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Effect of genotype, explant and culture medium on organogenesis in brinjal

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ABSTRACT

Brinjal were regenerated from callus derived from hypocotyl, cotyledon and root explants of five genotypes, namely Punjab Barsati, Punjab Sadabahar, Jamuni Gola, PBSR-11 and BB-93C on MS medium containing different concentrations of IAA and BAP. A combination of 2.5 mg/l IAA + 0.5 mg/l BAP was found optimum for adventitious shoot induction from all explants. Genotype, explant and genotype x explant interaction showed highly significant effects on organogensis. Among genotypes, PBSR-11 showed maximum response for organogenesis (79.43%). However, among explants, cotyledon was significantly better than hypocotyl and root. Plants regenerated via adventitious shoots were rooted on half-strength MS basal medium *in vitro*.

Key words: Egg plant, plant regeneration, organogenesis, shoot induction.

INTRODUCTION

Brinjal (*Solanum melongena* L.), also known as eggplant, aubergine and guinea squash is an economically important vegetable crop of India. It is a good source of vitamins and minerals and is compared with tomato in terms of total nutritional value. Eggplant has been used in traditional medicines for treatment of asthma, bronchitis, cholera, dysuria and lowering blood cholesterol (Khan, 7). The regeneration ability of brinjal has allowed the application of biotechnology, particularly for exploitation of somaclonal variation, hapliody, hybridization and genetic transformation (Collonier *et al*., 3). It is also a good system for *in vitro* studies, because plant regeneration can be achieved via organogenic pathway from different explants. Organogenesis from hypocotyl (Sharma and Rajam, 11; Yu-Bolan *et al*., 14), leaf, cotyledon (Sarker *et al*., 10; Dobariya and Kachhadiya, 4) and root (Franklin *et al*., 6) explants have been reported. However, genotypic differences and intra-genotype variation among explants has also been observed (Alicchio *et al*., 1; Sharma and Rajam, 18), which is further affected by cultural conditions (Mukherjee *et al*., 14). Ultimately, successful application of *in vitro* techniques rest upon the ability to regenerate plants from the desired tissues and the genotype. For this, a suitable culture protocol with fairly high regeneration potential for a particular genotype in question is required. The present study was therefore undertaken to generate information on five cultivated brinjal varieties for organogenic potential induced by using different explants on variable cultural conditions.

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MATERIALS AND METHODS

Healthy seeds of five brinjal varieties, *viz*., Punjab Barsati, Punjab Sadabahar, Jamuni Gola, BB-93C and PBSR-11 were obtained from the Department of Vegetable Crops and cultured in Dr G. Khush Laboratories, Punjab Agricultural University, Ludhiana. Seeds were washed five times in running tap water and then pre-soaked in water for 2 h. After surface sterilization with 0.1% (w/v) mercuric chloride for 2 min., seeds were rinsed with sterile water and germinated aseptically on MS basal medium (Murashige and Skoog, 9) with 3% (w/v) sucrose and 0.8% (w/v) agar, pH 5.8 at 25°C ± 2°C under dark. From 15-day-old seedlings, hypocotyl and root segments of 8-10 mm length and cotyledon of 100 mm^2 size were used as explants in all *in vitro* experiments.

The phytohormones (IAA and BAP) were added to MS basal medium and pH was adjusted to 5.8 before autoclaving at 120°C for 20 min. Explants were grown on medium solidified with 8 gl^{-1} agar and kept under a 16 h photoperiod illuminated with cool white flourescent light (40 µE $m^{-2}s^{-1}$) at 25 ± 2°C. Hypocotyl, cotyledon and root explants of Punjab Barsati were cultured on MS medium supplemented with different combinations and concentrations of IAA (0.0-3.0 mgl^{-1}) and BAP (0.0-0.5 mgl^{-1}) for callus induction and subsequent plant regeneration. An optimum phytohormone regime was determined for the regeneration of adventitious shoots (organogenic medium) from all the three explants. Then, it was used for further studies with hypocotyl, cotyledon and root explants using all the cultivars.

The regenerated adventitious shoots and somatic embryos obtained after 4-8 weeks of culture on appropriate medium were transferred to basal MS medium with one-third or half-strength mineral salts for rooting. The rooted plantlets could be successfully hardened for two weeks under high humidity (> 80% RH) conditions for transfer to the field.

Three replicates were maintained for each treatment and data were recorded after 4 weeks of culture. Data in percent were converted to Arc Sine value for analysis of variance (ANOVA) as per completely randomized design (simple and factorial) design. Least significant differences (LSD) at 5 per cent level of significance were calculated and interpretations were made using multiple range test.

RESULTS AND DISCUSSION

The results of callus cultures induced from hypocotyl, cotyledon and root explants of Punjab Barsati at different hormone concentrations and combinations are given in Table 1. Within a week of culture, explants started formation of callus predominantly from the cut end region having contact with the culture medium. Initially, when different explants were cultured on MS basal medium no change in morphology was observed. However, the addition of IAA (1.0 mg/l) promoted the callus formation, which was friable and brown. When explants were cultured on medium containing both IAA and BAP, the callus morphology was significantly better and callus induction was increased. However, differences were non significant between 1.5 mg/l IAA + 0.5 mg/l BAP, 2.5 mg/l IAA + 0.5 mg/l BAP and 3.0 mg/l IAA + 0.5 mg/l BAP combinations. Among explants, hypocotyl and cotyledon did not differ (83.33%), whereas, roots induce lower callusing (73.65%). The interaction between explant and hormone concentration was significant and showed 100% callusing in hypocotyl and cotyledon on all the media having IAA and BAP.

The effect of explant and different organogenic media on shoot induction (%) after four weeks of culture in variety Punjab Barsati is given in Table 2. Different phytohormone combinations significantly affected the shoot regeneration. Though, different explants formed calluses and shoots on a wide range of hormonal combinations, but best response for shoot induction were observed in medium containing 2.5 mg/l IAA + 0.5 mg/l BAP (70.88%). Among explants, cotyledon was the best (58.32%), followed by hypocotyl (55.20%) and root (38.32%) for shoot induction. For obtaining maximum adventitious shoots, sub culturing of explants was needed after every 15-20 days of incubation. If it was suspended, an adverse effect on shoot formation was observed either by seize in growth or by excessive callus formation. The differential responses for callus initiation and growth on different media may be due to varying level of phytohormones in the media, which plays an essential role in growth and differentiation. The exogenous application of phytohormones supplements the endogenous levels, which either establish or disrupt the balance that is essential for normal ontogeny and resulted into callus formation (Ammirato, 2).

It was observed that adventitious shoots could be regenerated on IAA alone, or in combination with BAP (Fig. 1). However, shoots obtained from medium containing both IAA and BAP could only be rooted and regenerated into whole plants. This is in agreement with earlier observations in brinjal (Kamat and Rao, 6; Sharma and Rajam, 11). IAA-cytokinin combinations have earlier been used to induce adventitious shoots

Table 1. Effect of explant and media on callus induction (%) in eggplant variety Punjab Barsati.

MS + hormone (mg/l)		Explant			Mean
IAA	BAP	Hypocotyl	Cotyledon	Root	
0.0	0.0	0.0 (0.00)*	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)^{d}
1.0	0.0	100 (89.96)	100 (89.96)	73.60 (59.08)	91.2 (79.66)^{c}
1.0	0.5	100 (89.96)	100 (89.96)	78.46 (62.35)	92.82 (80.76)^{b}
1.5	0.5	100 (89.96)	100 (89.96)	95.68 (78.53)	98.56 (86.15)^{a}
2.5	0.5	100 (89.96)	100 (89.96)	96.19 (78.98)	98.73 (86.30)^{a}
3.0	0.5	100 (89.96)	100 (89.96)	100 (89.98)	100 (89.96)^{a}
Mean		83.33 (74.96)^{a}	83.33 (74.96)^{a}	73.65 (60.16)^{b}	

$CD_{0.05}$: Explant = 1.04
Hormone conc. = 1.48
Explant × hormone conc. = 2.56

*Figures in parenthesis indicates Arc Sine transformed of values

Table 2. Effect of explant and media on shoot induction (%) in eggplant variety Punjab Barsati.

MS + hormone (mg/l)		Explant			Mean
IAA	BAP	Hypocotyl	Cotyledon	Root	
0.0	0.0	0.0 (0.00)*	0.0 (0.00)	0.00 (0.00)	0.00 (0.00)^{f}
1.0	0.0	47.82 (43.73)	55.23 (47.98)	31.76 (34.27)	44.93 (41.99)^{e}
1.0	0.5	60.90 (51.28)	64.42 (53.36)	40.5 (39.5)	55.27 (48.04)^{d}
1.5	0.5	71.30 (57.60)	76.36 (60.89)	47.5 (43.54)	65.05 (54.01)^{c}
2.5	0.5	76.19 (60.79)	78.57 (62.41)	57.89 (49.52)	70.88 (57.57)^{a}
3.0	0.5	75.00 (60.00)	77.31 (61.54)	52.3 (46.30)	68.20 (55.94)^{b}
		55.20 (45.56)^{b}	58.32 (47.70)^{a}	38.32 (35.52)^{c}	

$CD_{0.05}$: Explant = 1.02
Hormone conc. = 1.45
Explant × hormone conc. = 2.51

*Figures in parenthesis indicates Arc Sine transformed of values

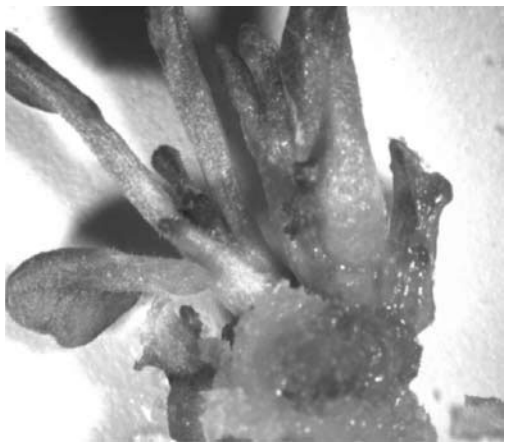

Fig. 1. Shoot induction on MS basal medium + 2.5 mg/l IAA + 0.5 mg/l BAP in egg plant.

in eggplant from immature (Yamada *et al*., 13) and mature embryos (Swamy *et al*., 12).

The optimum phytohormone combination, i.e. MS + 2.5 mg/l IAA + 0.5 mg/l BAP found suitable for regeneration of adventitious shoots from hypocotyl, cotyledon and root of Punjab Barsati was further used on other genotypes, namely Punjab Sadabahar, Jamuni Gola, PBSR-11 and BB-93 (Table 3). Among explants, cotyledon showed significantly highest response (81.78%) than hypocotyl (79.28%) and root (56.73%). These results are in accordance with the findings of Alicchio *et al.* (1), where callus derived from leaf and cotyledon was high in regenerative potential. Interexplant variation has also been reported by Dobariya and Kachhadiya (4). Comparison of genotypes for organogenic response depicted significant differences for shoot induction. The maximum

Table 3. Effect of explant and genotype on shoot induction (%) on MS basal medium + 2.5 mg/l IAA + 0.5 mg/l BAP.

Genotype	Explant			Mean
	Hypocotyl	Cotyledon	Root	
Punjab Barsati	76.19 (60.78)*	78.57 (62.43)	57.89 (49.52)	70.88 (57.58)^{c}
Pb. Sadabahar	79.41 (63.00)	82.35 (65.18)	58.33 (49.78)	73.36 (59.32)^{b}
Jamuni Gola	70.31 (56.96)	73.75 (59.17)	49.12 (44.47)	64.39 (53.54)^{d}
PBSR-11	88.31 (70.02)	90.00 (71.66)	60.0 (50.76)	79.43 (64.15)^{a}
BB-93C	82.19 (65.03)	84.26 (66.66)	58.33 (49.78)	74.92 (60.49)^{b}
Mean	79.28 (63.16)^{b}	81.78 (65.02)^{a}	56.73 (48.86)^{c}	

$CD_{0.05}$: Explant = 1.55
Genotype = 2.00
Explant × genotype = NS

*Figures in parenthesis indicates Arc Sine transformed of values

induction was in PBSR-11 (79.43%), followed by BB-93C (74.92%), Punjab Sadabhar (73.36%) and Punjab Barsati (70.88%), whereas, Jamuni Gola was at lowest. Genotypic differences have earlier also been reported for organogenesis in brinjal by Yamada *et al*. (13), and Dobariya and Kachhadiya (4). All the regenerated shoots (3-4 cm) of all the genotypes were successfully rooted when transferred to hormone-free half-strength MS medium *in vitro*. Rooting on MS medium without hormonal supplements was also observed by Sarker *et al*. (10).

The present findings have clearly demonstrated the potential of excised segments of hypocotyl, cotyledon and root explants of brinjal to form shoot buds and regenerate into whole plants under culture conditions. The optimum regeneration of shoot buds was obtained on 2.5 mg/l IAA + 0.5 mg/l BAP supplemented medium. Genotype PBSR-11 (recently released as Punjab Nagina) was the best for organogenesis and cotyledon explants yielded the maximum number of shoots. Apparently, the potential for regeneration is immense and therefore this technique may be used as a tool or suitable culture protocol in applying *in vitro* techniques, especially genetic transformation, haploidy and somaclonal variation.

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