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Full Length Research Paper

Effect of media composition and explant type on the regeneration of eggplant (*Solanum melongena* L.)

Mohinder Kaur[1]*, Ajmer Singh Dhatt[1], Jagdeep Singh Sandhu[2] Amrik Singh Sidhu[3] and Satbir Singh Gosal[2]

[1]Department of Vegetable Crops, Punjab Agricultural University, Ludhiana-141004, India.
[2]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141004, India.
[3]Indian Institute of Horticultural Research, Bangalore, India.

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Two as well as three way interactions of three eggplant genotypes, media compositions and explants (hypocotyl, cotyledon and leaf) showed significant differences for plant regeneration. Among three explants, hypocotyl induced highest percent callusing, but cotyledon showed best results for somatic embryogenesis on all the media compositions. For three way interactions, cotyledon of BSR-27 induced significantly highest somatic embryogenesis (94.47%) on Murashige and Skooge (MS) fortified with 1.5 mgl^{-1} indole butyric acid (IBA) + 1.0 mgl^{-1} 6-benzyl aminopurine (BAP). However, hypocotyl of BR-16 was not able to induce somatic embryogenesis on MS media fortified with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP. Moreover, the embryogenic callus from cotyledon of BSR-27 (72.24%) achieved highest plant regeneration from somatic embryos on MS supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1}kin + 0.2% activated charcoal. Therefore, the performance of cotyledon explant of BSR-27 is the best on MS fortified with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP and 2.5 mgl^{-1} BAP + 1.0 mgl^{-1}kin + 0.2% activated charcoal for somatic embryogenesis and plant regeneration, respectively.

Key words: Callus, somatic embryogenesis, hypocotyl, cotyledon, leaf.

INTRODUCTION

Eggplant (*Solanum melongena* L., 2n = 2x = 24) is an economically important vegetable crop in South Asia, especially in Indian subcontinent. It suffers from various biotic and abiotic stresses such as insect pest, disease attack (Singh et al., 2000; Kaur et al., 2004). For development of intrinsic plant resistance and quality improvement conventional breeding approaches face problems of non-availability of resistance in cultivated, cross incompatibility with wild relatives (*S. mammosum*, *Solanum incanum* and *Solanum grandiflorum*) and inadvertent linkage drag of undesirable genes (Baksh and Iqbal, 1979). Combination of conventional and biotechnological techniques is need of the hour to develop intrinsic plant resistance against these stresses.

*Corresponding author. E-mail: mkaur97@rediffmail.com. Tel: 919463664452.

The successful application of these *in-vitro* techniques for improvement of a crop depends upon its efficient regeneration protocol (Razdan, 2000). In eggplant, somatic embryogenesis was first reported from immature seed embryos of two different cultivars, when cultures on MS medium supplemented with IAA (Indole-3-acetic acid) (Yamada et al., 1967). Although, this crop is most amenable to *in vitro* culture methods, still its genetic make-up, explant type and culture media affect its regeneration potential (Kantharajah and Golegaonkar, 2004). Genotype is the most important factor affecting somatic embryogenesis and its further regeneration as endorsed by many researchers (Afele et al., 1996; Dobariya and Kachhadiya, 2004; Huda et al., 2007; Mir et al., 2008). Differential response among explants (hypocotyl, cotyledon, leaf and root) has also been substantiated (Sharma and Rajam, 1995; Franklin et al., 2004).

The response of growth hormones in the culture media

was also variable within genotype and explant for somatic embryogenesis and organogenesis (Slater et al., 2003), but the response of indole butyric acid (IBA), a weak auxin, for somatic embryogenesis is not much published in this crop. Moreover, standardization of regeneration potential in different explants of local genotypes is necessary. Therefore, present study was conducted to find out best combination of genotype, media composition and explant for the somatic embryogenesis and plant regeneration in eggplant.

MATERIALS AND METHODS

The investigation for interaction effects of different explants of three eggplant genotypes viz: BSR-27 (small round fruited), BR-16 (round fruited) and BL-7 (long fruited) was carried out on MS (Murashige and Skooge, 1962) medium fortified with different concentrations and combinations of IBA, 6-benzyl aminopurine (BAP) and Kin (Kinetin) for the development of regeneration protocol.

Seed disinfection

Seeds were first washed with teepol^{TM} (Labolene) and then disinfected with 50% Commercial Bleach (Sodium hypochlorite 4%, Sodium hydroxide 1%, Amine oxide 1%) for 20 min, and again washed to remove traces of bleach. The disinfected seeds were cultured on half strength MS medium solidified with 0.8% agar for germination at 25 ± 2°C in dark for 20 days.

Callus induction and somatic embryogenesis

Cotyledon and hypocotyl explants were excised from 15-day old *in vitro* grown seedlings of each genotype, whereas, leaf explants were excised from 20-day old seedlings. The explants were cultured aseptically on MS medium fortified with the different concentrations of IBA (0.5 to 1.5 mgl^{-1}) and BAP (1.0 mgl^{-1}) for callus induction and somatic embryogenesis and incubated in dark at 25 ± 2°C. The observations for callus induction (%) and somatic embryogenesis (%) were taken after 20 days from the number of explants showing callus induction and somatic embryogenesis over the total number of explants cultured, respectively.

Plant regeneration

The compact, friable, nodular and embryogenic callus of different explants from the best media combination was further used for regeneration on the MS medium fortified with 2.5 mgl^{-1} BAP+1.0 mgl^{-1} Kin along with 0.2% activated charcoal and incubated with 16 h light/ 8 h dark cycles at 25 ± 2°C. The regeneration (%) was calculated from the number of embryogenic calli regenerated over the total number of calli cultured for regeneration.

Statistical analysis

Statistical analysis was done in CRD factorial design using CPCS-1 soft ware package (Cheema and Singh, 1990). First, second and third factors used for the study were genotype, media and explants, respectively. Three repeats were maintained for each treatment and data were recorded and compiled for each observation. Results were compared at 5% level of least square differences (LSD) and interpreted.

RESULTS AND DISCUSSION

Callus induction and somatic embryogenesis

The interactions of genotype and media for callus induction and somatic embryogenesis were significant. BSR-27 induced statistically highest callusing (91.30%), followed by BR-16 (88.03%) and BL-7 (86.85%) where later two were at par, on MS medium fortified with 1.5 mgl^{-1}IBA + 1.0 mgl^{-1} BAP (Figure 1A). For somatic embryogenesis (Figure 1B), again BSR-27 was the best (65.55%) on MS medium supplemented with 1.5 mgl^{-1}IBA + 1.0 mgl^{-1}BAP, followed by BL-7 (59.92%) on 1.0 mgl^{-1} IBA + 1.0 mgl^{-1} BAP and BR-16 (58.47%) on 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP. The induction of embryogenic callus indicates a balance of auxins and cytokinins in the plant tissue that is required for the phenomena to occur.

The callus induction ability of plant depends upon the level of auxins and cytokinins in the plant tissue itself. However, differences in the callus induction potential of various eggplant genotypes on a particular medium may be due to the different level of auxins/cytokinins in their tissue, as reported by Centeno et al. (1996). The genotypic differences for hormone levels can be evidenced from the callus quality also. Compact nodular callus was obtained from BSR-27 and BL-7 that was better than the proliferated callus of BR-16. Significant differences for genotype and explant interactions for callus induction (Figure 1C) indicated cent percent callusing in hypocotyls of all the three genotypes, while, leaf was at lowest ebb with 55.80 and 55.81% in BSR-27 and BR-16, respectively. The cotyledon (Figure 1D) depicted 82.85% response for somatic embryogenesis in BSR-27 and 81.84% in BR-16, whereas, leaf explant induced maximum of 65.85% somatic embryogenesis in BL-7.

In contrast, hypocotyl receded lowest in all the genotypes. Different response for Somatic embryogenesis among explants has been correlated with the studies of Huda et al. (2007). Media and explant interactions also depicted 100% callusing in hypocotyl on all the MS media combinations (Figure 1E). It was followed by the cotyledon and leaf explant with maximum of 92.16 and 74.01% callus induction, respectively, on 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP media combination. However, cotyledon induced significantly highest somatic embryogenesis (Figure 1F) on MS media fortified with 1.5 mgl^{-1}IBA + 1.0 mgl^{-1} BAP (89.62%) followed by 1.0 mgl^{-1}IBA + 1.0 mgl^{-1} BAP (82.20%). In leaf, it was also the maximum on 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP (69.60%). However, hypocotyl achieved the maximum of 38.41% somatic embryogenesis on 0.5 mgl^{-1} IBA + 1.0 mgl^{-1}BAP. The differentiation of somatic embryos in callus depends upon the proportion of inbuilt auxins and cytokinins in the plant tissue as reported by Fobert and Webb (1988).

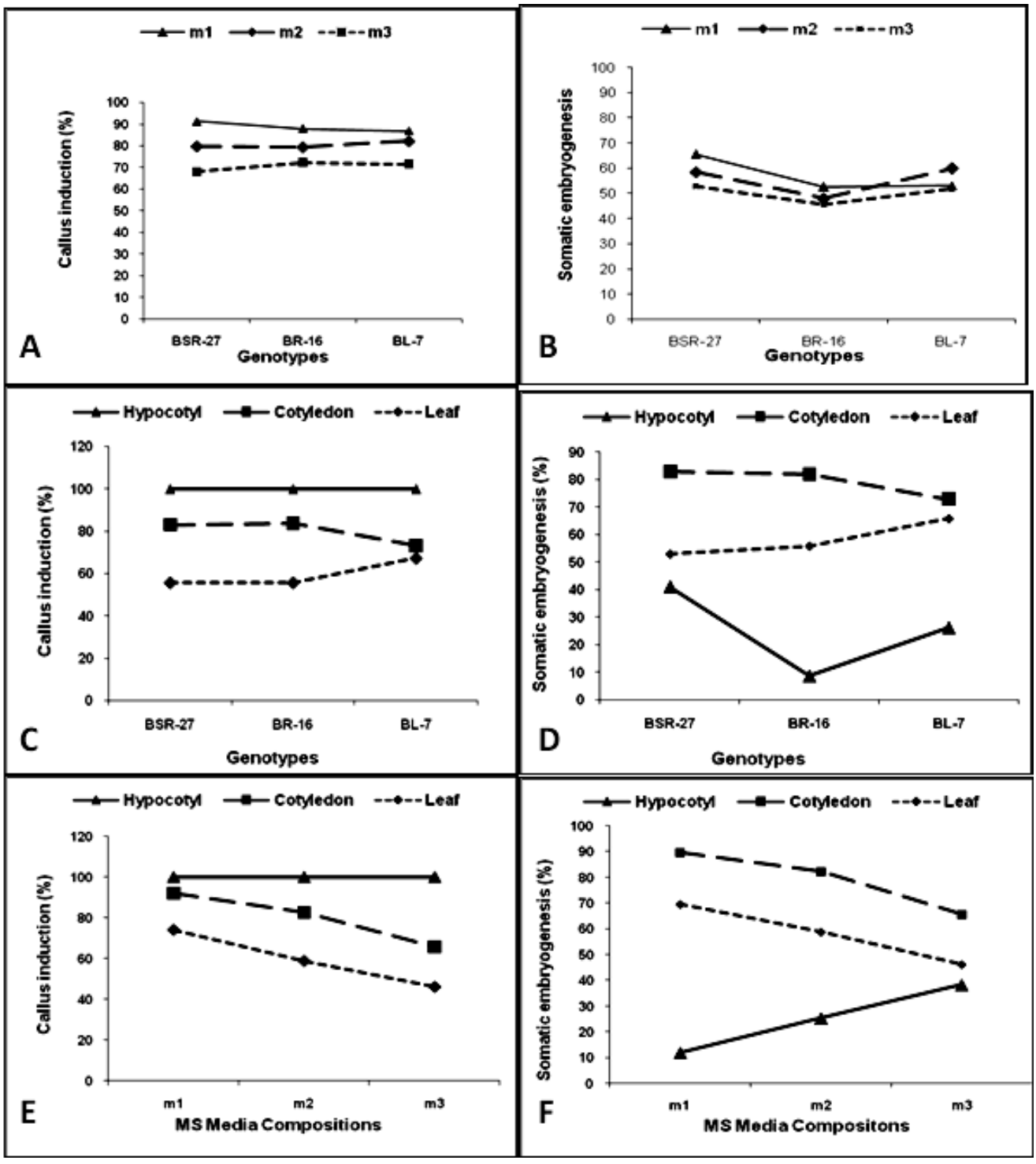

Figure 1 A). Effect of genotype and media on callus induction and **B**) Effect of genotype and media on somatic embryogenesis, **C)** Effect of genotype and explant on callus induction and **D)** Effect of genotype and explant on somatic embryogenesis in eggplant, **E)** Effect of media and explant on callus induction and **F)** Effect of media and explant on somatic embryogenesis in eggplant.
Noted: m1: (1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP), m2: (1.0 mgl^{-1} IBA + 1.0 mgl^{-1} BAP), m3: (0.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP Callus induction: LSD (P=0.05): Genotype × Media: 1.39; Genotype× Explant: 1.37; Media × Explant: 1.39. Somatic embryogenesis: LSD (P=0.05): Genotype × Media: 0.81; Genotype × Explant: 0.79; Media × Explant: 0.81.

Three way interactions for callus induction and somatic embryogenesis of genotype, media and explants (Table 1) also differed significantly. The hypocotyl of all the genotypes induced cent percent callusing on all the MS

Table 1. Effect of genotype, media and explant on callus induction and somatic embryogenesis in eggplant.

Genotype		Callus induction					Somatic embryogenesis				
		BSR-27	BR-16	BL-7	Media Mean	Explant Mean	BSR-27	BR-16	BL-7	Media Mean	Explant Mean
Media	**Explant**										
1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP	Hypocotyl	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	88.72 (75.20)	Hypocotyl: 100 .00 (89.96) Cotyledon: 80.17 (65.28) Leaf: 59.66 (50.79)	31.45 (34.09)	0.00 (0.00)	4.75 (12.57)	57.10 (47.95)	Hypocotyl: 25.29 (27.44) Cotyledon: 79.17 (63.69) Leaf: 58.19 (49.81)
	Cotyledon	94.47 (76.39)	98.71 (86.19)	83.31 (65.86)			94.47 (76.39)	92.43 (74.01)	81.97 (64.86)		
	Leaf	80.43 (63.04)	65.38 (53.93)	77.24 (61.48)			70.75 (57.23)	65.38 (53.93)	72.68 (58.46)		
1.0 mgl^{-1} IBA + 1.0 mgl^{-1} BAP	Hypocotyl	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	80.48 (68.55)		37.26 (37.60)	5.69 (13.78)	33.22 (35.18)	55.47 (48.06)	
	Cotyledon	87.57 (69.33)	83.79 (66.25)	76.56 (61.02)			86.24 (68.23)	83.79 (66.25)	76.56 (61.02)		
	Leaf	51.90 (46.07)	55.92 (47.61)	69.98 (56.75)			51.90 (46.07)	54.59 (47.61)	69.98 (56.75)		
0.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP	Hypocotyl	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	70.61 (62.28)		54.51 (47.57)	20.18 (26.68)	40.52 (39.52)	50.08 (44.94)	
	Cotyledon	67.83 (55.42)	69.30 (56.33)	59.98 (50.73)			67.83 (55.42)	69.30 (56.33)	59.98 (50.73)		
	Leaf	36.06 (36.89)	47.46 (43.53)	54.90 (47.79)			36.06 (36.89)	47.46 (43.53)	54.90 (47.79)		
Genotype Mean		79.69 (68.56)	79.91 (69.30)	80.21 (68.17)			58.94 (51.05)	48.76 (42.46)	54.95 (47.43)		
LSD (P=0.05)		Genotype 0.79; Media 0.81; Explant 0.79; Genotype × Media 1.39; Genotype × Explant 1.37; Media × Explant 1.39; Genotype × Media × Explant 2.38					Genotype 0.45; Media 0.47 ; Explant 0.45; Genotype × Media 0.81; Genotype × Explant 0.79; Media × Explant 0.81; Genotype × Media × Explant 1.37				

* Figures in parenthesis indicate arc sine transformation of values.

media combinations, while, cotyledon (98.71%) of BR-16 and leaf (80.43%) of BSR-27 induced maximum callus on MS fortified with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP. The lowest (36.06%) callus induction was observed on 0.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP media combination in leaf explant of BSR-27. With distinction, the cotyledon of BSR-27 induced significantly highest somatic embryogenesis (94.47%) followed by the cotyledon of BR-16 (92.43%) on MS medium fortified with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP, however, among leaf explants, BL-7 had maximum (72.68%) somatic embryogenesis on the same medium composition. Here, hypocotyl of BR-16 was not able to induce somatic embryogenesis on MS media fortified with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP. The results are corroborated with the findings of Slater et al. (2003) for somatic embryogenesis.

The overall performance for callus induction according to Table 1 was 80.21, 79.91 and 79.69% with non-significant differences in BL-7, BR-16 and BSR-27, respectively. Contrarily, somatic embryogenesis was highest in BSR-27 (58.94%) and lowest in BR-16 (48.76%). Matsuoka and Hinata (1979) also endorsed genotype as the most important factor for affecting the somatic embryogenesis.

In present study, differences in somatic embryogenesis of various eggplant genotypes may be due to inherent differences of auxins and cytokinins. BR-16 induced voluminous callus with pale yellow colour, whereas, BSR-27 and BL-7 produced pale coloured, compact, granular and embryogenic callus at cut ends in about 10 to 12 days on the medium supplemented with IBA and BAP. The genotypic differences for somatic embryogenesis have also been confirmed by Dobariya and Kachhadiya (2004); Huda et al. (2007); Mir et al. (2008). The degree of differences in somatic embryogenesis have also been correlated with developmentally regulated genes (Momiyama et al., 1995), polyamine content distribution and metabolism with position effects (Sharma and Rajam, 1995) and differences in mRNA expression in different cultivars (Afele et al., 1996) in eggplant. In this investigation, media means indicated MS fortified with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP was the best for callusing (87.72%) and somatic embryogenesis (57.10%)(Figure 2A), followed by 1.0 mgl^{-1} IBA + 1.0 mgl^{-1} BAP (80.48, 55.47%) and 0.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP (70.61, 50.08%).

Somatic embryogenesis is favoured by intermediate level of auxins, while higher concentration leads to callogenesis (Fobert and Webb, 1988). Comparison of explants means demonstrated 100.00% callusing in hypocotyl with significant edge over cotyledon (80.17%) and leaf (59.66%), while, cotyledon ranked at top with

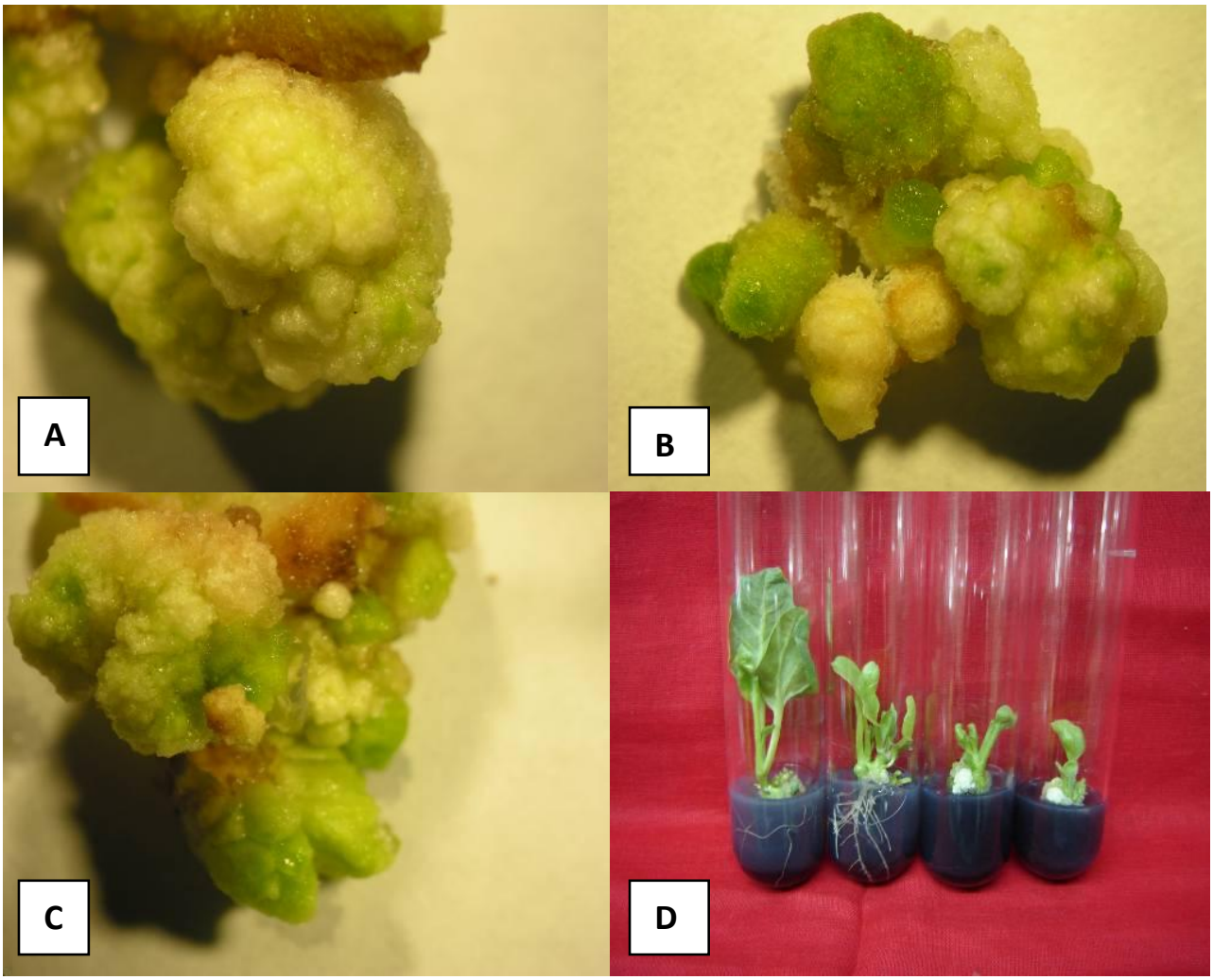

Figure 2. *In vitro* somatic embryogenesis and regeneration in eggplant: (A) Callus induction in cotyledon explant, (B) Embryogenic callus with initiation of regeneration. (C) Regenerating shoot buds in cotyledonary callus. (D) Regenerated eggplant shoots.

79.17% somatic embryogenesis, followed by leaf (58.19%) and hypocotyl (25.29%). The differentiation of somatic embryos in callus depends upon the proportion of inbuilt auxins and cytokinins in the plant tissue. But here, hypocotyl with quite less embryogenesis seems to have higher auxin level than cytokinins, whereas, high somatic embryogenesis in cotyledon and leaf indicates a balanced proportion. In different studies, explants like hypocotyl (Mir et al., 2011) cotyledon (Huda et al., 2007, Mir et al., 2008, Swamynathan et al., 2010), leaf (Rao and Singh, 1991) and root (Franklin et al., 2004, Mir et al., 2008) have been used for induction of somatic embryogenesis in eggplant. Even the quality differences for embryo induction among different sections of explants have been experienced (Sharma and Rajam, 1995; Magioli et al., 2001) that may have resulted from gradient phytohormones (Ulvskov et al., 1992).

Plant regeneration

Plant regeneration is the ability of the embryogeneic callus to convert into plantlets. The mode of regeneration depends upon the type and concentration of cytokinin, where the high concentrations of BAP and all concentrations of kinetin promoted the organogenesis and low concentrations of BAP induced somatic embryogenesis in addition to organogenesis as reported earlier by Reynolds (1986). Similar results with lower and higher concentrations of BAP have also been observed in this study.

Concentrations of BAP lower than 2.5 mgl^{-1} resulted callusing along with regeneration of a few buds, while higher concentrations lead to the browning of embryogenic callus. Embryogenic callus cultured on MS medium supplemented with 2.5 BAP + 1.0 mgl^{-1}kin + 0.2%

Table 2. Effect of genotype and explant on plant regeneration in eggplant.

Explant Genotype	Hypocotyl	Cotyledon	Leaf	Genotype Mean
BSR-27	13.88 (21.85)*	72.24 (58.19)	60.71 (51.16)	48.49 (43.73)
BR-16	7.61 (16.00)	58.08 (49.63)	44.90 (42.05)	36.86 (35.89)
BL-7	8.19 (16.62)	61.58 (51.67)	49.54 (44.71)	39.77 (37.67)
Explant Mean	9.89 (18.16)	63.97 (53.16)	51.72 (45.98)	

LSD (P=0.5): Genotype 0.71; Explant 0. 71; Genotype × Explant 1.23. *Figures in parenthesis indicate arc sine transformation of values

activated charcoal showed significantly higher plant regeneration in different genotypes and explants (Table 2).

The genotype and explant interactions resulted into the highest regeneration with cotyledon explant of BSR-27 (72.24%), followed by cotyledon of BL-7 (61.58%) that was at par with leaf (60.71%) of BSR-27 The highly embryogenic callus from cotyledons first changed into green colour (Figure 2B), then started organogenesis of buds (Figure 2C), which later on elongated into shoots (Figure 2D). The hypocotyl was found very poor in response to plant regeneration. The developmental differences among explants showed apparent by Swamynathan et al. (2010). Similar effects of genotype, explant and genotype-explant interaction on plant regeneration via somatic embryogenesis has been described earlier by Sharma and Rajam (1995); Dobariya and Kachhadiya (2004). As a whole, BSR-27 had highest plant regeneration (48.49%) followed by BL-7 (39.77%) and BR-16 (36.86%). The genotypic differences for organogenesis have also been reported (Huda et al., 2007; Mir et al., 2008) earlier.

In the present study, genotypes forming compact, nodular and embryogenic callus had good potential for regeneration, whereas, those with watery and too much proliferating callus were poor in organogenesis. Excessive proliferation of callus inhibited its differentiation into buds and their further elongation into plantlets. The explants also had different regeneration potential from embryogenic calli induced on the callus induction medium. The average performance of cotyledon (63.97%) was at the top among different explants.

Similarly, compact, nodular and embryogenic callus from cotyledon had highest regeneration potential. The different regenerative potential exhibited by the calluses isolated from different organs is due to the reason that different endogenous growth regulators may have important role in regenerative ability (Alicchio et al., 1982) and this differential response of the embryogenic calli from different explant for plant regeneration has also been reported by Huda et al. (2007). The regenerated plantlets after hardening with 0.2% solution of bavistin for 15 days were first transferred to polythene bags and then to earthen pot for further growth and flowering.

Conclusion

The investigation concluded that cotyledon of BSR-27 had high potential somatic embryogenesis and plant regeneration on MS medium fortified with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP and 2.5 mgl^{-1} BAP + 1.0 mgl^{-1}kin + 0.2% activated charcoal, respectively. As successful application of *in vitro* techniques, especially genetic transformation, somatic hybridization and somaclonal variations for crop improvement, depends upon the thriving regeneration protocol from the most responding tissue and the genotype. Hence, high regeneration potential of BSR-27 may be exploited further.

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