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PRODUCTION OF DIPLOID AND AMPHIDIPLOID INTERSPECIFIC HYBRIDS OF EGGPLANT AND *SOLANUM TORVUM*, AND POLLEN FERTILITY

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ABSTRACT

Eggplant (*Solanum melongena* L.) is susceptible to several pathogens and pests. However, *Solanum torvum* is resistant to *Verticillium*, bacterial wilts, root-knot nematode and some mycoplasmas. Sterile interspecific hybrids have been obtained from sexual or somatic hybridization studies of these species. The objective of this study is identifying a protocol that allows production a large number of interspecific hybrid plants between *S. melongena* and *S. torvum* by using *in vitro* embryo rescue and increasing the fertility by chromosome doubling to overcome the interspecific incompatibility problem. Seventy-seven interspecific hybrid genotypes from eggplant cultivar Faselis F_1 × *S. torvum* crossing were produced by *in vitro* embryo rescue from the seeds or underdeveloped ovules. Pollen viability and germination of the 50 interspecific hybrid genotypes were examined. The lowest and highest pollen viabilities in interspecific hybrid genotypes were 0.0% and 11.11%, respectively. The *in vitro* pollen germination of interspecific hybrids was by 0.0-1.36%. However, there was no fruit setting when the interspecific hybrid genotypes were backcrossed as a female with the parental species. The amphidiploid plants of some interspecific hybrid genotypes were obtained by 0.03% colchicine application. The pollen viability and germination percentages of the amphidiploid genotype were 11.57 and 9.43 fold of its diploid origin genotype, respectively.

Key words: Amphidiploid, Interspecific Hybridization Barrier, *Solanum melongena*.

INTRODUCTION

Cultivated eggplant (*Solanum melongena* L.) is susceptible to numerous pathogens and pests (Deng-Wei *et al.*, 2014; Yang *et al.*, 2014). It is known that these pathogens and pests cause significant yield reduction in eggplant. Chemical control of pathogens and pests are not preferred because they are ineffective after a period of time, also increase the cost of production and adversely affect the environment. The using of resistant rootstocks to control these pathogens and pests are also expensive. It has indicated that *S. melongena* has a narrow genetic base in commercial varieties, especially within the F_1 hybrid cultivars (Muñoz-Falcón *et al.*, 2009). Therefore, to control the soil-borne pathogens and pests, it is necessary to transfer the resistance genes to the eggplant cultivars from the resistant relative species. Wild eggplant (*Solanum torvum* Sw.) was notified to be resistant to *Verticillium*, bacterial wilt and *Meloidogyne* nematodes (Aribaud *et al.*, 2014; Yang *et al.*, 2014). Fruits and underdeveloped seeds were obtained from hybridization of eggplant cultivars (female) with *S. torvum* (male), however, these seeds did not germinate in a potting mix (Kumchai *et al.*, 2013). The inability to germinate may be due to seed abortion owing to incompatibility after the fertilization. Interspecific hybrids were obtained by *in vitro* embryo rescue from ovules harvested 15-27 days after pollination, but they were infertile (Bletsos *et al.*, 1998). Consequently, it could be stated that the interspecific hybrid plants of these species were sterile or self-incompatible according to reports by Bletsos *et al*. (1998), Kumchai *et al*. (2013) and Plazas *et al*. (2016). However, one study has showed the high pollen viability (90%) and fertility in the interspecific hybrid plants of E-35 (inbred line) and *S. torvum* (Bi-Hao *et al.*, 2009).

Pollen viability of the somatic hybrids between *S. torvum* and *S. melongena* that have been obtained by Collonnier *et al.* (2003) was varied from 2 to 20%, however, no fruit was achieved when the hybrid plants were selfed. On the other hand, Jarl *et al.* (1999) obtained somatic hybrid plants by fusing irradiated *S. torvum* protoplasts with *S. melongena* protoplasts by asymmetric hybridization method. The authors have reported that they have selected 12 (for use in subsequent breeding programs) somatic hybrid plants which have been detected as resistant to *Verticillium*. Nevertheless, to our knowledge, no commercial eggplant cultivars that were produced by somatic hybridization of these species are known. These studies further suggested that interspecific tetraploid somatic hybrid plants exhibit incompatibility or low fertility.

It was shown that fertile plants could be achieved by *in vitro* chromosome doubling of sterile hybrids between eggplant and its relative species *S. macrocarpon* using 0.05% colchicine (Khan *et al.*, 2013). However, successful results were not reported in

producing amphidiploids by colchicine application to interspecific hybrid plants of *S. melongena* and *S. torvum*.

The objective of this study is to identify a protocol that allows production of a large number of interspecific hybrid plants between *S. melongena* and *S. torvum* by using *in vitro* embryo rescue. To overcome the interspecific incompatibility problem, the present study also aims to increase the fertility by chromosome doubling of the interspecific hybrid.

MATERIALS AND METHODS

The plants of eggplant cultivar, Faselis F_1 (De Ruiter Seeds, The Netherlands) and three years old *S. torvum* were grown in a glasshouse (in the southeastern Mediterranean Region of Turkey) and crossed as female and male, respectively. One day before the flower bud was opened, immature male organs of Faselis F_1 flowers were emasculated, and these flower buds and unopened flowers of *S. torvum* were closed. After 15-24 h, the emasculated flowers of FaselisF_1 were pollinated with pollens from the flowers of *S. torvum*, and then the pollinated flowers were closed again. Totally, 68 flowers of FaselisF_1 were crossed with *S. torvum* pollens. The rate of fruit set was 55.88% (38/68), and 188.82 seeds or underdeveloped ovules were obtained per fruit. The seeds or underdeveloped ovules were extracted from the mature fruits and were kept at 4°C.

About 1070 seeds or underdeveloped ovules obtained from the crossing of Faselis F_1 and *S. torvum* were surface-disinfected in a solution of 0.8-0.9% sodium hypochlorite with two drops of Tween 20 per 100 ml, for 20 min. After rinsing with sterile distilled water 5 times, the seeds or underdeveloped ovules were germinated on MS (Murashige and Skoog, 1962) medium at 25±1°C, in the dark. The germinated seeds or underdeveloped ovules were transferred to the same medium in culture tubes (25x150 mm) and incubated in a growth room at 25±1°C, with a 16 h photoperiod and light intensity of 60-70 µmol m^{-2} s^{-1}. Media were solidified with 8 g L^{-1} agar, and were sterilized at 121°C for 15 min. Most of the germinated embryos of interspecific hybrids (Faselis F1 × *S. torvum*), grew and rooted in MS medium. However, growth and rooting of some interspecific shoots were poor on this medium. To induce growth, these shoots were transferred to MS medium containing 0.225 mg L^{-1} BA (N^6-Benzyladenine). Then, to stimulate the rooting, the elongated shoots were cultured on MS medium supplemented with 0.372 mg L^{-1} NAA (1-Naphtalenacetic acid). Nevertheless, again some of these shoots did not root. Thus, MS medium containing 0.2 mg L^{-1} IAA (Indole-3-acetic acid) was used for rooting the shoots (Moreira *at al.*, 2010). The 0.2 mg L^{-1} IAA caused much callus formation on the base of the shoots, similar to 0.372 mg L^{-1} NAA. Excessive callus formation at the base of shoots prevents or slows rooting and leads to poor quality root formation. Therefore, to stimulate direct root formation, these shoots were cultured on MS medium containing 250 mg L^{-1} cefotaxime, after they were incubated on MS medium supplemented either 0.372 mg L^{-1} NAA or 0.2 mg L^{-1} IAA for two weeks. Cefotaxime is known as an antibiotic that promotes rooting (Çürük and Meşe, 2012). The rooted plants were acclimated to the growth room conditions and were transplanted to the plastic pots containing 0.2 L substrate (66% peat Potgrond H, 34% perlite).

Thereafter, the plants were transferred to a glasshouse with steel structure (gutter height: 4 m, ridge height: 5.5 m, roof ventilation windows: 2 m, 4 exhaust fans). To propagate the interspecific hybrid genotypes, 3-8 cuttings of the 74 genotypes with 2-3 buds were planted to plastic pots containing 0.2 L substrate (above), in the glasshouse. Seedlings of parental species (FaselisF_1, *S. torvum*) were obtained from their seeds that had sown in plastic pots at the growth room specified above. These seedlings of parental species and their interspecific hybrids were transplanted to the plastic pots containing 16 L of the substrate and were placed in the glasshouse. The plant density was 2.5 plants m^{-2} (0.8x0.5 m), and the total applied N, P_2O_5 and K_2O were 400, 130 and 620 kg ha^{-1}, respectively. The temperature and relative humidity in the glasshouse were recorded every 30 min by HOBO U14 LCD Data Logger (Onset Computer Corporation) during the experiment. The highest, lowest and average temperature and relative humidity values recorded in the glasshouse were 27.74, 15.07, 20.21°C and 64.09, 36.23, 51.56%, respectively.

Backcrossing of interspecific hybrid genotypes with their parents: Flowers of 27 and 30 interspecific hybrid genotypes were crossed as described above with pollens from Faselis F_1 and *S. torvum*, respectively, and fruit set was examined.

Production of amphidiploid plants of interspecific hybrid genotypes: Both stem axillary buds and shoot tips of *in vitro* grown plantlets of interspecific hybrid genotypes were cultured in 90x15mm Petri dishes on solidified MS medium supplemented with 0.03% colchicine and 0.225 mg L^{-1} BA, for 3 or 6 days. Then the explants were transferred to the MS medium without colchicine. Each treatment consisted of three replicates and four explants (about 3 stem axillary buds and 1 shoot tip) per replicate (Petri dish). The regenerated shots were transferred to the medium in the culture tubes (25x150 mm) and then were *in vitro* grown and rooted and acclimated as mentioned above. The chromosome number was counted following Can and Hatipoğlu (1999) with two modifications (roots were hydrolyzed in 1N HCl at 60°C for 5 min, root tips were incubated for 1.5 h in Feulgen). The putative amphidiploid and its origin diploid interspecific plants were transplanted to a glasshouse with steel structure (gutter height: 2.5 m, ridge

height: 4.5 m, roof ventilation windows: 1 m, side ventilation windows: 1.2 m). These plants were cultured as mentioned above. The maximum, minimum and average air temperatures and relative humidity recorded by HOBO U14 LCD Data Logger in this glasshouse during the study were 28.39, 12.98, 19.12°C and 72.57, 37.00, 56.60%, respectively.

Pollen viability and germination: The pollen viabilities of interspecific hybrids (50 genotypes), *S. torvum*, FaselisF_1 and amphidiploid plants were assessed by staining fresh pollens in TTC (1% 2,3,5 triphenyl tetrazolium chloride, 60% sucrose) for 4-5h. The percentage of pollen viability was determined based on red stained, and red plus pink stained pollens in TTC. Dead pollens were not stained at all. The pollens were germinated in a medium composed of 5% sucrose, 50 mg L^{-1} boric acid, and 1% agar at 22-25°C for 5h (Khan and Isshiki, 2008). The viability or germination rates of the pollens were determined by observing about 200 pollens (4 replicates, 50 grains per replicate) under the light microscope.

Statistical analysis: The experiments were designed and analyzed according to a completely randomized design. All values expressed as percentages were transformed (arcsin (P=original percentage value)$^{0.5}$) (Bartlett, 1947), and analyzed by analysis of variance. Tukey's Honestly Significant Difference (HSD value) or Least Significant Difference (LSD) tests were used to compare the treatments at the 0.05 probability level.

RESULTS AND DISCUSSION

The germination ratio of the seeds or underdeveloped ovules of interspecific hybrids was 27% (289/1070). When the rooting of some interspecific shoots was poor, the shoots were cultured on MS medium containing 250 mg L^{-1} cefotaxime, after they were incubated on MS medium supplemented either 0.372 mg L^{-1} NAA or 0.2 mg L^{-1} IAA for two weeks. According to our visual observations, the incubation of the shoots on MS medium containing either NAA or IAA for two weeks improved the rooting of some recalcitrant interspecific hybrids. The effect of NAA or IAA on the rooting of the recalcitrant interspecific genotypes was similar. The ratio of the *in vitro* rooted interspecific plantlets was 14.77% (158/1070). The percentage of *in vitro* rescue in our study was greater than that of the ratio reported by Kumchai *et al.* (2013) for an interspecific hybrid between *S. melongena* and *S. torvum*. According to Kumchai *et al.* (2013), *in vitro* embryo rescuing of the interspecific hybrid genotypes was 7.2%. When the fruits of *S. melongena* × *S. torvum* were harvested 15 to 30 days after pollination, 80% of the rescued embryos were viable and developed into *in vitro* plantlets (Plazas *et al.*, 2016). However, the embryos have to be excised from immature seeds under stereomicroscope according to this protocol, which is a tedious procedure. In our study, 76.58% (121/158) of the transplanted plants (to the pots) survived. During growing period, 7.07% of the interspecific plants died because of the rotting at the roots or stems of the plants. Some interspecific hybrid genotypes were not propagated by cuttings in the glasshouse, due to weak growth, development and rooting. Consequently, 72 interspecific genotypes with 2 and 3 clones (28 and 44 genotypes, respectively) and 5 genotypes without clone were obtained.

Pollen viability and germination: Fifty genotypes with 2-3 clones that had produced flowers and pollens during the experiment were used to investigate pollen viability and germination. It was reported that 5 and 21 interspecific hybrid genotypes were produced through *in vitro* embryo rescue by Bletsos *et al.* (1998) and Kumchai *et al.* (2013), respectively.

The percentage of pollen viabilities based on red stained and red plus pink stained pollens in TTC and *in vitro* pollen germination are presented in Table 1. The differences among genotypes were significant with respect to viability and germination. The highest pollen viability and germination were observed in parental cultivar Faselis F_1, and then in *S. torvum*. Among interspecific hybrid genotypes, the genotype number 45 produced the most viable pollens both based on red, and red plus pink staining. Moreover, two genotypes (number 33 and 72) showed moderate viability compared to other genotypes based on red plus pink staining. The highest pollen viability (11.11%) obtained from genotype number 45 in our study was higher than the viability (1.2%) reported by Bletsos *et al.* (1998) and lower than the viability (90%) stated by Bi-Hao *et al.* (2009). This was probably due to the different genetic background of parental species used in hybridization. The highest pollen germination (1.36%) was obtained from the genotype number 69. The pollen germination of the genotypes numbers 41, 52, 69 and 70 was varied between 0.3 and 1.36%, although the staining of these genotypes was 0.0%. This might be due to low pollen production by these interspecific genotypes. When the tests were repeated, similar results were obtained. Besides, the pollen germination of some genotypes (number 10, 13, 65 and 74) was 0.0%, although these genotypes showed low red staining. This might be because of the un-optimized medium for *in vitro* germination of these interspecific hybrid genotypes.

The pollen homogeneity of pollen morphology, viability and germination were highest in Faselis F_1 (Table 1, Figure 1A, E), moderate in *S. torvum* (Table 1, Figure 1B) and lowest in interspecific genotypes (Table 1, Figure 1C, D, F). The poor pollen viability and germination observed in interspecific hybrid pollen was probably due to the meiotic abnormality in chromosome

behaviors as reported in *S. melongena* × *S. torvum* by Kumchai *et al.* (2013). The morphology of leaves, flowers, and inflorescence of the interspecific genotypes was intermediate to the parents (Figure 1G, H), however, there was variation among interspecific hybrids. Though there was no prickle on the leaves of the female parent Faselis F_1, there were prickles on the leaves of all interspecific hybrids as their male parent. Consequently, the morphological characteristics of the leaves, flowers, inflorescence, and pollens, and viability and germination ability of the pollens of the interspecific genotypes and their parents confirmed the interspecific hybridity.

Backcrossing of interspecific hybrid genotypes with their parents: Fruit set in the flowers of Faselis F_1 and *S. torvum* that were selfed for control, were 100% and 41.67%, respectively. However, there was no fruit set when the 227 flowers from 27 interspecific hybrid genotypes that were crossed with pollens of Faselis F_1, and 248 flowers from 30 interspecific hybrids crossed with that of S. *torvum*. Also, no fruit was obtained from the flowers of interspecific plants under random pollination condition. These results indicate that the obtained interspecific hybrids are highly sterile as reported by Kumchai *et al.* (2013) and Plazas *et al.* (2016), although they are in contrast with the results of Bi-Hao *et al.* (2009). Bi-Hao *et al.* (2009) have reported that the pollen viability of the interspecific hybrids between inbreeding line E-35 (eggplant) and *S. torvum* was 90%, and the fertile seed number was 120-150 per fruit.

Production of amphidiploid plants of interspecific hybrid genotypes: There was no significant difference in amphidiploid plant production between 6 or 3 days of colchicine application. Growth of one amphidiploid plant obtained from 12 stem axillary buds or shoot tips of interspecific hybrids that were cultured on MS medium supplemented with 0.03% colchicine and 0.225 mg L^{-1} BA for 6 days, was very slow which may be due to a physiological disturbance, resulting in a reduced rate of cell division, and the plant did not survive when it was transplanted to the pot in the growth room. These symptoms were infrequent in the plants obtained from the colchicine treatments for 3 days. The abnormal plant growth might be caused by colchicine application (Huang *et al.*, 2010; Dhooghe *et al*., 2011). According to Huang *et al.* (2010) and Sajjad *et al.* (2013), colchicine-treated explants had significantly shorter shoots than untreated explants. In many plant species, colchicine causes side effect such as abnormal growth (Dhooghe *et al*., 2011; Sajjad *et al.*, 2013). It has been reported that the regenerated shoots from colchicine treated explants of *Petunia hybrida* have thick abnormal dark colored leaves, and they were short and grew slowly (Abu-Qaoud and Shtaya, 2014).

Ten out of 12 explants (stem axillary buds or shoot tips) of interspecific hybrids (83.33%) sprouted when they were cultured for 3 days on the *in vitro* medium with 0.03% colchicine. These 10 shoots rooted *in vitro*, and 4 of them (40.00%) survived in the growth room. Two of the 4 rooted plants were amphidiploid. Overall, 16.67% of 12 explants that were cultured on the colchicine concentration for 3 days produced amphidiploid plants. The chromosome numbers of the plants were determined by counting the chromosomes of 25 metaphase cells and found to be 48 (Figure 2A). However, only one out of 2 amphidiploid plant survived and grown in the glasshouse. The flower color (pale violet), the number of the calyx (5), corolla (5) and stamen (5) of the amphidiploid plant were same as its diploid origin plant. The sizes of the plant, leaf (Figure 2B) and pollen of the plant were greater than those of its diploid counterparts. The flower width of the amphidiploid plant was 39% higher than that of its diploid origin. These results are confirming the amphidiploidy of the plant obtained by colchicine application. The interspecific amphidiploid plant flowers produced more pollens than diploid ones.

The differences between amphidiploid interspecific hybrid and its diploid origin plant were significant with respect to pollen viability and germination (Table 2). The amphidiploid interspecific hybrid pollen viability percentage was 2.66% and germination percentage was 2.17%, which were 11.57 and 9.43 fold of its diploid origin genotype, respectively. However, there was no fruit set when the flowers of amphidiploid interspecific plants were selfed. Tetraploid interspecific hybrids of *S. melongena* and *S. torvum* that have been produced by somatic hybridization were also generally incompatible or low fertile. Although they showed low fertility, it has been stated that the fertility level under field conditions was still high enough to allow normal fruit and seed set (Jarl *et al.*, 1999). But, as far as we know, there are no commercial eggplant cultivars originated from somatic hybridization studies. Collonnier *et al.* (2003) reported that somatic hybridization of the Dourga eggplant cultivar and *S. torvum* resulted in 26 somatic hybrids, and that the pollen viability varied from 2 to 20%, but that the hybrids did not produce fruit.

In our study, the pollen viability and germination rates of amphidiploid interspecific hybrid genotypes were not high enough to overcome interspecific cross infertility problem, although the pollen viability and germination percentages increased considerably by chromosome duplication. On the other hand, the interspecific fertility restoration was accomplished using 0.05% colchicine *in vitro* chromosome doubling of sterile hybrids of *S. melongena* × *S. macrocarpon* by Khan *et al.* (2013). The reason may be the distinctness of the species used in the hybridization or fewness of amphidiploid

plants. *S. torvum* belongs to the tertiary gene pool of wild relatives of eggplant, while *S. macrocarpon* is the member of the sister "anguivi grade" (secondary gene pool) of eggplant and known as cultivated species (Vorontsova *et al.*, 2013; Plazas *et al.*, 2016; Syfert *et al.*, 2016). We have shown that production of a large amount of interspecific hybrid genotypes, and their amphidiploid plants are possible by using *in vitro* colchicine application in solidified MS medium, ignoring the step of shaking the explants in liquid MS medium as reported by Khan *et al.* (2013). However, a low number of amphidiploid interspecific hybrid plants was obtained from a small number of their diploid shoot tips or axillary buds that were treated with colchicine. To overcome the interspecific infertility problem, more amphidiploid genotypes of distinct interspecific hybrids and their parental species should be produced so that backcrossing and recovering diploid individuals after subsequent anther culture could be possible. In this respect, further studies are underway in our lab.

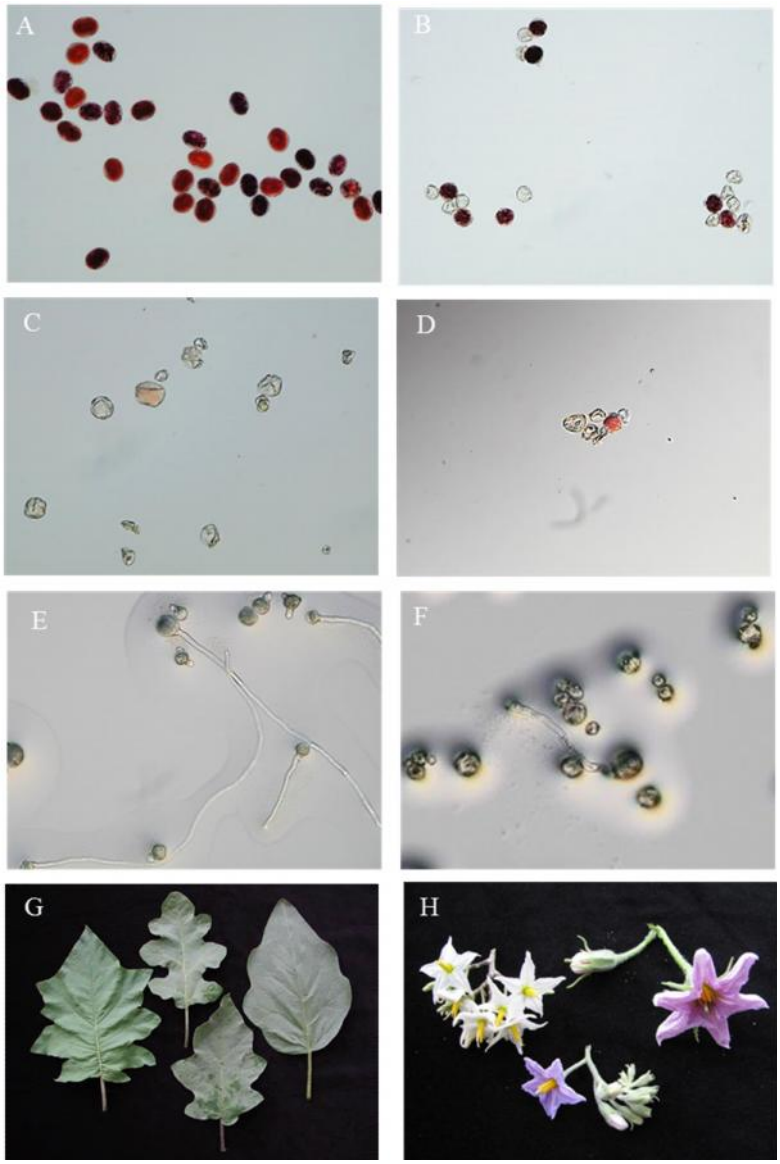

Figure 1: Pollen staining and *in vitro* germination, leaf and flower morphology of parental species and their interspecific hybrids. A. Red viable pollens of female parent, Faselis F_1 (82x), B. Red viable or unstained dead pollens of male parent, *S. torvum* (82x), C. Pink viable (36 µm), or unstained dead pollens of interspecific hybrid number 45, D. Light red viable (27 µm), or unstained dead pollens of interspecific hybrid number 72, E. Germinated or un-germinated pollens of Faselis F_1 (82x), F. Germinated or un-germinated pollens of interspecific hybrid number 72 (82x), G. Leaf of *S. torvum* (left), interspecific hybrid (center) and Faselis F_1 (right), H. Flower of *S. torvum* (above left), interspecific hybrid (lower center) and Faselis F_1 (above right)

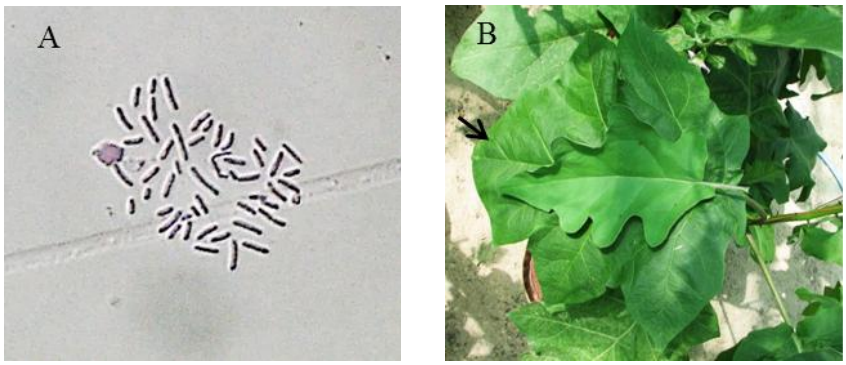

Figure 2: A. Chromosome number of interspecific amphidiploid plants obtained from *in vitro* application of 0.03% colchicine (1600x), and B. Sixth leaf (from the shoot tip) of the interspecific amphidiploid plant (lower) and of its origin diploid plant (upper).

Table 1. Pollen viability and germination of parental species and their interspecific hybrids.

Genotype number	Viability (red) (%)	Viability (red plus pink) (%)	Germination (%)
1	43.84 b	57.01 b	35.74 b
2	92.93 a	98.20 a	70.18 a
3	0.00 e	0.00 g	0.00 d
4	0.00 e	0.00 g	0.00 d
5	0.00 e	0.00 g	0.00 d
6	0.00 e	0.38 g	0.29 cd
8	0.00 e	0.00 g	0.00 d
9	0.00 e	0.00 g	0.00 d
10	0.41 de	3.85 def	0.00 d
11	0.00 e	1.06 efg	0.00 d
12	0.00 e	0.00 g	0.00 d
13	0.31 de	0.31 g	0.00 d
16	0.00 e	0.21 g	0.43 cd
17	0.00 e	0.00 g	0.00 d
18	0.00 e	0.00 g	0.00 d
21	0.00 e	0.46 fg	0.00 d
22	0.00 e	0.00 g	0.00 d
24	0.00 e	0.00 g	0.00 d
25	0.00 e	0.00 g	0.00 d
26	0.00 e	0.00 g	0.00 d
27	0.00 e	0.00 g	0.00 d
30	0.00 e	0.00 g	0.00 d
32	0.00 e	0.00 g	0.00 d
33	1.11 de	5.31 cde	0.28 cd
37	0.00 e	0.34 g	0.00 d
39	0.00 e	0.00 g	0.00 d
41	0.00 e	0.00 g	0.30 cd
42	0.00 e	0.00 g	0.00 d
43	0.00 e	0.00 g	0.00 d
45	3.01 c	11.11 c	0.78 cd
46	0.00 e	0.80 efg	0.90 cd
50	0.00 e	0.00 g	0.00 d
51	0.00 e	0.49 fg	0.00 d
52	0.00 e	0.00 g	0.65 cd
55	0.00 e	0.00 g	0.00 d
56	0.00 e	0.00 g	0.00 d
60	0.00 e	0.00 g	0.00 d

61	0.00 e	0.34 fg	0.40 cd
62	0.00 e	0.00 g	0.00 d
63	0.00 e	0.00 g	0.00 d
64	0.00 e	0.00 g	0.00 d
65	0.46 de	0.46 fg	0.00 d
66	0.00 e	0.00 g	0.00 d
67	0.00 e	0.32 g	0.31 cd
69	0.00 e	0.00 g	1.36 c
70	0.00 e	0.00 g	0.90 cd
71	0.00 e	0.00 g	0.00 d
72	1.80 cd	8.12 cd	0.25 cd
73	0.00 e	0.00 g	0.00 d
74	0.38 de	1.15 efg	0.00 d
76	0.45 de	0.45 fg	0.93 cd
79	0.00 e	0.00 g	0.00 d

Genotype no 1: *Solanum torvum*, genotype no 2: Faselis F_1, genotypes no 3-79: interspecific hybrids. Transformed values were used to compare the means, and untransformed values of the means were presented. Values of each parameter, followed by same letters in the columns, do not differ by Tukey's Honestly Significant Difference (HSD) test ($P<0.05$).

Table 2. Pollen viability and germination of parental species, Faselis F_1, amphidiploid and its diploid origin interspecific hybrid genotype.

Genotype	Viability (red plus pink) (%)	Germination (%)
Faselis F_1	96.99 a	52.16 a
Interspecific amphidiploid	2.66 b	2.17 b
Diploid origin genotype	0.23 c	0.23 c

Means of each parameter followed by same letters in the columns do not differ at Least Significant Difference (LSD) test ($P<0.05$).

Conclusion: The protocol identified in this study can be used for the production of large amount of diploid interspecific hybrid genotypes between *S. melongena* and *S. torvum*. The pollen viability and germination percentages of diploid interspecific hybrid plants of *S. melongena* and *S. torvum* increased considerably by duplication of their chromosomes with *in vitro* colchicine application. However, more interspecific hybrid genotypes with different genome and their amphidiploids should be produced to overcome the interspecific hybridization barrier so that production of fertile amphidiploid plants may be possible.

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