substitution of cytoplasm of *S. grandifolium* with that of *S. melongena* 'Uttara' by continuous backcrossing. Progenies were selected in the two directions of male fertile and sterile types during backcrossing.

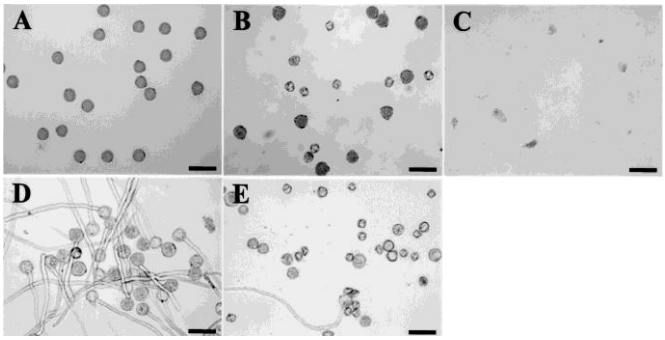

Fig 2. Acetocarmine-stained (A-C) and *in vitro* germinated pollens (D-E). *Solanum melongena* 'Uttara' (A and D), a male fertile BC_5 plant (B and E) and a male-sterile BC_5 plant (C). Scale bar = 50 µm.

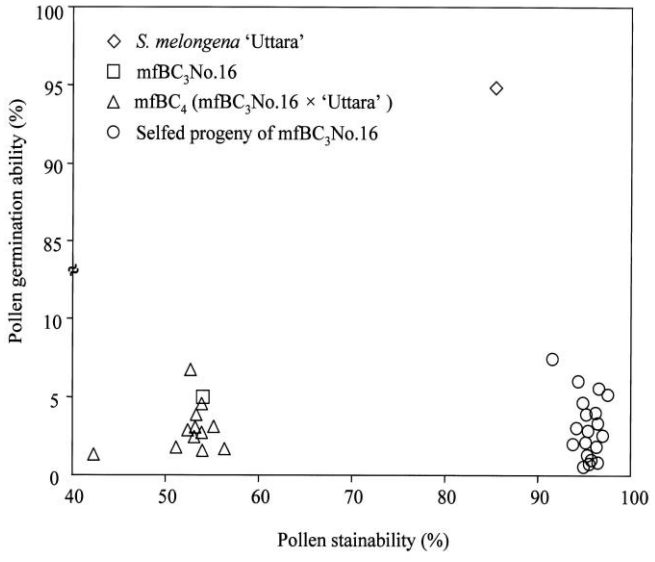

Fig 3. Comparison of pollen stainability and germination ability of *S. melongena* 'Uttara', $mfBC_3No.16$, $mfBC_4$ and selfed progeny of $mfBC_3No.16$.

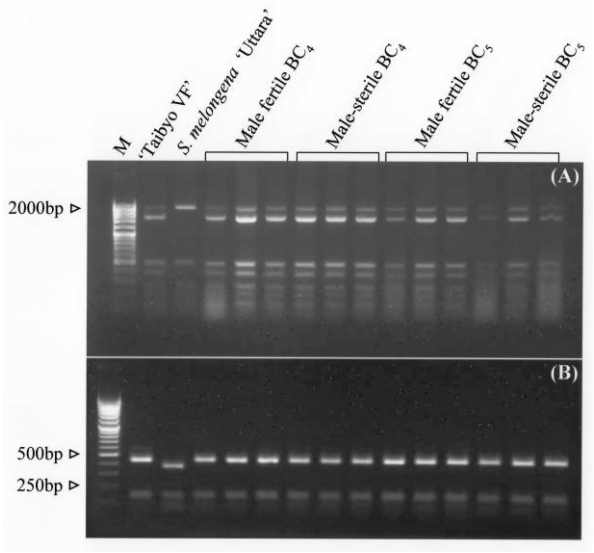

Fig 4. Restriction patterns of the (A) *Alu*I digested *rbc*L-ORF106 region of cpDNA and the (B) *Scr*F I digested V7 region of mitochondrial small ribosomal subunit RNA gene in 'Taibyo VF', *S. melongena* 'Uttara', and the male fertile and sterile BC_4 and BC_5 plants. The male fertile and sterile BC_4 and BC_5 plants had the restriction patterns identical to those of 'Taibyo VF' indicating maternal inheritance of both cpDNA and mtDNA. M is a 0.1-2 kbp DNA ladder.

(pollen formation) and sterile (pollen non-formation) types and both types of the backcross progenies at each generation were continuously backcrossed to 'Uttara', respectively (Table 1 and Fig. 1). Furthermore, selfed progenies of the mf BC_3 and BC_4, and the progenies obtained from crossing between ms and mf BC_3 progenies were also produced for genetic analysis of fertility restoration systems. All the plant materials were cultivated in pots in a glasshouse during summer season from April to November. Minimum and maximum temperatures for that period were 15 ºC and 38 ºC, respectively. All unopened flower buds were emasculated, bagged, and then hand pollinated during crossing to avoid unintentional pollination (Khan and Isshiki, 2011).

Pollen formation ability

To investigate pollen formation ability, anthers from freshly opened flowers were squashed in acetocarmine and examined for presence of pollen (Fig. 2). Anthers from 15 flowers were examined for each plant material. The plants that did not produce any pollen grains were defined as male-sterile ones.

Pollen fertility

Stainability of pollen with acetocarmine and *in vitro* germination rate of pollen were investigated for assessing the fertility of pollen in the mf plants. Pollen grains were extracted by dissecting anthers from freshly opened flowers. Pollen stainability was determined by staining fresh pollen in a drop of acetocarmine solution using the method described by Singh (2002). *In vitro* germination of pollen was determined by using a germination medium consisted of 1% agar, 5% sucrose and 50 mg/l boric acid. Germination rate was determined after incubation at 25ºC for four hours. At least 500 pollen grains per

flower were observed in 5 flowers per plant for assessing pollen fertility. One plant of *S. melongena* 'Uttara' and 'Taibyo VF', two BC_1, six BC_2, nine BC_3, 13 BC_4, 19 BC_5, seven progenies from the cross $msBC_3$No.10 × $mfBC_3$No.16, 19 progenies from selfing $mfBC_3$No.16 and 36 progenies from selfing $mfBC_4$No.18 were examined for pollen fertility.

Seed fertility

Seed fertility was estimated from the fruit set percentage, number of seeds per fruit and seed germination rate. The F_1 hybrid and the backcross plants were pollinated with the pollen of *S. melongena* 'Uttara'. The *S.melongena* 'Uttara' was selfed. At least 10 flowers for each plant were hand pollinated. For seed germination, seeds were sown in soil in a glass house with controlled minimum and maximum temperatures of approximately 15ºC and 30ºC, respectively. Seed germination was recorded 30 days after sowing.

Analyses of organelle DNAs

To identify the cytoplasm of backcross progenies, cpDNA and mtDNA were analyzed in 'Taibyo VF', *S. melongena* 'Uttara' and 6 plants from each of the BC_4 and BC_5 progenies. Total DNA was isolated from fresh leaves using the CTAB method described by Murray and Thompson, (1980). The cpDNA was analyzed by RFLP analysis of a PCR amplified region between *rbc*L and ORF106 following the method described by Isshiki et al. (1998). Sequences of the primers for the PCR amplification were 5'-ATGTC-ACCACAAACAGAAACTAAAGCAAGT-3' (*rbc*L) and 5'-ACTACAGATCTCATACTACCCC-3' (ORF106). The PCR product was digested with restriction enzyme *Alu* I. The mtDNA was analyzed by RFLP analysis of a PCR amplified V7 region of mitochondrial small ribosomal subunit RNA gene following the method described by Khan and Isshiki (2008). Sequences of the primers for the PCR amplification were 5'-TATGAACAACAAAACCTGTCTT-TAACGGGATGG-3' ($mtV7_{P1}$) and 5'-GCGGACTTGACG-TCATCCCCCACCTTCCTCCAG-3' ($mtV7_{P2}$). The PCR product was digested with *Scr*FI. The digested PCR products both of cpDNA and of mtDNA were electrophoresed on 1.5% agarose gel containing ethidium bromide and detected under a UV transilluminator.

Statistical analyses

A chi-square ($\chi 2$) goodness of fit test was performed on the test cross progenies against a possible theoretical segregation ratio using the formula: $\chi 2 = \Sigma (O - E)^2 / E$, where O and E are the observed and expected values, respectively (Steel and Torrie, 1980).

Acknowledgments

This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN), Japan.

References

Chauhan SVS (1984) Studies in genic male-sterile *Solanum melongena* L. Indian J Genet 44:367–371

Fang M, Mao R, Xie W (1985) Breeding of cytoplasmically inherited male sterile lines of egg-plant (*Solanum melongena* L.). Acta Hortic Sin 12: 261–266

Isshiki S, Uchiyama T, Tashiro Y, Miyazaki S (1998) RFLP analysis of a PCR amplified region of chloroplast DNA in eggplant and related *Solanum* species. Euphytica 102:295–299

Isshiki S, Kawajiri N (2002) Effect of cytoplasm of *Solanum violaceum* Ort. on fertility of eggplant (*S. melongena* L.). Sci Hortic 93:9–18

Jasmin JJ (1954) Male sterility in *Solanum melongena* L.: preliminary report on a functional type of male sterility in eggplants. Proc Amer Soc Hort Sci 63: 443

Kashyap V, Kumar SV, Collonnier C, Fusari F, Haicour R, Rotino GL, Sihachakr D, Rajam MV (2003) Biotechnology of eggplant. Sci Hortic 97:1–25

Khan MMR, Isshiki S (2008) Development of a male sterile eggplant by utilizing the cytoplasm of *Solanum virginianum* and a biparental transmission of chloroplast DNA in backcrossing. Sci Hortic 117:316–320

Khan MMR, Isshiki S (2009) Functional male-sterility expressed in eggplant (*Solanum melongena* L.) containing the cytoplasm of *S. kurzii* Brace & Prain. J Hortic Sci Biotech 84:92–96

Khan MMR, Isshiki S (2010) Development of the male-sterile line of eggplant utilizing the cytoplasm of *Solanum aethiopicum* Aculeatum Group. J Jpn Soc Hortic Sci 79:348–353

Khan MMR, Isshiki S (2011) Development of a cytoplasmic male sterile line of eggplant (*Solanum melongena* L.) with the cytoplasm of *S. anguivi*. Plant Breeding 130:256–260

Koizuka N, Imai R, Iwabuchi M, Sakai T, Imamura J (2000) Genetic analysis of fertility restoration and accumulation of ORF125 mitochondrial protein in the kosena radish (*Raphanus sativus* cv. Kosena) and a *Brassica napus* restorer line. Theor Appl Genet 100:949–955

Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK (2009) Potential of Molecular Markers in Plant Biotechnology. Plant Omics J 2:141–162

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nuttall VW (1963) The inheritance and possible usefulness of functional male sterility in *Solanum melongena* L. Can J Genet Cytol 5:197–199

Pahwa RS, Banga SK, Gogna KPS, Banga SS (2004) Tournefortii male sterility system in *Brassica napus*. Identification, expression and genetic characterization of male fertility restorers. Plant Breeding 123:444–448

Phatak SC, Liu J, Jaworski CA, Sultanbawa AF (1991) Functional male sterility in eggplant: inheritance and linkage to the purple fruit color gene. J Hered 82, 81–83.

Phatak SC, Jaworski CA (1989) UGA 1-MS male-sterile eggplant germplasm. HortScience 24:1050

Reboud X, Zeyl C (1994) Organelle inheritance in plants. Heredity 72:132–140

Saito T, Matsunaga H, Saito A, Hamato N, Koga T, Suzuki T, Yoshida T (2009) A novel source of cytoplasmic male sterility and a fertility restoration gene in eggplant (*Solanum melongena* L.) lines. J Jpn Soc Hortic Sci 78:425–430

Sambandum CN (1962) Heterosis in eggplant (*Solanum melongena* Linn.) prospects and problems in commercial production of hybrid seeds. Econ Bot 16:71–76

Sattari M, Kathiresan A, Gregorio G, Virmani SS (2008) Comparative genetic analysis and molecular mapping of fertility restoration genes for WA, Dissi, and Gambiaca cytoplasmic male sterility systems in rice. Euphytica 160:305–315

Singh RJ (2002) Plant Cytogenetics. 2nd edn. CRC Press, pp 21-22

Steel RGD, Torrie JH (1980) Principles and procedures of statistics: A biometrical approach. New York, McGraw-Hill

Zhang X, Wang X, Jiang P, Zhu W (2010) Inheritance of fertility restoration for cytoplasmic male sterility in a new *Gossypium barbadense* restorer. Agricultural Sciences in China 9:472–476