Plant Cell Tiss Organ Cult (2015) 120:379–386
DOI 10.1007/s11240-014-0591-4

RESEARCH NOTE

Development of interspecific F_1 hybrids (*Solanum melongena* × *Solanum khasianum*) in eggplant through embryo rescue technique

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Received: 25 April 2014 / Accepted: 30 July 2014 / Published online: 12 August 2014

Abstract The wild relatives of eggplant possess number of unexploited resistant genes against biotic and abiotic stresses. These wild relatives are either not easily crossable and produces sterile F_1 when crossed with cultivated genotypes. The present study was executed with the objective of standardization of protocol and obtaining hybrids of *Solanum melongena* and *Solanum khasianum* through embryo rescue technique. The cross pollination between indegenous colloections of *S. melongena* and locally collected *S. khasianum* was carried out in a reciprocal manner. Out of 25 indegenous genotypes, IC 203585, IC 261767, IC 261797, IC 305129, IC 354611, IC 099736 and IC 261888 showed good fruit setting when *S. khasianum* was used as male. However, no fruit setting was observed when *S. khasianum* used as female parent and most of the fruits dropped within 35 days after pollination. The hybrid combinations, viz. IC 203585 × *S. khasianum,* IC 261767 × *S. khasianum,* IC 261797 × *S. khasianum* and IC 305129 × *S. khasianum* gave the best results when inoculated after 25 days after inoculation. Maximum shoot regeneration was observed to the tune of 80.01 ± 0.108 in IC 261797 × *S. khasianum* when inoculated on MS media supplemented with 2 mg/l BAP + 0.5 mg/l IAA. However, the highest root regeneration was observed in IC 203585 × *S. khasianum* to the extent of 78.45 when inoculated on media MS + 2.5 mg/l kinetin + 1 mg/l IAA.

Keywords Interspecific hybridization · F_1 hybrids · Callus · Regeneration · Embryo rescue technique

Abbreviations

MS	Murashige and Skoog
BAP	6-benzylaminopurine
IAA	Indole acetic acid
IC	Indigenous collection
IBA	Indole butyric acid
NAA	Naphthalene acitic acid
NBPGR	National Bureau of Plant Genetic Resources

Introduction

Eggplant (*Solanum melongena* L.), 2n = 24 a member of solanaceous family is an agronomically important non-tuberous crop grown primarily for its large oval fruit. It is a perennial but grown commercially as an annual crop. The brinjal is of much importance in the warm areas of Far East, being grown extensively in India, Bangladesh, Pakistan, China and the Philippines besides in Egypt, France, Italy and United States. In India, it is one of the most common, popular and principal vegetable crops grown throughout the country except higher altitudes. It is a versatile crop adapted to different agro-climatic regions and can be grown throughout the year. It is good source of

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proteins and minerals, containing 4.8 g proteins, 525 mg calcium, 6 mg iron, and 0.4 mg vitamin A/100 g of servings. In popular medicines, eggplant is indicated for the treatment of several diseases (Claudia and Elisabeth 2005), including diabetes, arthritis, asthma and bronchitis.

Although eggplant is an important nutritional crop and helps in treatment of diabetes, arthritis, asthma and bronchitis but it is susceptible to several insect pests and diseases that cause serious crop losses (Claudia and Elisabeth 2005). Among various insects, fruit and shoot borer has been a major threat to the crop which causes losses up to 70 % (Dhandapani et al. 2003). Farmers are prone to an indiscriminate use of chemical pesticides around 25–80 sprays and this involves heavy expenditure and results in blemished fruits. Excessive chemical use also leads to a build up of pesticide residues in the produce, destruction of beneficial insects, pest resurgence, exposure of farm workers to pesticides and environmental pollution. Genetic resources of eggplant have been assessed for resistance against its most serious pest of present i.e. shoot and fruit borer. Attempts at crossing eggplant with its wild relatives resulted in limited success due to sexual incompatibilities and difficulties in obtaining fertile progenies (Gleddie et al. 1986). However, the ability of eggplant to respond well in tissue culture, notably plant regeneration, has allowed the application of biotechnology to produce improved varieties, such as embryo rescue, in vitro selection, somatic hybridization and genetic transformations (Kashyap et al. 2003; Collonier et al. 2001). The interspecific hybrids between cultivars of eggplant (*Solanum melongena* L.) and its wild relative *S. torvum* have been successfully obtained by through embryo rescue technique (Kumchai et al. 2013). In addition, traditional the insect, impairing the abstention of resistant varieties (O'Brien 1983; Melo and Costa 1985; Lin and Xiao 1995).The application of in vitro methodologies to eggplant has resulted in considerable success. The aim of the study is to transfer the resistant genes from *Solanum khasianum* to cultivated and susceptible varieties of *Solanum melongena.* Keeping these points in view the present problem was executed with the objective of obtaining hybrids of *S. melongena* and *S. khasianum* through embryo rescue technique.

Materials and methods

Plant material and hybridization

Twenty-five indigenous lines of *Solanum melongena* viz. IC 203585, IC 261767, IC 261797, IC 305129, IC 354611, IC 099736, IC 261888, IC 090126, IC 090958, IC 099665, IC 099680, IC 104101, IC 249357, IC 249374, IC 261828, IC 261836, IC 261850, IC 261852, IC 261249, IC 310886, IC 316223, IC 316232, IC 343145, IC 345360 and IC 354611 were obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. The wild genotype namely *S. khasianum* (wild resistance source for fruit and shoot borer) was collected through local explorations made in the of North Western Himalyan regions of Jammu and Kashmir, India. Indigenous collections (IC) collected from NBPGR, New Delhi, India were crossed with *S. khasianum* in reciprocal manner. Flowers of female parent were selected and emasculated a day prior to anthesis at ball on stage at and were bagged to avoid contamination. Next day pollination was performed using opened flowers as source of pollen.

Embryo rescue and regeneration of shoots and roots

The immature fruits of *S. melongena* and *S. khasianum* were harvested at 15, 25 and 35 days after pollination. The embryos were cultured on MS medium containing different concentrations and combinations of auxins and cytokinines for emergence of embryo or callus formation. After the emergence of embryo they were transferred to MS media supplemented with different concentrations and combination of auxins and cytokinines such as NAA, IBA, BAP and kinetin for further growth and development.

The emerged embryos when grew up to 5 cm and attained two true leaf stage were subcultured on fresh media containing different combinations and concentrations of auxins and cytokinines such as MS + 2 mg/l BAP + 0.5 mg/l IAA, MS + 2.5 mg/l BAP + 0.5 mg/l IAA, MS + 2 mg/l kinetin + 0.5 mg/l IAA and MS + 2.5 mg/l kinetin + 0.5 mg/l IAA for shoot multiplication. After culturing the cultures were kept at 26 ± 2 °C for 30 days and then process of sub culturing was followed on fresh medium for further shoot multiplication. The regenerated shoots which were developed from hypocotyl explants were transferred to root regeneration medium having MS medium with different concentration of auxins so as to obtain the complete plantlets. Different combinations used for root regeneration were basal MS medium +0.5 mg/l IBA and MS medium +1.0 mg/l IBA.

Hardening of the rooted shoots

In vitro developed plantlets were taken out and washed gently with distilled water to remove adhering agar medium in the laminar flow. The plants were then transferred in the tissue culture pots containing mixture of cocopeat: vermiculite: perlite (1:1:1) which was pre-sterilized by autoclaving at 15 lbs/inch^2 pressure for 1 h at 121 °C. The plantlets were then watered with ½ MS medium biweekly and were kept under varying conditions of humidity and light intensity and were observed for growth.

Results

Development of F_1 hybrids

Cross compatibility

In the present study, *S. melongena* and *S. khasianum* genotypes were hybridized in a reciprocal manner. Hundred crosses attempted in which *S. melongena* genotypes were used as females and twenty crosses attempted by using *S. khasianum* as male. The fruit setting between *S. melongena* and *S. khasianum* was successful with the use of growth regulator sprays after pollination. The effect of spray of growth hormone was ineffective till the month of June, 2013 due to high temperature i.e. more than 35 °C during this period at the time of pollination. But with the onset of monsoon, when temperature dropped below 35 °C, the effect of spray of 2,4-D was noticed quite effectively in the form of hybrid fruit setting.

Fruit setting

Out of 25 indigenous genotypes, IC 203585, IC 261767, IC 261797, IC 305129, IC 354611, IC 099736 and IC 261888 showed good fruit setting when *S. khasianum* was used as male. However, no fruit setting was observed when *S. khasianum* used as female parent and most of the fruits dropped within 35 days after pollination (Table 1). The F_1 hybrids viz. IC 203585 × *S. khasianum*, IC 261767 × *S. khasianum,* IC 261797 × *S. khasianum* had the good fruit setting up to 35 days after pollination and even after (Fig. 1).

Stage of the F_1 embryo for culturing

The F_1 hybrids between *S. melongena* × *S. khasianum* were harvested at 15, 25 and 35 days after pollination. These fruits were dissected under aseptic conditions and their immature seeds (embryos) were inoculated in the MS media

Table 1 Cross compatability of *S. melongena* and *S. khasianum*

Hybrid combinations	No. of crosses attempted	Fruit setting	Fruit drop within 35 DAP	Fruits obtained after 35 DAP
IC 203585 × *Solanum khasianum*	100	28	5	23
IC 261767 × *Solanum khasianum*	100	18	10	8
IC 261797 × *Solanum khasianum*	100	27	10	17
IC 305129 × *Solanum khasianum*	100	5	3	2
IC 354611 × *Solanum khasianum*	100	5	2	3
IC 099736 × *Solanum khasianum*	100	3	1	2
IC 261888 × *Solanum khasianum*	100	5	3	2
IC 090126 × *Solanum khasianum*	100	–	–	–
IC 090958 × *Solanum khasianum*	100	–	–	–
IC 099665 × *Solanum khasianum*	100	–	–	–
IC 099680 × *Solanum khasianum*	100	–	–	–
IC 104101 × *Solanum khasianum*	100	–	–	–
IC 249357 × *Solanum khasianum*	100	–	–	–
IC 249374 × *Solanum khasianum*	100	–	–	–
IC 261828 × *Solanum khasianum*	100	–	–	–
IC 261836 × *Solanum khasianum*	100	–	–	–
IC 261850 × *Solanum khasianum*	100	–	–	–
IC 261852 × *Solanum khasianum*	100	–	–	–
IC 261249 × *Solanum khasianum*	100	–	–	–
IC 310886 × *Solanum khasianum*	100	–	–	–
IC 316223 × *Solanum khasianum*	100	–	–	–
IC 316232 × *Solanum khasianum*	100	–	–	–
IC 343145 × *Solanum khasianum*	100	–	–	–
IC 345360 × *Solanum khasianum*	100	–	–	–
IC 354611 × *Solanum khasianum*	100	–	–	–
Solanum khasianum × *Solanum melongena*	20 each	–	–	–

IC: Indigenous collection, DAP: Days after pollination

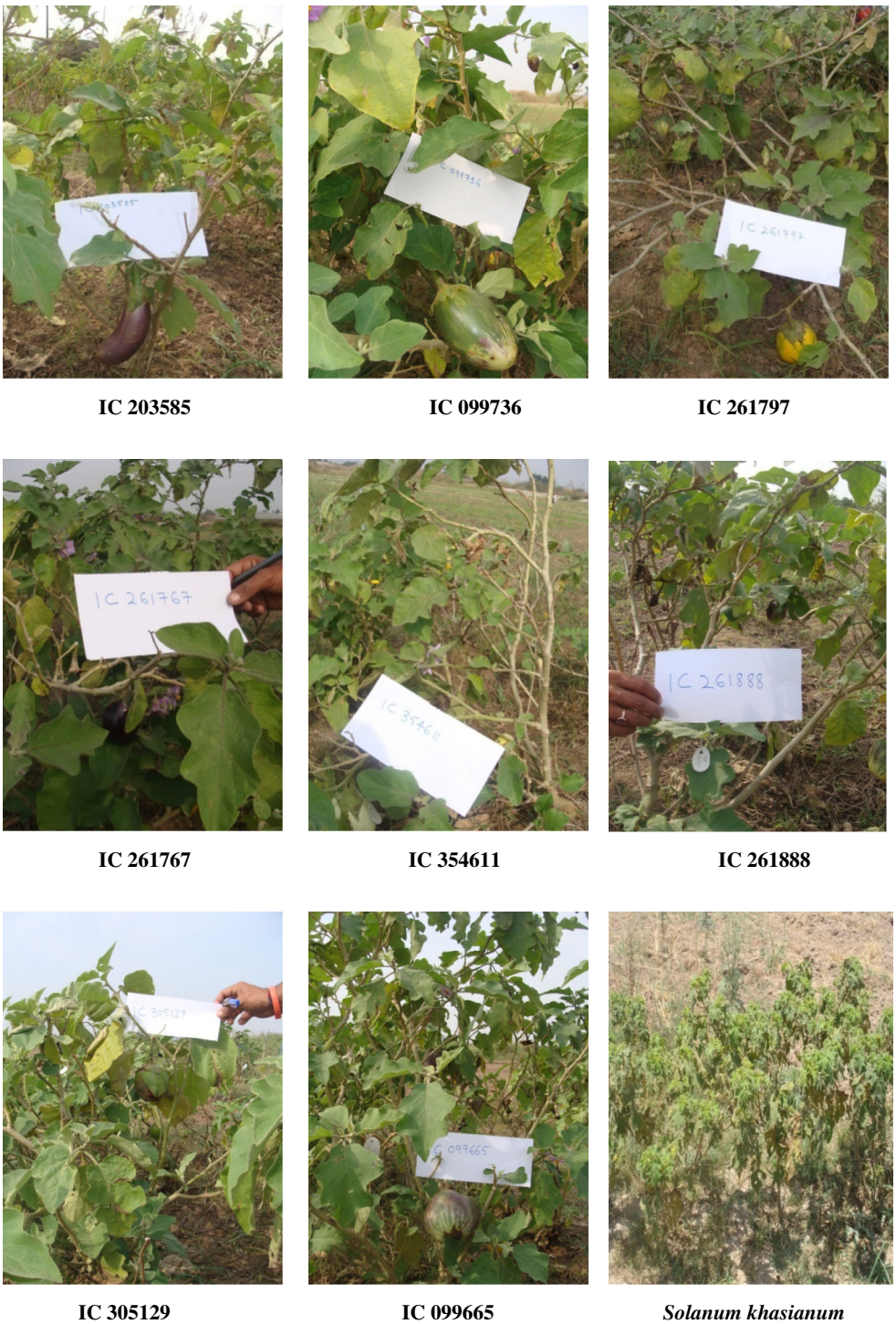

Fig. 1 Performance of different parents

containing different concentration and combinations of auxins and cytokinines viz. MS + 1 mg/l NAA + 1 mg/l BAP, MS + 2 mg/l NAA + 2 mg/l BAP, MS + 1/l 2,4-D + 0.5 mg/l kinetin, MS + 2/l 2,4-D + 1 mg/l kinetin. Some of the hybrid combinations such as IC 354611 × *S. khasianum,* IC 099736 × *S. khasianum,* IC261888 × *S. khasianum* and IC 261888 × *S. khasianum* did not show any growth or germination, irrespective of the age of hybrid embryo. However, hybrid combinations, viz. IC 203585 × *S. khasianum,* IC 261767 × *S. khasianum,* IC 261797 × *S. khasianum,* IC 305129 × *S. khasianum* gave the best results when inoculated after 25 days after inoculation.

Effect of media

The F_1 hybrid embryo of IC 203585 × *S. khasianum* showed the best response for embryo emergence on MS media supplemented with 2 mg/l NAA +2 mg/l after 15 days of inoculation (Table 2). The cross combination IC 261767 × *S. khasianum* showed embryo emergence after 35–40 days on MS media supplemented with 1 mg/l 2,4-D + 0.5 mg/l kinetin. While there was direct callusing on the MS media supplemented with 2 mg/l 2,4-D + 1 mg/l kinetin and MS media supplemented with 2 mg/l NAA + 2 mg/l BAP after 30-35 and 20–25 days after inoculations, respectively. The hybrid combination IC 261797 × *S. khasianum* produced callus after inoculation on the MS media supplemented with 1 mg/l NAA + 1 mg/l BAP and 2 mg/l 2,4-D + 0.5 mg/l kinetin after 60 days of inoculation. The emergence of embryo from immature ovules of hybrid IC 261797 × *S. khasianum* was observed after 20–25 and 15–20 days after inoculations on MS media supplemented with 2 mg/l 2,4-D + 1 mg/l kinetin and 2 mg/l NAA + 2 mg/l BAP, respectively.

Plantlets regeneration and hardening of the rooted shoots

Regeneration of shoot

The emerged embryos at two true leaf stage were sub cultured on fresh media containing different combinations and concentrations of auxins and cytokinines such as MS + 2 mg/l BAP + 0.5 mg/l IAA, MS + 2.5 mg/l BAP + 0.5 mg/l IAA, MS + 2 mg/l kinetin + 0.5 mg/l IAA and MS + 2.5 mg/l kinetin + 0.5 mg/l IAA for shoot multiplication. The F_1 hybrid embryo of IC 203585 × *S. khasianum* showed highest average number of shoots per explants (0.72 ± 0.102) and highest percentage of shoot regeneration (70 ± 0.150) on MS media supplemented with 2 mg/l BAP + 0.5 mg/l IAA. The cross combinations IC 261767 × *S. khasianum* and IC 261797 × *S. khasianum* also produced highest average number of shoots per explant on MS + 2 mg/l BAP + 0.5 mg/l IAA to the mark of 0.65 ± 0.128 and 0.88 ± 0.152, respectively. In the same manner highest shoot regeneration of 62.02 and 80.01 % was observed in cross combination IC 261767 × *S. khasianum* and IC 261797 × *S. khasianum,* respectively on media MS medium supplemented with 2 mg/l BAP + 0.5 mg/l IAA (Table 3).

Regeneration of roots

The regenerated shoots were sub cultured in the rooting media which contained different concentrations of auxins. Root initiation started after 15–20 days of inoculation irrespective of cross combination and well developed roots were obtained in 4 weeks. In cross combinations IC 203585 × *S. khasianum,* IC 261767 × *S. khasianum* and IC 261797 × *Solanum,* maximum root regeneration of 78.45, 62.00 and 52.08 %, respectively was observed on MS media supplemented with 0.5 mg/lt IBA. After root regeneration the completely formed plantlets were transferred to pre sterilized mixture of perlite: cocopeat: vermiculite (1:1:1) in pots (Fig. 2).

Table 2 Response of different hybrid combinations on growing media

Hybrid combinations	MS + 1 mg NAA + 1 mg BAP	MS + 2 mg NAA + 2 mg BAP	MS + 2 mg 2,4-D + 0.5 mg kinetin	MS + 2 mg 2,4-D + 1 mg kinetin
IC 203585 × *Solanum khasianum*	No response	Emergence of embryo was observed after 15 days of inoculation	Emergence of embryo and calluasing after 25 days of inoculation	Emergence of embryo after 30 days of inoculation
IC 261767 × *Solanum khasianum*	No response	Callusing after 20–25 days of inoculation	Emergence of embryo after 30–35 days of inoculation	Callusing after 30–35 days of inoculation
IC 261797 × *Solanum khasianum*	Callusing after 60 days of inoculation	Emergence of embryo was observed after 15–20 days of inoculation	Callusing after 60 days of inoculation	Emergence of embryo and calluasing after 20–25 days of inoculation
IC 305129 × *Solanum khasianum*	No response	No response	No response	No response
IC 354611 × *Solanum khasianum*	No response	No response	No response	No response
IC 099736 × *Solanum khasianum*	No response	No response	No response	No response
IC 261888 × *Solanum khasianum*	No response	No response	No response	No response

Table 3 Effect of different growing media on shoot regeneration

Hybrid combinations	MS + 2 mg/l BAP + 0.5 mg/l IAA	MS + 2.5 mg/l BAP + 1 mg/l IAA	MS + 2 mg/l kinetin + 0.5 mg/l IAA	MS + 2.5 mg/l kinetin + 1 mg/l IAA
IC 203585 × *Solanum khasianum*				
Callus formation	+++	+++	+++	+++
Av. no. of shoot/explant	0.72 ± 0.102	0.45 ± 0.100	0.12 ± 0.112	0.13 ± 0.132
% shoot regeneration	70 ± 0.150	42.6 ± 0.118	36.08 ± 0.142	30 ± 0.162
IC 261767 × *Solanum khasianum*				
Callus formation	++	++	+	+
Av. no. of shoot/explant	0.65 ± 0.128	0.38 ± 0.162	0.16 ± 0.101	0.11 ± 0.106
% shoot regeneration	62.02 ± 0.111	36.90 ± 0.120	12.08 ± 0.146	10.06 ± 0.142
IC 261797 × *Solanum khasianum*				
Callus formation	+++	+++	++	++
Av. no. of shoot/explant	0.88 ± 0.152	0.38 ± 0.100	0.10 ± 0.114	0.11 ± 0.102
% shoot regeneration	80.01 ± 0.108	30.06 ± 0.101	9 ± 0.128	8.02 ± 0.008

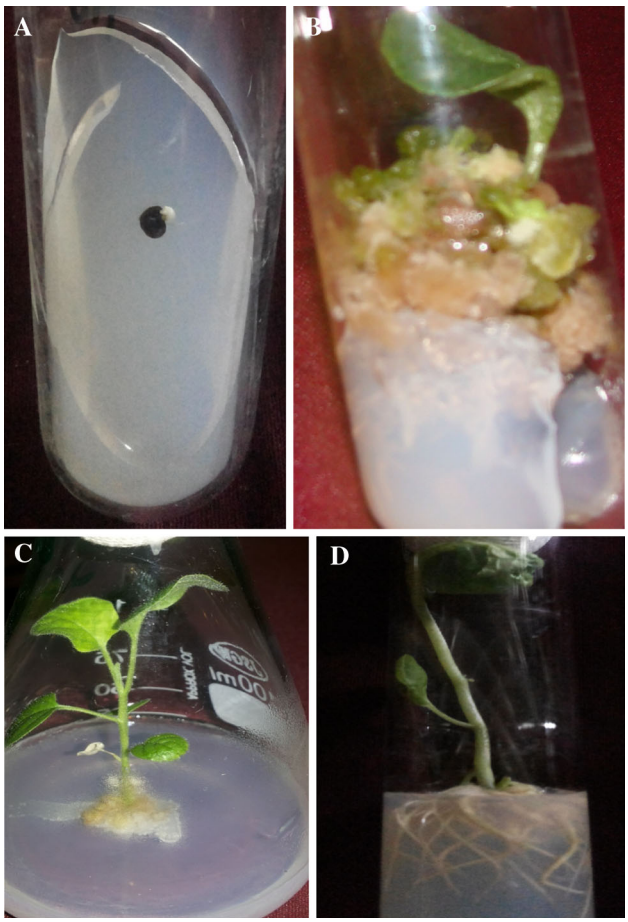

Fig. 2 a–d: Embryo rescue studies in brinjal **a** Emeregence of embryo from the immature ovule. **b** Multiple shoot regeneration from the embryo on shoot regeneration medium **c** Fully developed shoots explant on shoot regeneration medium **d** Emergence of roots on the root regeneration medium

Discussion

Cross compatibility of *Solanum melongena* and *Solanum khasianum*

The quantitative and qualitative breeding efforts in eggplant have resultued in introduction of many improved varieties into cultivation (Kashyap et al. 2003). In the present study, the cross combinations of IC 203585, IC 261767, IC 261797, IC 305129, IC 354611, IC 099736 and IC 261888 with *S. khasianum* showed fruit setting when *S. khasianum* was used as male, however no fruit setting was observed when the later was used as female. These findings are in line with the findings of Sharma and Rajam (1995) who also observed that when *S. melongena* was crossed with *S. khasianum* some of the ovaries got fertilized and the hybrids were rescued through embryo rescue technique. Similar finding were obtained by Kumchai et al. (2013) in their study in which interspecific hybrids between cultivars of eggplant (*Solanum melongena* L.) and its wild relative *S. torvum* were obtained by cross-hybridization and embryo rescue.

Fruit setting

Seed production in brinjal is highly influenced by environment, particularly temperature, which has a significant effect on all stages of plant growth and development (Sun et al. 1990; Kowalska 2003). Day and night temperature plays a crucial role in flowering, fruit setting and maturity in brinjal. The optimum temperature for pollen germination is 20–27 °C and the pollen grain is unable to germinate when temperature reaches 30 °C and above due to dryness

of the pollen grain (Dobromilska and Fawceet 1999). Besides environmental factors, the presence of heterostyled phenomenon also plays an important role brinjal fruit setting. In the present study F_1 hybrids were obtained in *S. melongena* × *S. khasianum*, when temperature dropped below 35 °C followed by spray of 2, 4-D @ 2 mg/lt after 3rd day of pollination. It was observed that 2, 4-D enhanced the production of true styled flowers in brinjal. These findings combat with the findings of Nothmann et al. (1983) who also reported the improvement in the fruit setting in brinjal after application of 2,4-D.

Effect of age of the hybrid embryo

It was observed that irrespective of the parent of the hybrid, the embryos rescued from the fruits harvested at 25 days after pollination gave the best results in shoot and root regeneration. This may be due to the fact that the F_1 hybrids harvested at the age of 15 days after pollination were quite immature for the regeneration. However, the F_1 hybrids harvested at the age of 35 days after pollination would have lost their viability as no regeneration was observed. Kharkongar et al. (2013) also reported no growth or germination of F_1 hybrid embryos at 15 and 35 days after pollination.

Effect of media on embryo emergence

Different response of hybrid combinations to various combinations of media was observed in the present study. Earlier researchers also suggested that the regeneration efficiency is influenced by explants type, genotype and other morphogenetic responses (Sharma and Rajam 1995). However in general, irrespective of the genotype of the hybrid the best response was observed on MS media supplemented with 2 mg/l NAA +2 mg/l BAP. Similar findings were reported by Kharkongar et al. (2013) in their studies. Growth hormones such as 2, 4-D was functional in order to optimize callus induction for undifferentiated splitting up of cells for the explant used. There was profound callusing when MS medium was supplemented with 2, 4-D. Similar finding were reported by Muhammad et al. (2013) in their study.

Development of complete plantlet

Shoot multiplication

The emerged embryos at two true leaf stage were sub cultured on fresh media containing different combinations and concentrations of auxins and cytokinines such as MS + 2 mg/l BAP + 0.5 mg/l IAA, MS + 2.5 mg/l BAP + 0.5 mg/l IAA, MS + 2 mg/l kinetin + 0.5 mg/l IAA and MS + 2.5 mg/l kinetin + 0.5 mg/l IAA for shoot multiplication. Maximum shoot regenerations were observed in most of the F_1 hybrid combinations on MS medium supplemented with 2 mg/l BAP + 0.5 mg/l IAA due use of medium concentrations of BAP and kinetin. Zayova et al (2012) and Bhat et al. (2013) had also observed higher shoot regeneration in brinjal with increased BAP concentrations i.e. 0.5, 1 and 2 mg/l. However, they reported that higher BAP level had negative effect on organogenesis leading to shoots vitrification. Kaur et al. (2011) observed that the addition of kinetin decreased the regeneration capability and number of buds on all the explant. Sagare and Mohanty (2012) also observed the shoot regeneration mediated through callus on medium containing BAP@ 1.0–2.0 mg/l with the highest shoot regeneration at 2.0 mg/l BAP. They observed that the hypocotyl when cultured on MS medium supplemented with kinetin, an increase in regeneration efficiency of shoot was resulted. These findings are in line with Sarkar et al. (2006) and Bhat et al. (2013) who also observed the increase in shoot regeneration with increase in kinetin level using cotyledonary leaves as explant.

Root regeneration

The highest root regeneration was observed in MS media supplemented with 0.5 mg/lt IBA with root generation of 78.45, 62.00, and 52.08 % in IC 203585 × *S. khasianum,* IC 261767 × *S. khasianum* and IC 261797 × *Solanum,* respectively. IBA is widely used for efficient root regeneration in brinjal (Zayova et al. 2012; Shivraj and Rao 2011) although several suggested IAA as efficient root regeneration hormone (Bardhan et al. 2012). These findings are similar to findings of Shivraj and Rao (2011), Chakravarthi and Prabavathi (2009), Hossain et al. (2007) and Rahman et al. (2006), who also observed higher root regeneration on MS medium supplemented with addition of IBA.

Acknowledgments The authors are thankful to Govt. of India, Ministry of Science and Technology, Department of Biotechnology, New Delhi, India for funding and to carry out the research project "Androgenesis mediated introgression of fruit and shoot borer (*Leucinodes orbonalis*) resistant genes into cultivated eggplant (*Solanum melongena*)" under the Bio-CARe scheme for women. The authors are also thankful to NBPGR, New Delhi for supplying germplasm for the present study.

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