Wide Hybridization and Embryo-Rescue for Crop Improvement in Solanum

Herbert P. Kharkongar, V.K. Khanna*, W. Tyagi, M. Rai, and N.T. Meetei

College of Post-Graduate Studies, CAU, Umiam-793103, Meghalaya, India

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 pimpinellifolium. 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. peruvianum 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. lycopersici), 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

*Corresponding author: V. K. Khanna, College of Post-Graduate Studies, CAU, Umiam-793103, Meghalaya, India, E-mail: khannavk@rediff.com

Received December 06, 2012; Accepted December 20, 2012; Published December 27, 2012

Citation: Kharkongar HP, Khanna VK, Tyagi W, Rai M, Meetei NT (2013) Wide Hybridization and Embryo-Rescue for Crop Improvement in Solanum. Agrotechnol S11:004. doi:10.4172/2168-9881.S11-004

Copyright: © 2013 Khanna VK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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% Carbenazim 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 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 glacial acetic acid-ethyl alcohol for 24 hours, after which they were washed in distilled water three times. The pistils were then transferred to Acetocarmine solution and observed under the microscope. The number of stigmas pollinated, and multiplied by 100. The germinated embryos were acclimatized by keeping 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 1). 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                                       | Exclusion 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/l) | BAP (mg/l) | GA3 (mg/l) | 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*.

**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 Murashige 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 Murashige 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.
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 Batram [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 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 stigmas increased with time. At 8 hours after pollination, the germination of the pollen was very less, and only a few pollen 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 stigma. 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 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

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

| Sl. No. | Primer’s name | Sequence (5’--3’) | Tm(°C) | GC content (%) |
|---------|---------------|------------------|--------|----------------|
| 1.      | OPAB-3        | TGCCGCACAC       | 43.1   | 70             |
| 2.      | OPAB-4        | GCCACCGGTT       | 45.6   | 70             |
| 3.      | OPAB-5        | CCGAGAGGGA       | 45.7   | 70             |
| 4.      | OPAB-7        | GTAACCCGCC       | 34.6   | 60             |
| 5.      | OPAB-15       | CCTCCTCTCTC      | 25.7   | 60             |
| 6.      | OPAB-16       | CCCGATGGGT       | 42.7   | 70             |
| 7.      | OPAB-17       | TCGAAACCAG       | 37.0   | 60             |
| 8.      | OPAB-18       | CTGCGGTGTC       | 35.3   | 70             |
| 9.      | OPAB-19       | ACACCGATGG       | 33.9   | 60             |
| 10.     | OPAB-20       | CTCTCGGAGC       | 27.3   | 60             |

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

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 var. 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.

**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 Amir, 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. Bhattachar 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 GA₃@ 0.5 mg l⁻¹ and NAA@ 0.05 mg l⁻¹ 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

| Table 4: Correlation among various characters in interspecific crosses of the three species of Solanum used in the experiments. |
|---|---|---|---|
| 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
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. Crossovers barriers between S. lycopersicum and S. peruvianum have hindered the efficient introgression of important characteristics into the cultivated tomato gene pool. Both pre-nuclear 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.

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
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%.

Acknowledgments

The authors would like to acknowledge funding received from College of PG Studies, Central Agricultural University (CAU), Imphal, Manipur for conducting the experiments. Mr Herbert P. Kharkongar was funded student fellowship by CAU.

References

1. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15: 473-479.
2. Hore DK (2010) Important plant genetic resources of North Eastern India: their sustainable utilization and conservation. In: Nagachan SV, Mishra A, Kadirvel G, Das A, Saikia K (Eds) Conservation of natural resources for sustainable hill agriculture. ICAR Res Complex for NEHR, Umiam, Meghalaya, India.
3. http://faostat.fao.org.
4. Sharma A, Zhang L, Niño-Liu D, Ashrafi H, Foolad MR (2008) A Solanum lycopersicum×Solanum pimpinellifolium linkage map of tomato displaying genomic locations of R-genes, RGA, and candidate resistance/defense-response ESTs. Int J Plant Genomics 2008: 926090.
5. Kallio G (1986) Tomato. In: Breeding habits selection bases and breeding methods. Allied Publisher Private Limited.
6. McGuire DC, Rick CM (1954) Self-incompatibility in species of Lycopersicon sect. Eriopersicon and hybrids with Lycopersicon esculentum. Hilgardia 23: 101-112.
7. Sirohi M, Kaushik N, Khanna VK (2004) Pollen tube behaviour and effect of wheat genotypes on embryo induction in wheat×maize crosses. In: Proceedings of the 4th International Crop Science Congress at Brisbane, Australia.
8. Singh RK, Choudhary BD (1985) Elementary Statistics. In: Biometrical Methods in Quantitative Genetics Analysis. Kalyani Publishers, New Delhi, India.
9. Panse VG, Sukhatme PV (1985) Correlation and regression. In: Statistical methods of agricultural workers, ICAR, New Delhi, India.
10. Anand A, Gupta AK, Teshale ET, Mishra A, Khanna VK (2008) Phylogenic relationship to study the ploidy status and resistance to Karnal Bunt in Indian wheat cultivars using RAPD technique. Biotechnology 7: 430-438.
11. Saxena P, Jaiswal JP, Khanna VK (2009) Assessment of genetic diversity at molecular level in wheat and its wild relatives with DNA markers using RAPD. Panth Nagar Journal of Research 7: 38-43.
12. Prasad A, Batham RD (1975) Studies on pollen morphology, viability and germination producing F1 hybrid seed. Proc Am Soc Hort Sci 46: 277-283.
13. Pico B, Herrai J, Ruiz JJ, Nuez F (2002) Widening the genetic basis of virus resistance in tomato. Sci Hort 94: 73-89.
14. Diane F, Hunter AG, Chambles OL (1991) Fruit set, pollen fertility, and combining ability of selected tomato genotypes under high temperature field conditions. J Am Soc Hortic Sci 116: 906-910.
15. Abdul-Baki AA, Stommel JR (1995) Pollen viability and fruit set of tomato genotypes under optimum- and high- temperature regimes. Hort Science 30: 115-117.
16. Lush WM, Griesser F, Wolters-Arts M (1998) Directional guidance of Nicotiana alata pollen tubes in vitro and on the stigma. Plant Physiol 118: 733-741.
17. Martin FW (1967) The genetic control of unilateral incompatibility between two tomato species. Genetics 56: 391-398.
18. Chen L, Adachi T (1992) Embryo abortion and efficient rescue in interspecific hybrids, Lycopersicon esculentum and the ‘peruvianum-complex’. Japan J Breed 42: 65-77.
19. Kozik EU, Dyki B (2001) Compatibility studies in three wild species of Lycopersicon. Acta Physiol Plant 23: 65-72.
20. Bhattacharai SP, de la Pena RC, Midmore DJ, Palchamy K (2009) In vitro culture of immature seed for rapid generation advancement in tomato. Euphytica 167: 23-30.
21. Hosseins MA, Minami M, Nemoto K (2003) Immature embryo culture and interspecific hybridization between Capsicum annuum L. and Crutescens L. via embryo rescue. Japanese Journal of Tropical Agriculture 47: 9-16.
22. Smith PG (1944) Embryo culture of a tomato species hybrid. Proc Amer Soc Hort Sci 44: 413-416.
23. Imanishi S, Watanabe Y, Hiura I (1985) A simple and efficient method for the interspecific hybridization between Lycopersicon esculentum and L. peruvianum. Journal of the Yamagata Agriculture and Forestry Society 42: 13-15.
24. Thomas BR, Pratt D (1981) Efficient hybridization between Lycopersicon esculentum and L. peruvianum via embryo callus. Theor Appl Genet 59: 219-219.
25. Kuriyama T, Kuniyasu K, Mochizuki H (1971) Studies on the breeding of disease-resistant tomato by interspecific hybridization. Influence of the environment and crossing factors on the frequency of interspecific hybrids. Bull Hort Res Station (Ministry of Agr. and For. Japan) 10: 51-90.
26. Yamakawa K (1971) Effect of chronic gamma radiation on hybridization between Lycopersicon esculentum and L. peruvianum. Gamma Field Symposium 10: 11-30.
27. Kuriyama T, Mochizuki H (1971) Studies on the breeding of disease-resistant tomato by interspecific hybridization. Fertility and disease resistance of the progenies of interspecific hybridization. Bull Hort Res Station (Ministry of Agri. and For. Japan) 11: 33-60.
28. Poysa V (1990) The development of bridge lines for interspecific gene transfer between Lycopersicon esculentum and L. peruvianum. Theor Appl Genet 79: 187-192.
29. Hogenboom NG (1972) Breaking breeding barriers in Lycopersicon. The genus Lycopersicon, its breeding barriers and the importance of breaking these barriers. Euphytica 21: 221-227.
30. Debbarma C (2011) Interspecific hybridization and embryo rescue in Capsicum. In: M.Sc. thesis, College of Post Graduate Studies, Central Agricultural University, Umiam, Meghalaya, India.
31. Ballester J, Vincente CD (1998) Determination of F1 hybrid seed purity in Pepper using PCR-based markers. Euphytica 103: 223-226.