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Wide Hybridization and Embryo-Rescue for Crop Improvement in *Solanum*

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Abstract

Tomato is known in the literature as *Solanum lycopersicum*, as well as *Lycopersicon esculentum*. In north eastern region of India, cultivation of tomato in rice fallow is becoming popular and may be helpful in increasing production of vegetables, which will not only increase per capita availability of vegetables, but also improve the economic condition of the farmers through employment generation. Tomato is highly prone to biotic stresses, especially diseases, insects and nematodes. Genes are available in different wild species, but it has not been easy to transfer these genes in cultivated species due to problems in crossability. *Solanum lycopersicum* was crossed with *S. peruvianum* and *Solanum pimpinellofolium*. 25 days after pollination was found to be the optimum time for rescuing the embryos. Murashige and Skoog's (MS) medium supplemented with 1 mg/l GA3, 0.1 mg/l NAA and 0.5 mg/l BAP was found to be the most effective for germination of the immature putative hybrid embryos. The confirmation of hybridity of the embryo rescued plants from the interspecific crosses of both *S. lycopersicum* var. MT-3 and *S. lycopersicum* var. Kashi Amrit with *S. peruvianum* (WIR-3957) was done using RAPD markers.

Keywords: Interspecific-hybridization; *Solanum* species; Embryo-rescue; RAPD

Introduction

North-East India [1] is one of the 12 mega bio-diversity hot spots in the world [2]. In vegetables, at least 12 species of *Solanum*, are consumed by the local people. Many wild relatives can also contribute as donors in the hybridization programme. Enormous diversities exist within *Solanum* at the interspecific level, and also in their landraces. Tomato is a self pollinated crop, which is a high demand vegetable crop in many parts of the world. Hybrid seed production from wide hybridization involves the fusion of the male and female gametes, where the aim of the crossing programme is to transfer important traits from the wild species to the already cultivated and popular species. However, in some of the wide crosses, the production of hybrid seeds is greatly hampered due to certain fertilization barriers. Thus, to meet the demand for hybrid tomato seed production and to overcome certain barriers to fertilization, the present research studies was conducted to study the crossability between the cultivated species and the wild accessions, pollen germination and pollen tube growth in the crosses performed, embryo rescue of the immature hybrid seeds, and the confirmation of hybridity of the rescued plants by RAPD markers.

Interspecific Hybridization and Embryo Rescue in *Lycopersicon*

India ranks third in terms of production worldwide, with an area of 0.69 million ha and production of 11.98 million tons of tomato, annually [3]. It is the second most important vegetable crop in Meghalaya. *Fusarium* wilt (*Fusarium oxysporum f.sp. lycopersicae*), late blight (*Phytophthora infestans*), and early blight (*Alternaria solani*) are its important diseases. Tomato fruit borer (*Helicoverpa armigera*) is an important insect pest of tomato. Wild species are reservoir of important genes, which when used in breeding programmes, can yield better quality tomato plants and fruits. According to Sharma et al. [4], *Solanum pimpinellifolium* is the only red-fruited wild species of tomato, and the only species from which natural introgression into the cultivated tomato has been self-compatible and bi-directionally cross-compatible with the cultivated tomato. Because of the close phylogenetic relationship between the two species, there is little or no difficulty in initial crosses in subsequent generations of pre-breeding and breeding activities. Furthermore, *S. pimpinellifolium* harbors numerous desirable genes for disease resistance, abiotic stress tolerance and good fruit quality. *S. pimpinellifolium* and *S. peruvianum* contain genes which confer resistance to *Fusarium* wilt and early blight [5]. Crossability barriers between *S. lycopersicum* and *S. peruvianum* have hindered the efficient introgression of important characteristics into the cultivated tomato gene pool. Both pre-zygotic and post-zygotic barriers prevent interspecific hybridization between these two distantly related species [6].

The present investigation was carried out on three species viz., *Solanum lycopersicum* (varieties Megha Tomato-3 and Kashi Amrit), *Solanum peruvianum* (WIR-3957) and *Solanum pimpinellifolium* (EC-521030) to study crossability among different species of Solanum (Synonym: *Lycopersicon*), to find out whether there is any difference in reciprocal crosses, the correlation between pollen germination, pollen tube growth, and abnormal pollen tubes with fruit set, to get hybrids between different species by embryo-rescue, and to confirm the hybridity. Embryo rescue was conducted on crosses of *Solanum lycopersicum* (Kashi Amrit)×*Solanum peruvianum* (WIR-3957) and *Solanum lycopersicum* (MT-3)×*Solanum peruvianum* (WIR-3957).

Materials and Methods

The experimental material used in the study comprised of three species viz., *Solanum lycopersicum* (varieties Megha Tomato-3 and Kashi Amrit), *Solanum peruvianum* (WIR-3957) and *Solanum pimpinellifolium* (EC-521030). Megha Tomato-3 variety was developed by ICAR Research Complex for NEH Region, India, and carries genes against bacterial wilt and can survive under low temperatures. Kashi

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Amrit is a determinate variety derived from interspecific cross between *S. lycopersicum* and *S. hirsutum* through backcross pedigree method.

Experimental strategies were developed by adopting various standard and self developed techniques. They were suitably modified, as per the need of the experiment. The complete course of the investigations was divided into the following parts:

Crossability studies

The seeds were germinated in germinating trays and transplanted in pots containing a mixture of soil, sand and Farmyard Manure (FYM), in the ratio of 1:1:1. Prior to transplanting, the soil was drenched with 0.1% Captan and 0.05% Carbendazim to prevent the occurrence of damping off disease. Transplanting was done one day after soil treatment. The seedlings were transplanted four weeks after sowing in the afternoon. The pots were kept inside a polyhouse, and light with frequent irrigation was given to the plants.

Selfing and inter-specific crosses: Crosses were made in the month of March-April, 2012. Flowers were allowed to self pollinate, and crosses between the various genotypes were made in all possible combinations. Flowers from healthy plants were selected for the process of emasculation. Flower buds of female parents were hand-emasculated one day before anthesis at 3.00 p.m. and the pistils were bagged using butter paper bags to avoid contamination from foreign pollen. Next morning, fresh pollen was collected from the male parent and dusted on the stigma of the female parent, and pollinated pistils were labeled. Pollination was performed from 7 a.m. to 10 a.m., using opened yellow colored flowers as the source of pollen.

Pollen viability, pollen germination, pollen tube growth and fruit set: Pollen were collected from opened flowers and stained with Acetocarmine solution and observed under the microscope. The viable pollen stained reddish in color, whereas those which were not viable did not take up the stain. The data of viable and non-viable pollen was recorded on ten samples and expressed in percentage, and compared with the percent fruit set.

In order to observe pollen germination on the stigma, pollen tube development in the style and the entry of tubes into ovules, the styles were collected 8, 16 and 24 hours, following hand pollination. At least three samples after different timings were removed and fixed in 1:3 glacial acetic acid-ethyl alcohol for 24 hours, after which they were washed in distilled water three times. The pistils were then transferred to aniline blue dye, and the method as reported by Sirohi et al. [7] was followed, to study the percentage pollen germination, which was compared with percent fruit set. The rate of pollen tube growth was measured with a microscope, with an ocular micrometer and the length of only the three largest pollen tubes was recorded. Pollinated styles were observed for fruit set till maturity, and the percent fruit set was expressed as the total number of fruit set in a cross divided by the number of stigmas pollinated, and multiplied by 100.

Correlation studies for various characters in inter-specific crosses of *Capsicum*

The mean data were subjected to statistical analysis and the standard errors of means of the characters were calculated following Singh and Choudhary [8]. Correlation coefficients between different pre- and post-fertilization characters were estimated following Panse and Sukhatme [9].

In vitro studies: embryo rescue

The crosses which yielded a low fruit set, i.e., *S. lycopersicum*×*S. peruvianum* were used for embryo rescue.

Effect of the age of the hybrid embryos on development, when cultured on media: The immature fruits of *S. lycopersicum*×*S. peruvianum* were harvested at 15, 25 and 35 days after pollination, and brought to the laminar hood. The embryos were extracted from the sterilized fruit and cultured on Murashighe and Skoog's [1] medium, supplemented with NAA, BAP and GA3 (Table 1). The best growth response was seen with the embryos which were taken out from immature fruits, after 25 days of pollination. Only these were used for further studies to get the putative hybrids.

Effect of media: The maximum germination of the embryos was seen in the Murashighe and Skoog's [1] media supplemented with 1 mg/ GA3, 0.1 mg/l NAA and 0.5 mg/l BAP (Table 2). The cultured embryos were kept in the culture room. A photoperiod of 16 hours light and 8 hours dark period was maintained. The temperature was maintained at 25 ± 2°C, with humidity of 65% and under a relative humidity of 60%. The cultures were observed at regular intervals for germination, and the embryo germination percentage was expressed as the number of germinated embryos divided by the total number of cultured embryos, and multiplied by 100. The germinated embryos were acclimatized by growing them in potting mixture of peat, vermiculite and lignite (1:1:1 ratio) in small pots in the culture room, until transplanted. Irrigation of the plantlets was done periodically. The acclimatized plantlets at 5-6 leaf stage were transferred to pots containing soil, FYM, and sand in the ratio of 1:1:1.

Confirmation of hybridity by RAPD markers

The determination of hybridity of embryo-rescued plants was carried out using RAPD markers. DNA from the parents and the hybrids was extracted from leaf samples, using CTAB simple mini-prep method of DNA extraction [10]. Ten decamer oligonucleotide primers selected from the literature were used for differentiating the hybrids from the parents (Table 3). The method followed was as per Saxena et al. [11]. Out of the ten primers, only 5 primers which showed polymorphism between the parents were selected for confirming the

Sl. No	Age of the embryo (days after pollination)	Growth response
1	15	Excision of embryos was difficult due to small size. Few embryos germinated.
2	25	Embryos showed best growth. Large number of healthy plantlets was rescued at this stage.
3	35	Only a few embryos could be excised. Most seeds had shriveled and only few seeds were present in a fruit.

Table 1: The effect of age of the hybrid embryos on development, when cultured on the MS medium.

Media	NAA (mg/lit)	BAP (mg/lit)	GA3 (mg/lit)	Number of embryos cultured	Number of embryos germinating	Germinating Embryos (%)
M-1	0.1	0.5	0.1	20	4	20
M-2	0.1	0.5	0.5	20	6	30
M-3	0.1	0.5	1.0	20	16	80
M-4	0.1	1.0	0.1	20	10	50
M-5	0.1	1.0	0.5	20	2	10
M-6	0.1	1.0	1.0	20	8	40

Table 2: Effect of media composition on germination of embryos of *S. lycopersicum*×*S. peruvianum*.

Sl. No.	Primer's name	Sequence (5'---3')	Tm(°C)	GC content (%)
1.	OPAB-3	TGGCGCACAC	43.1	70
2.	OPAB-4	GGCACGCGTT	45.6	70
3.	OPAB-5	CCCGAAGCGA	45.7	70
4.	OPAB-7	GTAAACCGCC	34.6	60
5.	OPAB-15	CCTCCTTCTC	25.7	60
6.	OPAB-16	CCCGGATGGT	42.7	70
7.	OPAB-17	TCGCAACCAG	37.0	60
8.	OPAB-18	CTGGCGTGTC	35.3	70
9.	OPAB-19	ACACCGATGG	33.9	60
10.	OPAB-20	CTTCTCGGAC	27.3	60

Table 3: List of decamer RAPD primers used to confirm the hybridity of putative hybrids.

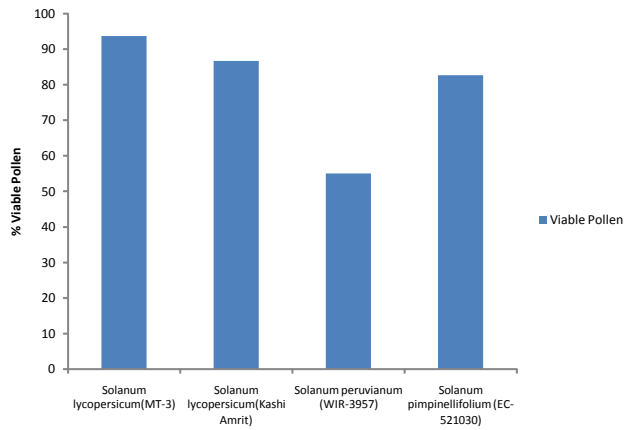

Figure 1: Percent pollen viability in the four parents and the three species of *Solanum* used in the experiments.

hybridity of the embryo rescued plants. These primers were OPAB-7, OPAB-17, OPAB-18, OPAB-19 and OPAB-20.

Results and Discussion

Pollen viability

In the present study, *S. lycopersicum* MT-3 (93.66 ± 0.88) had maximum number of viable pollen; whereas, *S. peruvianum* accession WIR-3957 (55 ± 1.15) had the least viable pollen percentage (Figure 1). The pollen viability in this study was recorded during the month of March, where the average temperature was 28°C. Pollen viability of 98.6% was recorded by Prasad and Batham [12] in Pusa Ruby in the month of December, when temperature was 15 ± 20°C and relative humidity of 82.5 ± 0.5%.

Pollen germination

At 24 hours after pollination, maximum number of germination was seen in the selfing of *S. lycopersicum* MT-3 (40.33 ± 2.40), and minimum pollen germination was seen in the cross of *S. lycopersicum* variety Kashi Amrit and *S. peruvianum* (WIR-3957) (20.66 ± 1.85), with the latter as the pollen donor (Figure 2). Normal pollen germination was recorded in selfings, interspecific crosses of cultivated species with wild species and in the reciprocal crosses. Similar normal pollen germination in the interspecific crosses of *S. lycopersicum* and the wild species (S. chilense, *S. peruvianum* and *S. hirsutum*) was reported by Pico et al. [13]. Dane et al. [14] noted reduced pollen fertility after prolonged periods of high temperature in the field. Response of pollen to heat treatments in tomato was genotype dependent, and not a general predictor of fruit set under high-temperature stress [15]. Pollen germination had started 8 hours after pollination.

In the present study, a positive and significant correlation between pollen germination and fruit set was established, 24 hours after pollination. The initial step for pollen–pistil interaction is the physical adhesion of the pollen grain to the stigma. Following physical contact with the stigma, pollen becomes hydrated and produces the pollen tube. In tobacco, lipids are thought to be essential for pollen hydration and tube growth [16].

Pollen tube growth

The germination of pollen showed a continuous increase, from 8 hours to 24 hours after pollination. At 24 hours after pollination, the pollen tubes had penetrated through the stigma hairs. The number of pollen tubes penetrating the stylar tissues increased with time. At 8 hours after pollination, the germination of the pollen was very less, and only a few pollens had germinated. Most of the pollen of *S. lycopersicum* had germinated at this hour, but had not entered through the style. A few pollen tubes had just entered the stigma. At 18 hours after pollination, most of the pollen had germinated and entered through the style. Some of the pollen had just penetrated through the stylar hairs. At 24 hours, the pollen tube was long and had moved a long distance through the style, but due to poor staining, the pollen tube movement could not be traced further. Pollen tube growth had a positive correlation with fruit set. The correlation was also found to be significant (Table 1).

The pollen tubes in the selfing of *S. lycopersicum* MT-3 were the longest (623.5 ± 0.76), and followed by *S. lycopersicum* var. KA (621.7 ± 0.83). The findings are in agreement with the results of Pico et al. [13], in which the pollen tubes in the interspecific cross of *S. lycopersicum* and the wild species showed a slower pollen tube growth. Pollen tubes traverse the length of the style by 24 h post-pollination in self pollinations of *S. lycopersicum* [16]. Martin [17] reported that pollen tubes of the *Lycopersicon esculentum* are inhibited in the upper portion of the styles, when it is crossed with *L. peruvianum*. Chen and Adachi [18] observed that the average pollen tube length of *Lycopersicon esculentum*×*L. peruvianum* and *Lycopersicon esculentum* selfing at 30 min, 1 hr and 3 hr after pollination were 50-90, 0.1-0.4 and 0.1-1 µm, respectively. The fluorescent microscopy of the reciprocal crosses showed that the tip of the pollen tube of *Lycopersicon esculentum* in *L. peruvianum* style looked swollen, and the growth was stopped. Kozik

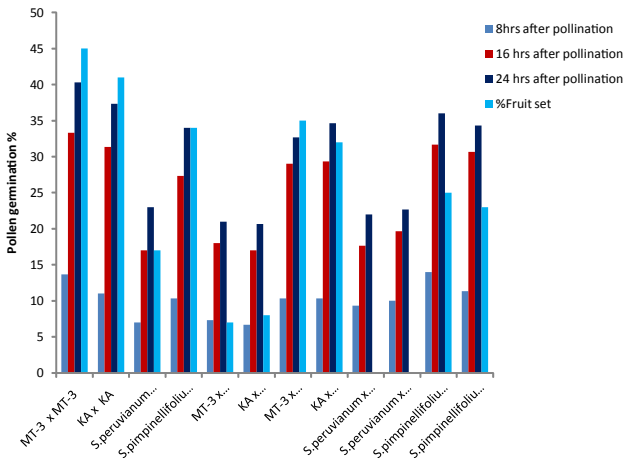

Figure 2: Pollen germination and fruit set at different timings on selfing and after inter-specific crosses of the four parents and the three species of *Solanum* used in the experiments.

and Dyke [19] studied the self and cross compatibility of *L. esculentum*, *L. penelli, L. chinense* and *L. hirsutum*, and observed that most of the pollen tubes in the former had entered the ovaries of the entire pistil analyzed.

Fruit set

Tomato seed production is highly influenced by environmental factors, particularly temperature, which has a significant effect on all stages of plant growth and development. Day and night temperature, and the variation between the two, has pronounced effect on flowering, fruiting and yield of fruits and seeds in tomato, but the night temperature is a critical factor for fruit set in tomato. The optimum temperature for fruit set in tomato ranges between 15-20°C [14]. Optimum moisture during flowering and fruit setting is essential for fruit set. Spraying of growth hormones, like NAA during flowering, is known to increase fruit set in tomato. The time of pollination also affects fruit set in crops, which may be due to their influence on pollen germination and pollen tube growth on the pistil. In our study, *S. lycopersicum* MT-3 (45%) and *S. lycopersicum* variety KA (41%) gave maximum fruit set (Figure 3). The fruit set on selfing *S. lycopersicum* was also good. This could be attributed to the higher pollen germination and pollen tube growth, which was recorded in the selfings of these species (Figures 2 and 4). Maximum fruit set in interspecific crosses was obtained in the cross between *S. lycopersicum* (MT-3)×*S. pimpinellifolium* (35%) and *S. lycopersicum* (KA)×*S. pimpinellifolium* (32%). The lowest fruit set was seen in *S. peruvianum*×*S. lycopersicum* var. MT-3 and *S. peruvianum*×*S. lycopersicum* var. KA. The interspecific cross of *S. lycopersicum* var. MT-3 with *S. peruvianum*, and the cross of *S. lycopersicum* var. KA with *S. peruvianum* gave 7% and 8% fruit set, respectively. However, the reciprocal crosses of these species with *S. peruvianum* as the female parent resulted in no fruit set. This was also reported by Pico et al. [13], when the wild species *S. peruvianum* was used as a female parent and *S. lycopersicum* as a pollen donor, which also resulted in zero fruit set.

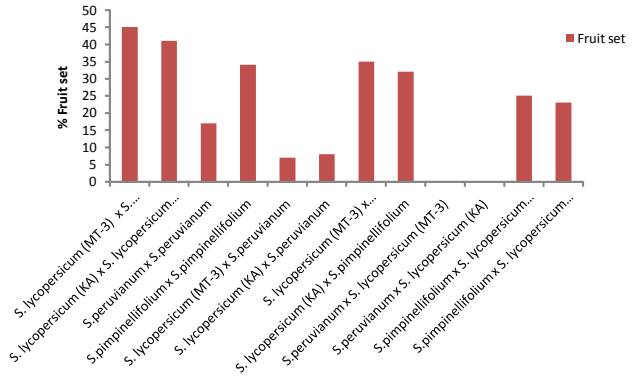

Figure 3: Percent fruit set on selfing and after inter-specific crosses of the four parents and the three species of *Solanum* used in the experiments.

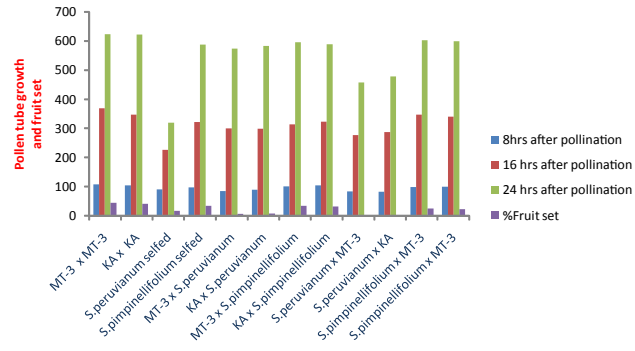

Figure 4: Pollen tube growth (µm) and fruit set at different timings on selfing and after inter-specific crosses of the four parents and the three species of *Solanum* used in the experiments.

Relationship of the different parameters with fruit set

The fruit set showed a positive correlation with viable pollen, which was non-significant (Table 2). The fruit set also depicted a positively significant correlation with pollen germination and pollen tube growth in the various selfings, and in the interspecific and reciprocal crosses of Solanum. However, in the interspecific crosses of *S. peruvianum* (WIR-3957)×*S. lycopersicum* variety Megha Tomato-3, and *S. peruvianum* (WIR- 3957)×*S. lycopersicum* variety Kashi Amrit, no fruit set was recorded (Figure 3), which might have resulted due to pre-fertilization barriers in the interspecific cross.

Correlation studies

A positive correlation of fruit set with pollen germination and pollen tube growth was found, which was significant at 5% level of significance. A positive correlation between fruit set and pollen viability was observed, which was not significant at 5% level of significance.

The immature hybrid embryos in the present study revealed that 25 days after pollination was the optimum stage for rescuing the immature embryo (Table 4). Chen and Adachi [18] in their studies reported that embryos excised from interspecific cross of *Solanum lycopersicum* and *Solanum peruvianum* gave a peak germination percentage at 19-22 days after pollination, when cultured on HLH medium supplemented with 1 g/l of yeast extract and 2 mg/l of BAP. Bhattarai et al. [20] found that MS medium supplemented with 0.5 mg l^{-1} GA3, 0.1 mg l^{-1} IAA and 0.5 mg l^{-1} BAP was most effective for germination (60%), and regeneration of 10 days old embryos. In the present study, MS medium supplemented with 1 mg l^{-1} GA3, 0.1 mg l^{-1} NAA and 0.5 mg l^{-1} BAP was most effective for germination (80%) of 25 days old embryos (Table 3). The age and correct composition of the growth has a great influence on the success of rescuing the hybrids. According to Hossain et al. [21], explants 28-33 days after pollination and GA_3@ 0.5 mg/l and NAA@ 0.05 mg/l showed good results. The breeding barriers in interspecific hybrids of tomato have been overcome using two given methods. Firstly, hybrid embryos have been rescued by embryo culture [22], ovule culture [23], and plant regeneration from ovule-derived callus [24]. Secondly, normal seed development has been obtained by adjusting the environment and crossing factors, during the crossing period and fruit growth [25], pollination with gamma-ray irradiated pollen grains [26], use of polyploidy [27], bridge crossing [28], and the selection of self-compatible species [29]. However, the plants obtained are very few. In our study, the low seed set in the hybrid fruits and the occurrence of brown wrinkled seeds inside the fruit suggested that post-fertilization abnormalities occurred in the interspecific hybrid fruits of *S. lycopersicum*×*S. peruvianum*. Also, the fruit set in this cross

Characters	Pollen tube growth	Percent viable pollen	Percent fruit set
Percent pollen germination	0.636	0.649	0.916*
Pollen tube growth		0.362	0.554*
Percent viable pollen			0.371

*Significant at 0.05 probability level

Table 4: Correlation among various characters in interspecific crosses of the three species of Solanum used in the experiments.

was 7-8%, when two different varieties were used in this cross (Figure 3). In the reciprocal crosses, no fruit set took place. In the other crosses and the selfing of the parents, fruit set varied from 17-45%. Therefore, embryo-rescue was tried only in the crosses between *S. lycopersicum*×*S. peruvianum*, in which there was very little fruit set. Crossability barriers between *S. lycopersicum* and *S. peruvianum* have hindered the efficient introgression of important characteristics into the cultivated tomato gene pool. Both pre-zygotic and post-zygotic barriers prevent interspecific hybridization between these two distantly related species [6]. More specifically, a unilateral incompatibility mechanism prevents the cross between *S. peruvianum* and *S. lycopersicum*, when the latter is used as the male parent. In this case, pollen tube usually stops, before fertilization can occur [29] (Plate 1 and 2).

Hybridity test by molecular markers

Confirmation of hybridity of the putative hybrids in this study was done using RAPD markers. A total of five 10-mer RAPD primers which showed polymorphism between the parents were used to confirm hybridity. The average scorable bands in the present study were 7 per primer. The average polymorphic bands were found to be 2.2 per primer. Similar results were also reported by Debbarma [30], using 8 RAPD primers for the confirmation of hybridity in *Capsicum* interspecific hybrids, where the average scorable bands per primer were 6.62, and an average of two polymorphic bands per primer was reported.

The dominant inheritance of RAPD markers does not pose a problem for checking the hybridity of the putative hybrids, because only the polymorphism which is associated with the male parent is considered for confirming the hybridity [31]. The hybridity in this study was also screened by first distinguishing the clear polymorphic bands between the male and the female parent. The hybridity of the putative hybrids was then confirmed by the presence or absence of the male specific bands. The plants which show the absence of the male specific bands, and resembling the female banding pattern are most likely to be the embryos resulting from selfing. The reproducibility of the primers which showed specific polymorphic bands was determined by repeating the amplification for three more times.

Plate 1: Embryo rescued plantlets of *S. lycopersicum* (Megha Tomato-3×*S. peruvianum*, 30 days after culturing in MS medium.

Plate 2: Acclimatization of embryo rescued plants from *S. lycopersicum* (Megha Tomato-3)×*S. peruvianum* inside the plant growth chamber.

Pico et al. [13] used RAPD markers to confirm the hybridity after *S. lycopersicum* and *S. peruvianum* crosses. RAPD markers were also successfully employed in confirming the hybrid nature of the somatic hybrids of *S. lycopersicum* cv. Pusa Ruby×*S. peruvianum* and *S. lycopersicum* cv. Pusa Ruby×*S. chilense*. In the present study, primer OPAB-7 showed a unique band at approximately 850 bp in the putative hybrid of *S. lycopersicum* (MT-3)×*S. peruvianum* (WIR-3957). Also, a unique band was scored in *S. lycopersicum* (KA)×*S. peruvianum* (WIR-3957) at 750 bp, which was absent in both the parents. The presence of unique bands specific to the hybrids and absent in both the parents can occur, which may be due to the DNA recombination or mutation. Chromosomal crossing-over during meiosis may have resulted in the loss of priming sites, and thus, bands may be present uniquely in the parents only. This was also recorded with primer OPAB-20, which produced a unique band at 1700 bp only in the male parent *S. peruvianum* (WIR-3957).

Putative hybrid of *S. lycopersicum* (KA)×*S. peruvianum* (WIR-3957) and *S. peruvianum* (WIR-3957), both gave unique bands at 1200 bp with primer OPAB-17. This unique band was also seen in *S. lycopersicum* cv. Pusa Ruby×*S. peruvianum* (WIR-3957) putative hybrid. Using primer OPAB-18, unique bands were seen in both the putative hybrids of *S. lycopersicum* (MT-3)×*S. peruvianum* (WIR-3957) and *S. lycopersicum* (KA)×*S. peruvianum* (WIR- 3957), which were also present in the male parent *S. peruvianum* (WIR-3957) at 350 bp and 550 bp. A unique band was scored in *S. lycopersicum* (KA)×*S. peruvianum* (WIR-3957) and *S. peruvianum* (WIR-3957) at 1900 bp, with primer OPAB-20. Primer OPAB-7 generated a unique band in putative hybrids of *S. lycopersicum* (KA)×*S. peruvianum* (WIR-3957), and also in the male parent *S. peruvianum* (WIR-3957) at 2000 bp. Thus, the unique bands specific to both the male and the putative hybrids of the above primers was supportive enough to confirm the hybridity of the immature embryo rescued plants.

Molecular markers has proven to be the most effective means to confirm the hybridity of the plants from various interspecific crosses, which is accurate, and at the same time can be carried out even when the plants are still in the juvenile stages. Contrary to this, the use of morphological parameters to confirm the hybridity of the interspecific hybrids is not the most reliable, since it is affected by the environment and the growth conditions of the hybrids and the parents. In addition, the resolving power of the morphological characters to distinguish hybrids from the parents is also weak, and becomes impractical when the plantlets are in the juvenile stages. Hence, molecular markers are highly regarded for use in hybrid confirmation, because of the quick, accurate and reliable results they produce and besides, they are not subjected to environmental effects (Plate 3).

Conclusion

In the present study, embryo rescue was successfully employed to get hybrids, and RAPD markers were used to confirm the hybrid nature

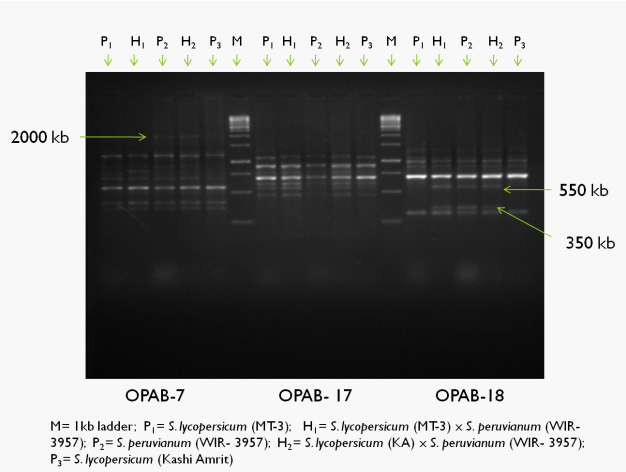

Plate 3: RAPD profile of *S. lycopersicum* and *S. peruvianum*, and their putative hybrids with primers OPAB-7, OPAB-17 and OPAB-18.

of the embryo rescued plants, from the interspecific crosses of both *S. lycopersicum* var. MT-3 and *S. lycopersicum* var. Kashi Amrit with *S. peruvianum* (WIR-3957), in which the fruit set was only 7-8%.

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