Production of Amphidiploids of the Hybrids between *Solanum macrocarpon* and Eggplant

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Abstract. To restore fertility of the F₁ between *Solanum macrocarpon* and eggplant, amphidiploids were produced through doubling of chromosomes by colchicine treatment. Shoot tips and axillary buds of F₁ plants were kept for 2 and 4 days in liquid Murashige and Skoog (MS) media with 0.05% colchicine. As a result of colchicine treatment, two amphidiploids were identified by observing root tip cell chromosome number, stomatal guard cell size, and pollen characteristics. The amphidiploids contained 48 chromosomes, twice the normal diploid number of 24. Stomata size and pollen diameter were significantly larger in amphidiploids than F₁. Flower diameter and length and width of anther, petal, and sepal were significantly larger in the amphidiploids than in F₁. Pollen stainability was 40% in amphidiploids but only 0.86% in the diploid F₁. The amphidiploids, after selfing and backcrossing with *S. macrocarpon*, set fruits with healthy seeds. Therefore, production of amphidiploids by colchicine treatment restored the pollen and seed fertility of F₁ between *S. macrocarpon* and eggplant. The amphidiploids produced in this study would be very useful in future breeding programs of eggplant.

The gboma eggplant, *Solanum macrocarpon*, belongs to the subgenus *Leptostemonum*, section *Melongena*, and series *Macrocarpon*. It was domesticated in Africa from the wild savanna species *S. dasyphyllum* (Lester et al., 1990). The leaves of *S. macrocarpon* are cooked like spinach and the fruits are stewed (Lester, 1998). The cultivated eggplant (*S. melongena*) is reported to be susceptible to the shoot and fruit borer (*Leucinodes orbonalis*) and spider mite (*Tetranychus urticae* Koch), whereas *S. macrocarpon* is reported as resistant (Bletsos et al., 2004; Gowda et al., 1990; Schaff et al., 1982). Interspecific hybridization is of great significance to incorporate desirable genes into the cultivated species. So, hybridization of the two species may provide a practical way of transferring resistance into eggplant cultivars. Although hybrids between *S. macrocarpon* and eggplant have been reported, they were sterile (Gowda et al., 1990; Magoon et al., 1961)

and produced either seedless fruits (Wanjari, 1976) or fruits with a few seed (Schaff et al., 1982). Formerly, we developed F₁ hybrids between *S. macrocarpon* and eggplant; however, those hybrids were completely sterile and set no seed on selfing or backcrossing. One of the common ways to restore fertility to many interspecific hybrids is to double their chromosomes artificially through colchicine treatment and production of relatively fertile amphidiploids. Restoration of fertility of sterile hybrids between eggplant and its related species, *S. violaceum* Ort. (*S. indicum* auct. non L.), *S. virginianum* L. (*S. surattense* Burm. f.), and *S. aethiopicum* Aculeatum Group (*S. integrifolium* Poir.), and *S. aethiopicum* L. Gilo Group (*S. gilo* Raddi), was achieved by chromosome doubling, and the progenies of these synthetic amphidiploids were studied for selection of economic types (Ali et al., 1992; Isshiki et al., 2000; Rajasekaran, 1970, 1971; Toppino et al., 2008a, 2008b). However, chromosome doubling has not been investigated yet in the interspecific hybrid between *S. macrocarpon* and eggplant, although it may have a great potential for practical breeding. The purpose of this study was therefore to restore fertility of the F₁ between *S. macrocarpon* and eggplant through chromosome doubling by colchicine treatment.

Induction of amphidiploids. Shoot tips and axillary buds of 1 to 3 mm were collected from plantlets of F₁ hybrids between *S. macrocarpon* (Accession BIRM S.0133) and *S. melongena* 'Uttara' regenerated in vitro on hormone free MS medium (Murashige and Skoog, 1962), which was supplemented with 10 g·L⁻¹ sucrose adjusted to pH 5.5 and solidified using 2.5 g·L⁻¹ Gelrite. The culture was maintained in a culture room under light (170 μmol·m⁻²·s⁻¹) for 16 h·d⁻¹ at 25 °C.

Filter-sterilized 0.05% colchicine solution was added in autoclaved liquid MS medium supplemented by 5 g·L⁻¹ sucrose at pH 5.5. Each 100-mL conical flask contained 30 mL medium and three explants. Five conical flasks were put on a rotary shaker having 100 strokes/min for each duration of 2 and 4 d in the culture room under the prescribed light and temperature conditions. After treatment, the explants were washed thoroughly with sterilized distilled water and then cultured on basal MS medium supplemented with 10 g·L⁻¹ sucrose and 0.5 mg·L⁻¹ 6-benzylaminopurine before solidifying by using 2.5 g·L⁻¹ Gelrite for shoot induction. After one month, the shoots were transferred to MS medium supplemented by 30 g·L⁻¹ sucrose and 3 mg·L⁻¹ indole-3-butyric acid and solidified by using 8 g·L⁻¹ agar for root induction. After another month, the rooted plantlets were transferred to pots filled with vermiculite and kept in the culture room for 2 weeks before the plants were transferred to a glasshouse.

Identification and characterization of amphidiploids. The number of chromosomes in the root tip cells, size of the stomatal guard cells, and pollen characteristics of F₁ and regenerated plants were investigated for ploidy determination. The ploidy levels of histogenic layers L_I, L_{II}, and L_{III} of the amphidiploids were identified on the basis of stomatal guard cell size, pollen characteristics, and root tip cell chromosome number, respectively, following Hermesen et al. (1981).

Roots were cut ≈1 cm from the tips and pretreated with 0.1% colchicine for 2.5 h at 20 °C and kept in the mixture of acetic acid and ethyl alcohol (1:3 v/v) before hydrolyzing at 60 °C for 10 min in 1 N HCL. The root tips were then stained with leucobasic fuchsin for 20 min and placed separately on a glass slide with the addition of a drop of 45% acetic acid on them. A coverslip was placed over the root tip and gentle pressure was applied to the coverslip to smear the root tip. The number of chromosomes in the root tip cells was observed and counted under a microscope.

The size of stomatal guard cells was measured from five well-expanded leaves of each plant. Three samples of epidermal cells were collected from the lower surface (abaxial side) by using the nail varnish technique. A small area of abaxial side of the leaves was covered with a thin layer of clear nail polish and left to dry. A piece of cellophane tape was firmly attached to one end of the nail

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polish and the polish was carefully pulled off the leaf by the tape. Each peel with tape was placed on microscope slides, then covered with a cover glass and observed under a microscope. Guard cells were randomly selected and their length and width were measured in units of an ocular micrometer that was calibrated with a calibration slide.

Pollen stainability in acetocarmine, pollen diameter of stained pollen, and in vitro germination ability of pollen were investigated for assessing the pollen characteristics. Pollen grains from freshly opened flowers were extracted from the anthers by dissection and then smeared in 1% acetocarmine to assess their staining ability following Singh (2002). In vitro germination ability of pollen was also investigated following Singh (2002) with a slight modification using a germination medium consisting of 1% agar, 5% sucrose, and 50 mg·L⁻¹ boric acid. The ability of pollen to germinate was determined after incubation for 4 h at 25 °C. For each plant, over 500 pollen grains were examined for assessing pollen fertility.

Flower characteristics. For flower diameter, we measured the distance between distal ends of two opposite petals. Petal and sepal

Fig. 1. Somatic chromosomes from a root tip of the amphidiploid ($2n = 4x = 48$) between *Solanum macrocarpon* and *S. melongena* 'Uttara'.

lengths were measured from the base of the receptacle to the apex and width was measured at the widest point of each of two opposite petals and sepals, respectively. Ten flowers from each plant were examined to study flower characteristics.

Seed fertility. Seed fertility was assessed by investigating fruit set, number of seeds per fruit, and seed germination rate. For these investigations, hand pollination was done. Seeds were sown in soil in a glass house with controlled temperature ranging from 15 to 30 °C. The germination rate of seeds was recorded 30 d after sowing.

Results and Discussion

Colchicine treatment resulted in the identification of two expected amphidiploids both for 2 and 4 d duration through observation of chromosome number in the root tip cells. The four expected amphidiploids ($2n = 4x = 48$) had 48 chromosomes (Fig. 1) in the root tips, whereas F_1 hybrid and parental species ($2n = 2x = 24$) had 24 chromosomes.

Among the four amphidiploids, the stomatal length and width and pollen diameter in amphidiploids No. 1 and 4 (Table 1) were significantly larger compared with that in F_1 and parental species. This result is in conformity with previous works (Griesbach and Bhat, 1990; Kadota and Niimi, 2002), who reported larger stomata of the induced amphidiploids and described this as a simple diagnostic feature for identifying the potential amphidiploids.

The pollen stainability was quite high both for *S. macrocarpon* and eggplant but very low for their F_1 (Table 1). It, however, increased significantly in amphidiploids No. 1 and 4 over that of the F_1 . Low pollen fertility in F_1 between *S. melongena* and several related *Solanum* species was also reported (Isshiki and Kawajiri, 2002; Rajasekaran,

1970; Rangasamy and Kadambavanasundaram, 1974). A number of investigators (Gowda et al., 1990; Magoon et al., 1961; Schaff et al., 1982; Wanjari, 1976) even reported sterility of F_1 between *S. macrocarpon* and eggplant. However, after doubling of chromosomes, the pollen fertility of F_1 was restored in this study that was high enough to fertilize its own ovules and to produce viable seeded fruits. It is well known that the allotetraploid status restored fertility in several other sexual interspecific *Solanum* hybrids (Rajasekaran, 1970, 1971; Rao, 1981).

Among the four amphidiploids, flower diameter and length and width of anther, petal, and sepal were significantly larger in amphidiploids No. 1 and 4 compared with that in F_1 (Table 2). From the observation of stomatal guard cell size, pollen characteristics, and root tip cell chromosome number, it is revealed that amphidiploids No. 1 and 4 had $4x$ ploidy in all three layers, whereas amphidiploid No. 2 and 3 were chromosome chimeras (Table 3). Similar results of chimeric plants developed by colchicine treatment were also found by Ali et al. (1992). Therefore, among the four expected amphidiploids, two were confirmed amphidiploids, one for each of 2 and 4 d duration of colchicine treatment.

The F_1 hybrid did not set any seed by selfing and backcrossing. The induced amphidiploid No. 1 set seeded fruits by selfing and by pollinating with the pollen of *S. macrocarpon* (Table 4). The seeds of the amphidiploid of both selfing and backcrossing germinated normally. Moreover, after selfing and backcrossing, fruiting with viable seeds confirmed that the pollen and seed fertility were restored in the identified amphidiploids. Similar results of fertility restoration of sterile hybrids between eggplant and related species were reviewed in the past (Ali et al., 1992; Isshiki et al., 2000; Isshiki and Taura, 2003; Rajasekaran, 1970, 1971; Toppino et al., 2008a, 2008b).

The amphidiploids developed in our experiment have created a new genetic material with the double genomes of both eggplant and *S. macrocarpon*. The high fertility of our amphidiploids can be attributed to the allotetraploid status of the nuclear genomes. As a result of high pollen fertility, obtaining dihaploids of our synthetic amphidiploids might be possible by anther culture. This dihaploids might be backcrossed to recurrent eggplant to introgress of resistance traits derived from *S. macrocarpon* into the cultivated eggplant. Dihaploids obtained by anther culture

Table 1. Chromosome number, stomatal guard cell size, and pollen characteristics of the F_1 between *Solanum macrocarpon* and *S. melongena* 'Uttara' and its amphidiploids.

Plant materials	Chromosome number ($2n$)	Stomatal guard cell size (μm)		Pollen diam (μm)	Pollen stainability (%)
		Length	Width		
<i>S. macrocarpon</i>	24	20.54 $\pm$ 0.28 a ²	14.91 $\pm$ 0.17 a	29.98 $\pm$ 0.16 a	96.04 $\pm$ 0.61 a
<i>S. melongena</i> 'Uttara'	24	22.22 $\pm$ 0.39 a	16.44 $\pm$ 0.24 ac	27.68 $\pm$ 0.13 b	95.44 $\pm$ 1.01 a
F_1	24	22.12 $\pm$ 0.51 a	14.93 $\pm$ 0.26 a	24.22 $\pm$ 0.24 c	0.86 $\pm$ 0.20 b
Amphidiploid No. 1	48	29.10 $\pm$ 0.54 b	19.90 $\pm$ 0.46 b	35.32 $\pm$ 0.27 d	40.28 $\pm$ 2.26 c
Amphidiploid No. 2	48	27.09 $\pm$ 0.46 b	17.90 $\pm$ 0.37 c	28.11 $\pm$ 0.33 b	3.22 $\pm$ 1.22 b
Amphidiploid No. 3	48	21.83 $\pm$ 0.50 a	16.85 $\pm$ 0.42 c	25.18 $\pm$ 0.19 c	2.72 $\pm$ 0.55 b
Amphidiploid No. 4	48	32.68 $\pm$ 0.59 c	21.24 $\pm$ 0.35 b	37.57 $\pm$ 0.26 e	40.44 $\pm$ 1.43 c

²Values represent the means $\pm$ SE, and those with the same letters within a column are not significantly different ($P \leq 0.05$) by Scheffe's multiple range tests.

Table 2. Flower characteristics of the F_1 between *Solanum macrocarpon* and *S. melongena* 'Uttara' and its amphidiploids.

Plant materials	Flower diam (mm)	Anther (mm)		Petal (mm)		Sepal (mm)	
		Length	Width	Length	Width	Length	Width
<i>S. melongena</i> 'Uttara'	31.44 $\pm$ 0.69 ab ²	6.29 $\pm$ 0.04 cd	1.49 $\pm$ 0.03 b	18.42 $\pm$ 0.69 a	13.67 $\pm$ 0.88 abc	7.27 $\pm$ 0.25 a	2.12 $\pm$ 0.06 a
<i>S. macrocarpon</i>	35.05 $\pm$ 2.55 ab	5.20 $\pm$ 0.08 ab	1.70 $\pm$ 0.03 c	25.19 $\pm$ 2.25 d	14.65 $\pm$ 0.79 bcd	14.87 $\pm$ 1.34 d	3.75 $\pm$ 0.26 c
F_1	31.75 $\pm$ 2.88 ab	5.52 $\pm$ 0.28 ab	1.38 $\pm$ 0.04 ab	19.26 $\pm$ 1.54 a	12.48 $\pm$ 0.28 ab	11.05 $\pm$ 1.40 bc	2.77 $\pm$ 0.15 b
Amphidiploid No. 1	44.01 $\pm$ 3.21 c	7.11 $\pm$ 0.35 e	1.91 $\pm$ 0.09 d	25.77 $\pm$ 2.09 d	15.90 $\pm$ 2.04 cd	15.40 $\pm$ 1.72 d	4.77 $\pm$ 0.30 d
Amphidiploid No. 2	30.07 $\pm$ 1.10 a	5.08 $\pm$ 0.10 a	1.25 $\pm$ 0.03 a	17.83 $\pm$ 0.82 a	11.65 $\pm$ 0.41 a	7.78 $\pm$ 0.25 ab	2.53 $\pm$ 0.20 ab
Amphidiploid No. 3	36.40 $\pm$ 1.76 b	5.79 $\pm$ 0.16 bd	1.38 $\pm$ 0.05 ab	19.90 $\pm$ 1.36 ac	13.67 $\pm$ 0.41 ac	8.17 $\pm$ 0.69 ab	2.68 $\pm$ 0.15 ab
Amphidiploid No. 4	42.62 $\pm$ 2.05 c	6.78 $\pm$ 0.37 ce	1.75 $\pm$ 0.10 c	23.86 $\pm$ 1.29 cd	16.46 $\pm$ 1.03 d	13.29 $\pm$ 2.24 cd	4.08 $\pm$ 0.44 c

²Values represent the means $\pm$ SE, and those with the same letters within a column are not significantly different ($P \leq 0.05$) by Fisher's exact test.

Table 3. Expected ploidy level of three histogenic layers (L) of the amphidiploids.

Plant materials	Ploidy level		
	L _I	L _{II}	L _{III}
Amphidiploid No. 1	4x	4x	4x
Amphidiploid No. 2	4x	2x	4x
Amphidiploid No. 3	2x	2x	4x
Amphidiploid No. 4	4x	4x	4x

Table 4. Seed fertility of the F₁ between *Solanum macrocarpon* and *S. melongena* 'Uttara' and amphidiploid No. 1.

Cross combination	Number of flower pollinated	Number of fruit set	Fruit set(%)	Number of seed per fruit	Seed germination (%)
F ₁ × <i>S. melongena</i> Uttara	100	2	2	1	0
F ₁ × <i>S. macrocarpon</i>	20	1	5	0	—
F ₁ selfing	30	0	0	0	—
Amphidiploid No. 1 × <i>S. melongena</i> 'Uttara'	20	5	25	3	0
Amphidiploid No. 1 × <i>S. macrocarpon</i>	10	4	40	4	43.75
Amphidiploid No. 1 selfing	10	2	20	14	57.14

of somatic hybrids between eggplant and the *S. aethiopicum* Gilo Group have been reported (Rizza et al., 2002), which were used in backcrosses for the introgression of desirable traits from the close relative into the cultivated eggplant (Toppino et al., 2008a, 2008b). In addition to their use in bridging the fertility gap, amphidiploids can be valuable tools for basic and applied research in genomic organization of the cultivated eggplant and its relatives. Probably, introgression of useful characters of *S. macrocarpon* into eggplant by the way of our synthetic amphidiploids may be achieved. Moreover, amphidiploids produced in the present study may generate variants that possess useful characteristic and may provide a wider germplasm base for future breeding programs of eggplant.

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