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RESEARCH ARTICLE

Development of inter-specific hybrids between *Solanum melongena* L. and *Solanum sisymbriifolium* Lam. through embryo rescue

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Abstract

In present investigation, inter-specific hybridization between two species was carried out using a three-way cross with the aim to study the response of heterotic hybrids for cross-compatibility and to improve the fertility traits of interspecific cross such as pollen viability and density for further introgression of agronomically important traits. The present investigation highlights genotypic variation of for cross-compatibility between *S. melongena* hybrids and *Solanum sisymbriifolium.* Among three hybrids, H3 x *Solanum sisymbriifolium* (4%) and H4 x *Solanum sisymbriifolium* (1.5%) showed compatibility for fruit set and developed immature seeds only. The embryo rescue from 25 day-old fruits and *in vitro* culture on ½ MS medium supplemented with 2mg/L^{-1}BAP showed germination. H5 x *S. sisymbriifolium* showed 49.4% embryo germination and produced four complete plantlets. Furthermore, all plants were morphologically variable for the presence or absence of spines, flower clustering, slightly curved/straight stigma tip, and flower color. The regenerated plantlets were intermediate to cultivated and wild parents for the quantitative traits. Two SSR markers *viz.,* emg01A17, emh11001 and emb01E03 also confirmed the hybridity of three-way cross plants. Among four interspecific plants, one established high pollen viability (78.5%), while others were partial-fertile (62.8%) and all carried low pollen density (69-299 pollen/ 1cm^2) at anthesis. Inbreeding and backcrossing of these plants to both cultivated and wild parents remained unsuccessful. This is the first report of developing a partially-fertile three-way cross between *S. melongena* and *S. sisymbrifollium* via embryo rescue.

Keywords: Eggplant, three-way cross, *Solanum sisymbriifolium*, hybridity, pollen viability, reproductive potential.

Introduction

Eggplant, *Solanum melongena* L. (2n = 2x = 24) is one of the most important non-tuberous, warm-season vegetable crops grown in India and other parts of the world. The primary and secondary centers of diversity of eggplant are India and China, respectively (Bagheri 2010). Eggplant is one of the vegetables with the highest antioxidant capacity because of its high fruit phenolics and flavonoids as an antidote for the cure of numerous diseases. Although it is an important nutritional crop, but it is susceptible to a wide array of insect pests, diseases and many abiotic stresses, causing reduced production potential (Rotino et al. 2014). Many wild relatives of primary, secondary and tertiary gene pool possess resistance against various biotic and abiotic stresses and are being used for grafting as well as breeding eggplants for adaptation to climatic change. South American wild species, *S. sisymbriifolium* Lam. (2n=2x-24) have been identified as resistant to several desirable agronomic traits, disease and insect pests resistance such as root-knot nematodes, and carmine spider mites, verticillium wilt, fruit and shoot borers and *Aphis gossypii* (Rotino et al. 1997; Collonnier et al. 2003). It belongs to the Cryptocarpum section of the Solanaceae family and also called sticky nightshade (Weese and Bohs 2007).

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Many researchers have attempted crosses between cultivated eggplant and *S. sisymbriifolium* Lam, but limited success has been obtained due to sexual incompatibilities (pre and post-pollination effects) and difficulties in obtaining fertile progenies (Gleddie et al.1986; Sihachakr et al.1994; Bletsos et al.1998). Interspecific hybridization between cultivated and wild species facilitated with embryo rescue technique has been a best way to introgress desirable agronomic traits. The compatibility of interspecific hybridization between eggplant and wild species and the resulting cross's reproductive potential depends on the genetic divergence and the direction of the cross (Plazas et al. 2016). In interspecific hybridization, the direction of the cross also affected compatibility, the number of seeds produced, as well as the dormancy of the seeds due to maternal effects (Morgan et al. 2011). *S. sisymbriifolium,* a tertiary gene pool relative, hybridized with difficulty and produced sterile or low-fertility hybrids with cultivated genotypes only after embryo rescue technique (Rotino et al. 2014). In already cited literature, unidirectional crossability of *S. sisymbriifolium* as male parent has been reported, where embryo rescue technique was used to produce interspecific plants that were either died before maturity or had been sterile (Sharma et al.1984; Blestsos et al. 1998). Mostly, the appearance of intermediate traits to both parents indicated the hybridity of the interspecific cross, but the similarity of hybrid plants with maternal parent needs molecular marker-based confirmation of heterozygosity for the alleles (Meena et al. 2017). The F_1 hybrids of a cultivated parent, generally showing improved performance for most of the agronomic traits, can be tested for their potential for cross-compatibility with the wild parent and for the improvement in fertility status of resulted crosses.

In view of the important traits present in *S. sisymbriifolium* and reports of sterile interspecific hybrids between cultivated and wild species, the present investigation was executed with the objectives of understanding the crossability behavior between cultivated F_1's and wild species, to improve the fertility status between two species through embryo rescue technique.

Materials and methods

Plant material

For the development of fertile three-way cross, F_1 hybrids of advanced breeding lines *viz;* MR-319 X SL-15-2(H1), SR-5 X SR-9322(H2), 219 X PSB-7-2(H3), PSB-7-2 X 319(H4) and SR-9322 X BLW-231(H5) showing hybrid vigour for most of the agronomic traits were used as female parents to test the compatibility behavior with the wild species. *Solanum sisymbriifolium* (2n=2x=24), producing flowers and fruit in both spring and rainy season and being maintained in open fields at the Department of Vegetable Science, Punjab Agricultural University, Ludhiana, Punjab was used as male parent in the present investigation.

Compatibility studies

First generation hybrids between different advanced breeding lines of *S. melongena* were raised during rainy season of 2018 following the standard package of practices. The wide hybridization was performed on healthy plants in field during the months of October-November. The F_1 crosses from *S. melongena* were used as female, while the wild species, *Solanum sisymbriifolium,* was used as male parent. Long-styled flower buds on the female parent were emasculated a day before anthesis and covered with butter paper bag to avoid contamination. On the day of anthesis the pollen grains were collected from the covered flowers of the wild parent, dusted onto the stigmatic surface of the emasculated flowers of the female parents and were covered again with cotton. Fifty crosses were attempted for each cross combination. The cross-compatibility rate (%) was computed from the number of fruit set over total number of attempted crosses.

Embryo rescue and germination

For embryo rescue, the immature fruits of interspecific cross (*S. melongena* × *Solanum sisymbriifolium*) with under developed seeds were collected at 15, 20 and 25 days after pollination (DAP). The fruit were washed thrice with distilled water, then surface-sterilized with 70% ethanol for 5 minutes and flame-sterilized for five seconds under a laminar airflow cabinet. The sterilized fruits were cut opened to separate the immature ovules from pulp, aseptically. After separation, ten ovules were cultured per jam jar containing half-strength solid Murashige and Skoog (Murashige and Skoog, 1962) medium with or without growth hormones (0.5–2 mg/l BAP) and incubated at 25° ± 2°C with a 16-h/8h (light/dark) photoperiod with a light intensity of 35-40 $\mu molm^{-2}s^{-1}$. The germinated seeds at two true-leaf stages were sub-cultured in the fresh half strength MS medium for further shoot elongation and root induction. The fully-developed plantlets were finally hardened on the moist cotton in the jam-jars for a week and further shifted into polythene-bags containing a mixture of soil-cocopeat-perlite (1:2:1, v/v/v) for initial growth. These poly-bags were kept in a controlled greenhouse chamber at 25 ± 2°C with relative humidity (80–90%) for about 30 days. These plants were finally shifted to bigger growing bags. Once acclimated in polybags, the putative hybrids were transferred into soil under field conditions to further characterize their morphological traits and study their reproductive potential.

Morphological and molecular confirmation

For the confirmation of hybridity, the morphological traits of resulting interspecific cross were compared with both the cultivated and wild parents. Secondly, two SSR markers viz; emg01A17 (FP-5'-ATAAGCCAAAGCAAGCACACTTGA-3' and RP-5'-GTTTGAGCTGAAGGTATGCAAGCTGGA-3') and emh11001 (FP-5'-ATTGTGTCGATGAGATTTTGGTCA-3' and

RP-5′-GTTTAGCTACGTTGGTTTGGTGCTGAA-3′) showing polymorphism between female and male parent were also used to ascertain the recombination of alleles. For molecular confirmation, the genomic DNA of both the parents and regenerated plants was extracted in triplicates from young growing leaves (1g approx.) as per CTAB method (Doyle and Doyle 1990). The leaf sample from each entry was first ground into fine powder in liquid nitrogen, dissolved in CTAB buffer, and incubated at 55°C for 5 min. A solution of Phenol, chloroform and isoamyl alcohol in 25: 24:1 proportion was added to the homogenates, mixed thoroughly followed by centrifugation (Eppendorf Centrifuge 5424) at 13,000 rpm for 5 minutes. DNA from the supernatant was precipitated by adding 1:1 volume of ice-cold isopropanol. The centrifuge tubes containing these mixtures were cooled down at -20°C for 20 min and then pelleted at 13,000 rpm for 25 minutes. The pellets were washed thrice with ice-cold 70% ethanol and spun for 5 minutes. The last washing of pellets was given with 90% ice-cold ethanol. The washed DNA pellets were air dried and then were dissolved in 50 µL 1X TE buffer. Each DNA sample was again treated with 5 µL 40 U/µl RNase and incubated for 1 hour in water bath set at 37°C. Thermo Scientific Nano-Drop™ 1000 Spectrophotometer was used to check quality and concentration of isolated DNA samples. After isolation and quantification, DNA dilutions of 50ng/ml for all the samples were prepared. For polymerase chain reaction, 12.4-µL reaction mixture containing 6µl master mix (Takara, Japan), 0.7µL $MgCl_2$ (25 mM), 1.5 µL each of forward and reverse primer (5 µM), 1.8 µL nuclease free water and 1.5 µL of total genomic DNA (50 ng /µL) was prepared. The reaction mixture was subjected to PCR (During stage-1, Initial denaturation at 95°C for 3 minutes, for stage-2, further denaturation of 20 cycles at 95°C for 45 seconds, annealing of primers at 58 (-0.5°C/ cycle) for 45 seconds, extension of DNA chain at 72°C for 45 seconds. and again in stage-3, denaturation of 10 cycles at 95°C for 45 seconds, annealing of primers at 50°C for 45 seconds, extension of DNA chain at 72°C for 45 seconds and at the end in stage-4 a final extension at 72°C for 7 minutes and hold at 4°C for infinite time) in an eppendorf thermo cycler. The amplified products were resolved on a 3% agarose gel containing 0.05 µL/mL ethidium bromide and run at 100 volts for 5 to 6 hours. Finally, the amplified bands were visualized under UV light and images were captured by Gel Documentation System (Alpha Imager HP, USA).

Reproductive potential

Next important goal was to check the fertility status of the developed three-way cross plants so that these can be used for further introgression breeding programmes especially related to various insect-pest and disease resistance traits available in *S. sisymbriifolium*. To check the reproductive potential of interspecific three-way plants, pollen viability and density was estimated as per the method described by Prasad et al. (2006). Freshly opened flowers were excised pollen viability test. Two anthers were squeezed completely in 1ml of 2% acetocarmine solution and observed under binocular compound (OLYMPUS Magnus MLX- DX microscope) microscope. Pollen grains with bright red stain were categorized as viable, pink as semi-sterile and unstained as sterile. Pollen viability (%) was calculated from the number of stained pollen over total pollen visible in the slide. For pollen density 100ul solution was spread on slide and total number of pollen was counted on $1cm^2$ area. All the three-way plants were self-pollinated and backcrossed to *S. melongena* again.

Statistical analysis

The data on number of embryos per fruit, number of embryos cultured and embryo germination percentage, and complete plant formation was taken. The data on morphological characters of both the parents and individual regenerant of three-way cross was collected and statistically analyzed using one factor analysis of variance. A test for significant differences between means was performed at $p \leq 0.05$ and used to differentiate different genotypes.

Results and discussion

Compatibility studies

The cross-compatibility behavior between F_1 hybrids of *S. melongena* genotypes and *S. sisymbrifolium* are given in Table 1. Among various cross-combinations for interspecific hybridization, the highest fruit set (4.0%) was observed from H4 x *S. sisymbriifolium* followed by H5 x *S. sisymbrifolium* (3.6%) and H3 x *S. sisymbriifolium* (1.5%). There was no fruit set, when H1 & H2 hybrids from *S. melongena* were used as female parent in an interspecific cross with *S. sisymbriifolium*. The literature also highlights various studies combining the genome of wild and cultivated species through somatic hybridization or interspecific hybridization and embryo rescue. Wide-hybridization and embryo rescue technique has been used to develop hybrid progenies between eggplant (*Solanum melongena* L.) and another wild relative *S. torvum* (Kumchai et al.2013). However, study reported failure of crosses between *S. melongena* and *S. sisymbriifolium* in any of the directions (Gleddie et al.1986). In support to our results, low fruit set (11.4%) between *S. melongena* and *S. sisymbriifolium* and parthenocarpic fruit development has been reported recently (Plazas et al.2016 and Rakha et al.2020). The variation of response for compatibility of different crosses in our results and the previous reports might be related to the different genetic backgrounds of *S. melongena* genotypes, irregular chromosome associations during cross-over or to different environmental conditions during interspecific hybridization (Nwofia and Eneoblong 2001; Kumchai et al. 2013).

Table 1. Compatibility behaviour for interspecific hybridization between *S. melongena* (F_1 hybrids) and *S. sisybriifolium*

Female parent	Interspecific cross	Total attempted crosses	Set fruits	Fruit set percent
MR-319 X SC-15-2 (H1)	H1x *S. sisymbriifolium*	50.0	0.0	0.0
SR-5 X SR-9322(H2)	H2x *S. sisymbriifolium*	87.0	0.0	0.0
219 X PSB-7-2 (H3)	H3 x *S. sisymbriifolium*	76.0	3.0	4.0
PSB-7-2 X 319 (H4)	H4 x *S. sisymbriifolium*	65.0	1.0	1.5
SR-9322X BLW-231 (H5)	H5 x *S. sisymbriifolium*	55.0	2.0	3.6

Embryo rescue and germination

During embryo rescue from compatible interspecific crosses, only the fruits collected at 25 days after pollination (DAP) responded to *in vitro* cultures of immature embryos on half-strength Murashige and Skoog (MS) medium supplemented with 2 mg/l^{-1}BAP+ 15 g/l^{-1} sucrose +8 g/l^{-1} agar and set at 5.8 pH (Fig 1). Among various interspecific crosses, the fruits obtained from H5 x *S. sisymbriifolium* had the highest average number of embryos per fruit (202) with an embryo germination percentage of 49.4% on half-strength Murashige and Skoog (MS) medium. While H3 x *S. sisymbriifolium* had lowest average number of embryos per fruit (149) that were directed towards callus formation without any plant regeneration any of the media combination. H5 x *S. sisymbriifolium* was the most successful cross with the direct germination of 4 complete plantlets from immature embryos (Table 2). These four putative interspecific hybrids took a week *in-vitro* and 15 days in small black polythene bags for acclimatization to outer environment. The slow growing acclimatized plants were first planted to bigger growing bags for vegetative growth and then to the field during rainy season 2020 to record morphological data and to check their reproductive behaviour.

Similar studies on embryo rescue and their germination in a modified MS medium at 24°C and a 16 hours photoperiod have been reported earlier for an interspecific cross between the eggplant cultivars and *S. torvum* (Bletsos et al.1998; Kumchai et al. 2013). The stage of embryo rescue (torpedo), type of species used for hybridization, the direction of cross and type of medium and growth hormones used for embryo germination also affected the success rate of regeneration efficiency in eggplant as established earlier by many researchers in *S. melongena* × *S. sisymbriifolium, S. melongena* × *S. incanum,* and *S. melongena* × *S. integrifolium* crosses (Singh et al. 2002; Verba et al. 2010; Rakha et al. 2020). Our results of successful embryo rescue at 25 day old torpedo stage and regeneration of plantlets from S. *melongena* × *S. sisymbriifolium* in present investigation were substantiated with the findings of Rattan et al. (2015) who developed interspecific crosses between *S. melongena* and *S. khasianum*.

Morphological characters

The morphological traits of the putative three-way plants obtained from cross between H5 (SR-9322 × BLW-231) and *S. sisymbriifolium* are presented in Tables 3 and 4 as well as in Fig 2. The hybridity of the three-way cross was mainly confirmed from the presence of spines on stem, leaf, petiole and calyx, leaf lobbing pattern and the color of the inflorescence. The semi-erect plant growth habit of two regenerants (Plant 1 and Plant 2) was similar to female parent H5, while third plant carried erect growth habit (Plant P3) from male parent *S. sisymbriifolium*. Plant 4 looked similar to *S. melongena*,

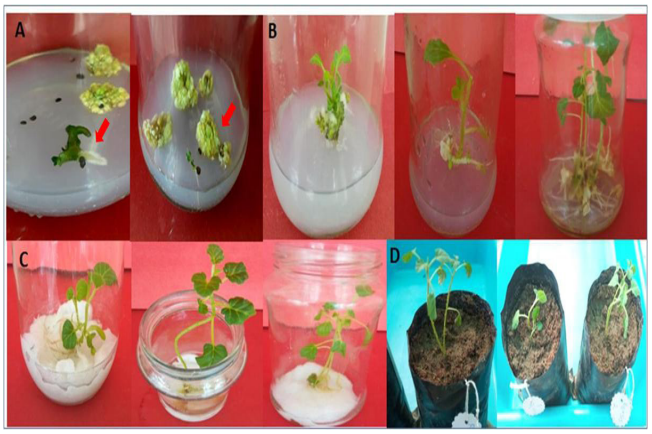

Fig. 1. Embryo rescue from S. *melongena* × *S. sisymbriifolium*: A. Direct germination of immature embryos on half strength MS Medium, B. Shoot and root growth on 1/2 MS+ 0.5 mg/l^{-1}IBA, C. plantlet acclimatization on moist-cotton & D. Plantlet acclimatization on growing media in polybags

Table 2. Embryo rescue and *in-vitro* germination of three-way cross between *S. melongena* (F_1) and *S. sisymbriifolium* (SS)

Three-way cross	Immature embryos per fruit	Embryos cultured	Embryo germination %	Days for germination	Complete plants	Remarks
H3 x *SS*	149	136	0.0	-	-	Embryo callusing
H4 x *SS*	172	145	0.0	-	-	Embryo callusing
H5 x *SS*	202	159	49.4%	12.0	4	Full grown plants

Table 3. Morphological characterization of three-way cross between *S. melongena* (F_1) and *S. sisymbriifolium* for qualitative traits

Traits	SR-9322 ×BLW-231	S. sisymbriifolium	Three-way cross		
			Plant 1	Plant 2	Plant 3
Plant growth habit	Semi erect	Erect	Semi erect	Semi erect	Erect
Leaf veins	Pigmented	Green	Green	Green	Green
Leaf lamina	Dark green	Light green	Light green	Dark green	Dark green
Stem pigmentation	Pigmented	Green	Green	Green	Green
Stem spines	Absent	Present	Present	Present	Present
Leaf spines	Absent	Present	Present (very minute)	Absent	Present
Calyx spines	Absent	Present	Absent	Absent or present	Present
Flower habit	Cluster	Cluster	Heavy cluster	Heavy cluster	Cluster
Flower colour	Purple	White	White	purple	Light purple
Type of flowers	Medium-styled	Exerted/long styled	Exerted- styled	Exerted-styled	Exerted-styled
Calyx colour	Green	Light green	Light green	Light green	Light green
Stigma tip	Straight	Curved	Slightly curved	Straight	Straight

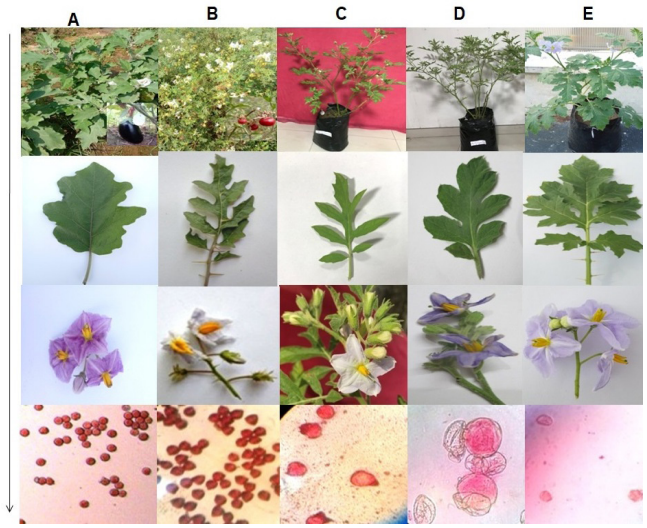

Fig 2: Morphological confirmation of inter-specific three-way cross describing plant, leaf, inflorescence and pollen fertility status of A. *Solanum melongena* H5 (♀), B. *Solanum sisymbriifolium* (♂), C-E. Plants 1-3 regenerated from three-way cross

but could not survive and reach to reproductive maturity. All the three-way cross plants behaved differently for most of the qualitative traits among each other. Plant 1 carried green stem with spines, light green leaves with green veins and presence of small -soft spines, light green calyx with absence of spines, and heavy inflorescence with exerted-styled white flowers (Fig 2C and Table 3). Plant 2 displayed green stem with spines, dark green leaves with green veins and absence of spines, light green calyx with presence of single spine on lower most bud, and heavy inflorescence with exerted-styled violet flowers (Fig 2D and Table 3). Plant 3 displayed green stem with spines, dark green leaves with green veins and many spines, light green spiny calyx, and clustered inflorescence with exerted-styled light-violet flowers (Fig 2E and Table 3). Spines on stem, leaf and calyx were totally absent in cultivated *S. melongena* parent (H5), but present in wild parent *S. sisymbriifolium*. Interestingly, spines on stem were present in these generated three-way cross plants**.** Only one plant showed absence of spines on the leaf. Calyx spines in two plants appeared very late in the winters. Appearance of spines on different plant parts, light green calyx and exerted-styled flowers in all th e three-way plants got inherited from *S. sisymbriifolium* (male parent) and confirmed the parental recombination in these plants.

The plants obtained from embryo rescue of three-way cross were significantly variable from each other for various quantitative traits (Table 4). The regenerates from three-way cross were taller (95–107cm) than the cultivated female parent H5 (60 cm) as well as wild male parent *S. sisymbriifolium* (88 cm). Other growth traits of three-way plants such as leaf length (9.3-17.2 cm), bud length (12.4–16.2 mm), bud width (4.9–6.6 mm), anther length (6.9–7.8 mm), anther width (1.1–1.7 mm), stigma length (9.2-15.5 mm) and stigma width (0.3–0.4 mm) represented an intermediate response to both the parents and could be easily differentiated through measurements and visual comparison with H5 and *S. sisymbriifolium.* The leaves of all three-way plants were thinner (5.8– 8.7 cm) and variable in length (9.3–17.2 cm) in comparison to both parents. The number of flowers per cluster also increased in interspecific regenerants (9.2-9.8) in comparison to female i.e. H5 (3.6) and male i.e. *S. sisymbriifolium* (6.0) parents.

The intermediate expression of morphological characters to the cultivated eggplant and *S. sisymbriifolium* provided preliminary evidence in favour of the hybridity of the produced regenerants. The presence of spines, white flower colour and deeply lobed leaves in the wild species and the inheritance of these traits to the first generation progeny

Table 4. Morphological characterization of three-way cross between *S. melongena* (F_1) and *S. sisymbriifolium* for quantitative traits and reproductive potential

Traits	SR-9322X BLW-231	Solanum sisymbriifolium	Three-way cross		
			Plant 1	Plant 2	Plant 3
Plant height (cm)*	60.0 ± 0.0	88.0 ± 0.0	107 ± 0.0	103 ± 0.0	95 ± 0.0
Leaf length (cm)	12.4 ± 1.7	14.4 ± 1.1	9.3 ± 0.7	10.9 ± 1.4	17.2 ± 0.9
Leaf width (cm)	13.2 ± 0.6	11.1 ± 0.9	5.8 ± 0.6	6.2 ± 0.6	8.7 ± 0.8
Flowers per cluster	3.6 ± 0.5	6.0 ± 1.2	9.4 ± 1.9	9.8 ± 0.8	9.2 ± 1.5
Bud length (mm)	19.5 ± 1.4	26.1 ± 1.1	16.2 ± 1.3	12.4 ± 0.8	13.6 ± 0.9
Bud width (mm)	8.3 ± 0.9	5.5 ± 0.6	6.2 ± 1.6	4.9 ± 0.4	6.6 ± 1.1
Number of anthers	5 ± 0.0	5.4 ± 0.2	5 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
Anther length (mm)	7.2 ± 0.7	9.1 ± 0.7	7.8 ± 0.5	7.1 ± 0.5	6.9 ± 0.7
Anther width (mm)	2.0 ± 0.2	1.3 ± 0.2	1.4 ± 0.0	1.7 ± 0.2	1.1 ± 0.1
Stigma length (mm)	10.7 ± 0.7	16.3 ± 1.4	15.5 ± 1.1	11.0 ± 1.3	9.2 ± 0.8
Stigma width (mm)	0.5 ± 0.0	0.2 ± 0.0	0.32 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Pollen density (no.)**	907 ± 1.2	1245 ± 0.9	299 ± 1.3	185 ± 0.8	69.0 ± 0.5
Pollen viability (%)	95.8 ± 5.2	97.43 ± 4.8	78.5 ± 3.0	62.9 ± 2.7	69.4 ± 3.2

The results were significant at $P < 0.05$; *Each observation was an average of five observations; ** Pollen density was observed on 18*18mm cover slip at 40X magnification

has been a morphological marker for the identification of interspecific cross (Fig 2A-D). In literature, somatic hybrids between *Solanum melongena* L. + *S. sisymbriifolium* and interspecific hybrids between *Solanum lycopersicum* L. and *S. sisymbriifolium* also showed intermediate morphological traits (Gleddie et al.1986; Piosik et al.2019). Madalageri and Gowda (1988) also highlighted the spinyness in the F_1 hybrid of *S. macrocarpon* x *S. melongena* on the upper surface of leaves. In our study, both the parents had clustered flowers, while the regenerated interspecific plants carried improvement in the number of flowers per inflorescence. This might have occurred due to hybrid vigour for this trait. Secondly, the violet and light violet colour of flowers also showed the inheritance of trait from female parent (H5) that was again a cross between purple (SR-9322) and white (BLW-231) flowers. In an earlier study on *S. melongena* (P-12) x *S. macrocarpon* this type of improvement in number of flowers and inheritance of purple colour from wild parent was also reported (Gowda et al.1990). Our study was also in line with the findings of Patel et al. (2001) and Kumchai et al. (2013) in the interspecific hybrid between the eggplant cultivars and *Solanum* species.

Molecular confirmation

The hybridity of plants regenerated through embryo rescue culture, in present investigation, was also confirmed through SSR molecular markers. Two SSR markers emg01A17 and emh11001, showed polymorphism between the alleles of the parents (P1, P2, H5 and *S. sisymbrifollium*) and also displayed the presence of all alleles in three-way interspecific regenerants (Fig 3). *S. melongena* parents and their hybrid (H5) did not show polymorphism, but all these genotypes amplified a different allele in comparison to *S. sisymbriifolium*. In our study, SSR markers showed Monomorphic results between the parents of S. melongena hybrid (H5), while H5 and *S. sisymbriifolium* were polymorphic and helped in the identification of interspecific cross. Similar to our results, the literature also highlighted that the limited numbers of SSR and SNP markers have proved useful for confirmation of hybridity between *S. melongena* and the wild species (Rakha et al. 2020).

Reproductive potential

The reproductive potential of an interspecific cross decides its further utilization in crop improvement programme. The results presented in Table 4 indicated that three-way cross unveiled intermediate behavior for the dimensions of male

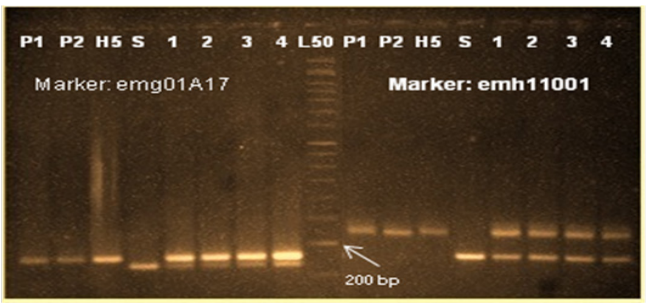

Fig. 3. Molecular confirmation of hybridity of four Three-way plants between *S. melongena* and *S.sisymbriifolium* (P1=SR-9322, P2=BLW231, H5= SR-9322X BLW-231, S= *S. sisymbriifolium,* 1-4 three-way plants, L = 50bp)

and female reproductive parts. The pollen density of these plants reduced (69-299) to a greater extent as compared to their male (1245) and female parents (907). The pollen viability indicating male fertility in three-way cross plants was also lower (62.9-78.5%) than *S. melongena* (H5) (95.8%) and *S. sisymbriifolium* (97.4%). Among three-way cross plants, the highest pollen viability was observed in plant 1 (78.5%) followed by plant 3 (69.4%) and plant 2 (62.9%). Pink stained pollen grains with acetocarmine dye of three-way cross plants showed partial-fertility with varying pollen density (Fig 2C-E). These plants did not set fruits either with self-pollination or backcross to *S. melongena*. With the best of our knowledge about the interspecific hybridization between *S. melongena* and *S. sisymbriifolium*, this is the first report describing the possibility of development three-way cross plants individually carrying differential behavior for male fertility. Partial fertility of pollen grains with poor pollen density might be the reason for no fruit set on selfing. Similar to *S .sisymbriifolium*, three-way cross could not set fruit as female parent during backcross. In previous studies also, few researchers succeeded interspecific cross between *S. sisymbriifolium* and cultivated eggplant, but there is no record of development of fertile progenies with good pollen viability (Bletsos et al. 1998, Rakha et al. 2020). The major problem had been the sterility of interspecific somatic cross because of less pollen production and low pollen viability (Collonnier et al. 2003). Although attempts have been made to restore fertility through the development of amphidiploids (Curuk and Dayan 2018), these plants again could not be directly utilized in introgression breeding due to differences in ploidy level (2n=4X=48). In another study for interspecific hybridization with *S. torvum*, we have developed fertile reciprocal backcross (study submitted). Therefore, the improvement in percentage pollen viability in diploid three-way cross in comparison to already available literature also suggested their use as male parent in reciprocal backcrosses and microspore culture for introgression of important agronomic traits.

Author's contributions

Conceptualization of research (MKS, NKS); Designing of the experiments (MKS, JK, NKS); Contribution of experimental materials (MKS, NKS, ASD); Execution of field/lab experiments and data collection (JKS); Analysis of data and interpretation (JK, MKS); Preparation of manuscript (JK, MKS).

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References

Bagheri M. 2010. Collection, conservation and breeding of Iranian eggplant landraces. Advances in Genetics and Breeding of Capsicum and Eggplant, Editorial de la Universitat Politècnica de València, Valencia, Spain, P421

Bletsos F.A., Roupakias D.G., Tsaktsira M.L., Scaltsoyjannes A.B., and Thanassoulopoulos C.C. 1998. Interspecific hybrids between three eggplant (*Solanum melongena* L.) cultivars and two wild species (*Solanum torvum* Sw. and *Solanum sisymbriifolium* Lam.). Plant Breed., **117**:159-164. https://doi.org/10.1111/j.1439-0523.1998.tb01471.x

Collonnier U., Mulya K., Fock I., Mariska I., Servaes A., Vedel F., Siljak-Yakovlev S., Souvannavong V. and Sihachakr D. 2003. Somatic hybrids between *Solanum melongena* and *S. sisymbriifolium* as a useful source of resistance against bacterial and fungal wilts. Plant Sci**., 164**: 849-861. DOI:10.1016/S0168-9452(03)00075-X

Curuk S. and Dayan A. 2018. Production of diploid and amphidiploid interspecific hybrids of eggplant and *Solanum torvum*, and pollen fertility. J. Anim. Plant Sci., **28**: 1485-1492.

Doyle J.J. and Doyle J.L. 1990. Isolation of plant DNA from fresh tissue. Focus, **12**: 3-15.

Gleddie S., Keller W.A. and Setterfield G. 1986. Production and characterization of somatic hybrids between *Solanum melongena*, L., and *S. sisymbriifolium* Lam. Theor. Appl. Genet., **71:** 613-621.

Gowda P.H.R., Shivashankar K.T. and Joshi S.H. 1990. Interspecific hybridization between *Solanum melongena* and *Solanum macrocarpon*: study of the F1 hybrid plants. Euphytica, **48**: 59–61.

Madalageri B.B. and Gowda P.H.R. 1988. Inheritance of characters in the progeny of *Solanum macrocarpon* and *S . melongena*. Proc. Conf. Cytol. and Genet., **1**: 199-202.

Meena, H. P., Sujatha, M., and Soni, P. K. 2017. Interspecific hybrid between cultivated sunflower (*Helianthus annuus* L.) and silver leaf sunflower H. argophyllus T. and G.: Cytomorphological and molecular characterization. Indian J. Genet. Plant Breed., **77:** 547–555. Retrieved from https://www.isgpb.org/journal/index.php/IJGPB/article/view/28

Morgan E.R., Timmermann-Vaughan G.M., Conner A.J., Griffing W.B. and Pickering R. 2011. Plant interspecific hybridization: Outcomes and issues at the intersection of species. Plant Breed. Rev**., 34**: 161-220. DO - 10.1002/9780470880579.ch5

Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant., **15**: 473-97.

Nwofia G.E. and Eneoblong E.E. 2001. Barriers to interspecific hybridization among non-tuberous *Solanum* species. J. Appl. Chem. Agric. Res., **7**: 37-41.

Patel D. A., Shukla P. T., & Jadeja G. C. 2001. Morphological studies on interspecific hybrids between *Solanum indicum* L. and *Solanum melongena* L. Indian J. Genet. Plant Breed., **61:** 180–182. https://doi.org/.

Piosik L., Ruta-Piosik M., Zenkteler M. and Zenkteler E.Z. 2019. Development of interspecific hybrids between *Solanum lycopersicum* L. and *S. sisymbriifolium* Lam. via embryo calli. Euphytica, **215**: 1-20.

Plazas M., Vilanova S., Gramazio P., Rodríguez-Burruezo A., Fita A., Herraiz F. J., Ranil R., Fonseka R., Niran L., Fonseka H., Kouassi B., Kouassi A., Kouassi A., and Prohens J. 2016. Interspecific hybridization between eggplant and wild relatives from different gene pools. J. Amer. Soc. Hort. Sci., **141:** 34-44. Retrieved Jun 1, 2023, from https://doi.org/10.21273/JASHS.141.1.34

Prasad P. P. V., Boote K. J. and Hartwell Allen L. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. Agric. For Meteorol. **139**: 3-4.

Rakha M., Namisy A., Chen J. R., El-Mahrouk M.E., Metwally E., Taha N., Prohens J., Plazas M. and Taher D. 2020. Development of interspecific hybrids between a cultivated eggplant resistant to bacterial wilt (*Ralstonia solanacearum*) and eggplant wild relatives for the development of rootstocks. Plants, **9**: 1-13. doi: 10.3390/plants9101405

Rattan P., Kumar S., Salgotra R. K., Samnotra R.K. and Sharma F. 2015. Development of interspecific F_1 hybrids (*Solanum melongena* x *Solanum khasianum*) in eggplant through embryo rescue technique. Plant Cell Tiss. Organ Cult., **120**: 379-86. https://doi.org/10.1007/s11240-014-0591-4

Rotino G. L., Perri E., Acciarri N., Sunseri F. and Arpaia S. 1997. Development of eggplant varietal resistance to insects and diseases via plant breeding. Adv. Hort. Sci., **11:** 193–201. http://www.jstor.org/stable/42883182

Rotino G.L., Sala T. and Toppino L. 2014 . Eggplant. In: Pratap A. and Kumar J. (eds.). Alien gene transfer in crop plants. Volume 2. Springer, New York, NY. , Pp 381–409.

Sharma D.R., Sareen P.K. and Chowdhary J. B. 1984. Cross-ability and pollination in some non-tuberous *Solanum* species. Indian J. agric. Sci., **59**: 514-516.

Sihachakr D., Daunay M.C., Serraf I., Chaput M.H., Mussio I., Haicour R., Rossignol L. and Ducreux G.1994. Somatic Hybridization of Eggplant (*Solanum melongena* L.) with Its Close and Wild Relatives. In: Bajaj Y.P.S. (eds) Somatic Hybridization in Crop Improvement I. Biotechnology in Agriculture and Forestry, vol 27. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-57945-5_17.

Singh M., Kumar S., Srivastava K., Banerjee M.K. and Kalloo G. 2002. Wide hybridization of eggplant (*Solanum* spp.) Capsicum Eggplant Newsl., **21**: 89-92.

Verba V.M., Mamedov M.I., Pyshnaya O.N., Suprunova T.N., and Shmykova N.A. 2010. Isolation of eggplant interspecific hybrids by the method of embryo culture. Agric. Biol., **5**: 66–71.

Weese T.L. and Bohs L. 2010. Eggplant origins: Out of Africa, into the Orient. Taxon, **59**: 49-56.