

Role of genotype, explant and growth hormones on regeneration in eggplant (*Solanum melongena*)

MOHINDER KAUR¹, AJMER SINGH DHATT², JAGDEEP SINGH SANDHU³,
AMRIK SINGH SIDHU⁴ and SATBIR SINGH GOSAL⁵

Punjab Agricultural University, Ludhiana 141 004

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ABSTRACT

Induction of somatic embryos in cultured hypocotyl, cotyledon and leaf explants of BL 5, BR 14 and BSR 23 genotypes of eggplant (*Solanum melongena*) was investigated on MS medium fortified with different concentrations of IBA and BAP. The statistical analysis showed significant differences among various interactions. Cotyledon of BL 5 exhibited 89.02 and 87.35% callusing and somatic embryogenesis, respectively with fortification of MS using 1.5 mg/l IBA + 1.0 mg/l BAP. Whereas, hypocotyl of all genotypes produced 100% callusing with poor somatic embryogenesis on all the MS media combinations (0.5–1.5 mg/l IBA + 1.0 mg/l BAP). Compact, nodular and friable cotyledonary callus of BSR 23 attained 64.86% plant regeneration on MS fortified with 2.5 mg/l BAP + 1.0 mg/l Kin + 0.2% activated charcoal, with significant differences from cotyledonary callus of BR 14 (57.33%) and BL 5 (52.83%). As a whole, the cotyledonary callus of BSR 23 exhibited somatic embryos with highest regenerative potential.

Key words: Cotyledon, Eggplant, Hypocotyl, Leaf, Regeneration, Somatic embryogenesis

Eggplant (*Solanum melongena* L. $2n=2\times=24$) is an economically important vegetable crop in South Asia, especially in Indian subcontinent. It is most amenable to cell, tissue organ culture methods. The successful application of *in-vitro* techniques depend on ability to produce embryogenic callus with good regeneration potential (Razdan 2000) that depend upon genetic makeup of genotype, type of explant and composition of growth hormones (Kantharajah and Golegaonkar 2004, Magioli and Mansur 2005). Among these factors, genotype had most significant effect on somatic embryogenesis (Matsuoka and Hinata 1979) and many researchers endorsed genotypic differences for it (Alicchio *et al.* 1982, Gleddie *et al.* 1983, Ali *et al.* 1991, Sharma and Rajam 1995, Afele *et al.* 1996, Dobariya and Kachhadiya

2004, Huda *et al.* 2007). Differential response for somatic embryogenesis of hypocotyl, cotyledon, leaf and root explants has also been observed (Macchia *et al.* 1983, Gleddie *et al.* 1986, Rao and Singh 1991, Sharma and Rajam 1995, Fari *et al.* 1995, Tarre *et al.* 2004, Franklin *et al.* 2004 and Huda *et al.* 2007). The growth hormones achieved variable response for somatic embryogenesis and regeneration with different genotype and explants (Kamat and Rao 1978, Slater *et al.* 2003), but the response of IBA (Indole Butyric Acid), a weak auxin for somatic embryogenesis is not much published in this crop. High regeneration protocol is the prerequisite for successful application of *in vitro* techniques especially genetic transformation and somatic hybridization for crop improvement. Therefore, present study deals with the role of genotype, explant and growth hormones on regeneration in eggplant (*Solanum melongena* L.).

MATERIALS AND METHODS

The investigation for regeneration through callus induction and somatic embryogenesis from hypocotyl, cotyledon and leaf explants of eggplant genotypes, namely BL 5 (long fruited), BR 14 (round fruited) and 'BSR 23' (small round) was carried out during 2005–08 using MS (Murashige and Skoog 1962) medium fortified with different concentrations and combinations of IBA (Indole Butyric Acid), BAP (6-Benzyl aminopurine) and Kin (Kinetin) for

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¹Assistant Professor of Horticulture (e mail: mkaur97@rediffmail.com), KVK, Ferozpur Cantt, Punjab, 152 001.

²Vegetable Breeder (email: ajmerdhatt@gmail.com), Department of Vegetable Crops; ³Assistant Biotechnologist (e mail: js_sandhu@hotmail.com), School of Agricultural Biotechnology;

⁴Director (e ail:amriksidhu@rediffmail.com), IIHR, Bangalore;

⁵Director (e mail: ssgosal@rediffmail.com), School of Agricultural Biotechnology

the development of regeneration protocol. Seeds were washed with teepol™ (Labolene), treated with 50% fabric whitener 'Ala Bleach' (containing 4% Sodium hypochlorite, 1% Sodium hydroxide, 1% Amine Oxide) for 20 min. and cultured on half strength MS medium solidified with 0.8% agar for germination at 25±2°C in dark for 20 days. 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 (0.5–1.5 mg/l) and BAP (1.0 mg/l) for callus induction and somatic embryogenesis and incubated in dark at 25±2°C. The observations for callus induction and somatic embryogenesis were recorded after 10 days of culturing. The callus induction (%) and somatic embryogenesis (%) was calculated from the number of explants cultured over the total number of explants showing callus and somatic embryogenesis, respectively. The compact, friable, nodular and embryogenic callus from the best media combination was further used for regeneration on the MS medium fortified with 2.5 mg/l+1.0 mg/l Kin along with 0.2% activated charcoal and incubated with 16 hrs light/8 hrs dark cycles at 25±2°C. The regeneration (%) was calculated from the number of calli regenerated over the total number of calli cultured for regeneration. Three repeats were maintained for each treatment and data were recorded. Statistical analysis was done in CRD factorial design using CPCS 1 soft ware package developed by Cheema and Singh (1990). Results were compared at 5% level of least square differences (LSD) and interpreted.

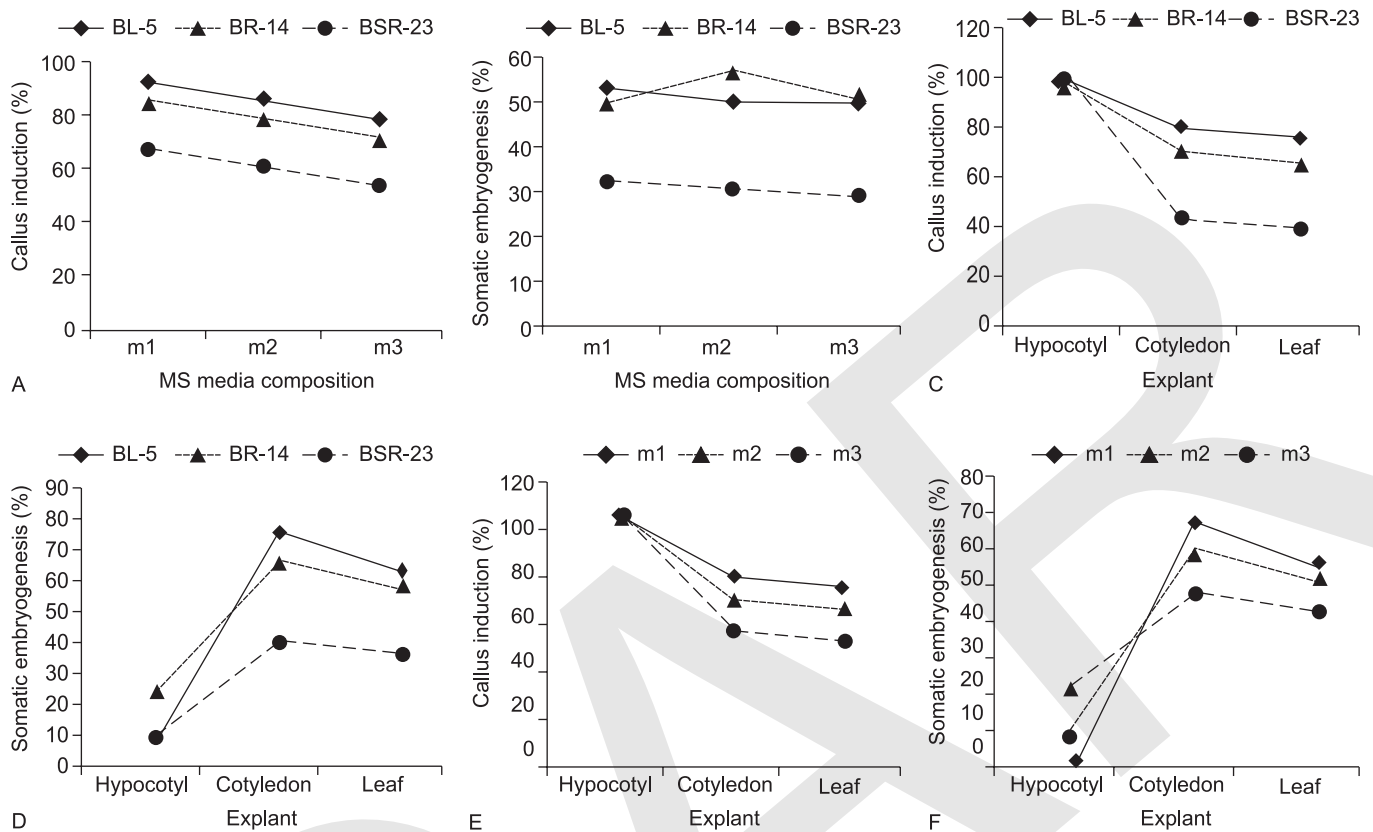
RESULTS AND DISCUSSION

Callus induction and somatic embryogenesis

After statistical analysis, two-way interactions of different factors, viz genotype, media and explant were observed significant for callus induction and somatic embryogenesis. The effect of genotype and media on callus induction given in Fig 1 showed significant differences, where BL 5 induced maximum callusing (92.40%) on MS medium supplemented with 1.5 mg/l IBA + 1.0 mg/l BAP, followed by MS + 1.0 mg/l IBA + 1.0 mg/l BAP (86.02%). BR 14 was at par with 85.76% callusing on MS + 1.5 mg/l IBA + 1.0 mg/l BAP. However the interaction for somatic embryogenesis in Fig 1 indicated BR 14 as the best genotype (56.77%) on MS medium fortified with 1.0 mg/l IBA+1.0 mg/l BAP that was significantly higher than BL 5 (52.62%) on 1.5 mg/l IBA+1.0 mg/l BAP and BSR 23 (50.03%) on 0.5 mg/l IBA+1.0 mg/l BAP. In present study, differences in somatic embryogenesis of various eggplant genotypes can be due to inherent differences of auxins and cytokinins. Thus, differences in the callus induction potential of various eggplant genotypes on a particular medium can be due to the different level of auxins or cytokinin in their tissue, as these differences in

Actinidia deliciosa has been reported (Centeno *et al.* 1996). The genotypic differences for hormone levels can be evidenced from the callus quality also. Although, BSR 23 induced callus in less number of plants, but it was small, compact, friable and nodular. BR 14 and BL 5 developed voluminous callus in more number of explants having somatic embryos in less intensity. That is why per cent somatic embryogenesis was greater in later genotypes. The intensity of somatic embryos in callus decreased with the increase in auxin concentration. Similar results have been reported for somatic embryogenesis by Fobert and Web (1988). Significant differences for genotype and explant interactions were also observed (Fig 1), where hypocotyl induced 100.00% callusing in all the three genotypes and leaf of BSR 23 was at lowest ebb with 39.25%. However, the interactions depicted 79.31% somatic embryogenesis in BL 5 cotyledon that was significantly higher than BR 14 (69.12%) cotyledon, while, leaf explants of BL 5 and BR 4 were able to achieve 65.73% and 62.15% somatic embryogenesis, respectively in Fig 1. With a difference, hypocotyl receded lowest in all the genotypes. Media and explant interactions also depicted cent per cent callusing with hypocotyl on all the MS media combinations as described in Fig 1. It was followed by the cotyledon and leaf explants with maximum of 75.05 and 70.25% callusing, respectively, on 1.5 mg/l IBA + 1.0 mg/l BAP media combination. The interaction also depicted that cotyledon developed significantly highest somatic embryogenesis on MS media fortified with 1.5 mg/l IBA + 1.0 mg/l BAP (72.27%), followed by 1.0 mg/l IBA + 1.0 mg/l BAP (65.43%), whereas, with leaf, it was also the highest (59.98%) on same medium. However, hypocotyl induced to the maximum of 26.56% somatic embryogenesis on 0.5 mg/l IBA+1.0 mg/l BAP as illustrated in Fig 1. Even the different intensities of embryo induction among different sections of cotyledon have also been experienced (Magioli *et al.* 2001). The different responses displayed by different segments may have resulted from gradient phytohormones (Ulvskov *et al.* 1992). Further, the differentiation of somatic embryos in callus depends upon the proportion of inbuilt auxins and cytokinins in the plant tissues. Compact, friable, and somatic callusing from cotyledon visible in Fig 2 can be due to balanced proportion of auxins and cytokinins. Even the quality differences for callusing of hypocotyl segments in eggplant have been reported by Sharma and Rajam (1995).

Three-way interaction of genotype, media and explant, being most important, denoted significant differences for callus induction and somatic embryogenesis (Table 1) in the eggplant, where hypocotyl of all the genotypes induced 100% callusing on all the MS media combinations (1.5 mg/l IBA + 1.0 mg/l BAP, 1.0 mg/l IBA +1.0 mg/l BAP and 0.5 mg/l IBA + 1.0 mg/l BAP), while, cotyledon (89.02%) and leaf (88.20%) of BL 5 induced maximum callus on 1.5 mg/l IBA + 1.0 mg/l BAP. The lowest callusing (29.38%) was observed on MS with 0.5 mg/l IBA + 1.0 mg/l BAP media combination



m1: (1.5 mg/litre IBA + 1.0 mg/litre BAP), m2: (1.0 mg/litre IBA + 1.0 mg/litre BAP), m3: (0.5 mg/litre IBA + 1.0 mg/litre BAP) Callus induction: LSD (P=0.05): Genotype \times Media: 0.63; Genotype \times Explant: 0.61; Media \times Explant: 0.63 somatic embryogenesis: LSD (P=0.05): Genotype \times Media: 0.83; Genotype \times Explant: 0.81; Media \times Explant: 0.83

Fig 1 (A) Genotype and media effects on callus induction, (B) genotype and media effects on somatic embryogenesis in eggplant, (C) genotype and explant effects on callus induction, (D) genotype and explant effects on somatic embryogenesis in eggplant, (E) media and explant effects on callus induction, (F) media and explant effects on somatic embryogenesis in eggplant

with leaf explant of BSR 23. Three way interactions also had significant differences for somatic embryogenesis, where, cotyledon of BL 5 induced 87.35 and 81.06% somatic embryogenesis on MS medium fortified with 1.5 mg/l IBA + 1.0 mg/l BAP and 1.0 mg/l IBA + 1.0 mg/l BAP, respectively. It was followed by cotyledon of BR 14 (78.49%) on MS having 1.5 mg/l IBA + 1.0 mg/l BAP. The leaf explant followed a similar trend on same culture media in all the genotypes for maximum somatic embryogenesis. However hypocotyl of BL 5 and BSR 23 was not able to induce somatic embryogenesis on MS medium fortified with 1.5 mg/l IBA + 1.0 mg/l BAP. The overall average for callusing was considerably highest in BL 5 (85.54%) followed by BR 14 (78.77%) and BSR 23 (60.88%), whereas somatic embryogenesis was highest in BR 14 (52.15%) and lowest in BSR 23 (29.95%). Matsuoka and Hinata (1979) endorsed that genotype is the most important factor for affecting the somatic embryogenesis. The genotypic differences for somatic embryogenesis have also been observed by Alicchio *et al.* (1982), Ali *et al.* (1991), Sharma and Rajam (1995),

Dobariya and Kachhadiya (2004), and Huda *et al.* (2007). Momiyama *et al.* (1995) reported degree of difference during somatic embryogenesis due to developmentally regulated genes. The polyamine content distribution and metabolism have also been correlated with position effects on the embryogenic competence in eggplant (Fobert and Webb 1988, Sharma and Rajam 1995, Yadav and Rajam 1997 and 1998). Afele *et al.* (1996) reasoned differences in mRNA expression in different cultivars for induction of somatic embryos. Media means indicated MS fortified with 1.5 mg/l IBA + 1.0 mg/l BAP as the best (81.77%) for callusing, followed by 1.0 mg/l IBA + 1.0 mg/l BAP (75.85%) and 0.5 mg/l IBA + 1.0 mg/l BAP (67.57%). However, MS medium with 1.0 mg/l IBA + 1.0 mg/l BAP induced highest somatic embryogenesis (45.68%), followed by MS with 1.5 mg/l IBA + 1.0 mg/l BAP (44.51%) and 0.5 mg/l IBA + 1.0 mg/l BAP (42.56%). Comparison of explants demonstrated that hypocotyl induced 100.00% callusing with significant edge over cotyledon (64.55%) and leaf (60.64%). But cotyledon ranked at top with 63.62% somatic embryogenesis, followed

Table 1 Effect of genotype, growth hormones and explant on callus induction and somatic embryogenesis in eggplant

| Genotype | | Callus induction (%) | | | | | Somatic embryogenesis (%) | | | | |
|--------------------------------|-----------|--|-------------------|-------------------|------------------|---------------------------------|---|------------------|------------------|------------------|--------------------------------|
| MS Media | Explant | BL 5 | BR 14 | BSR 23 | Media Mean | Explant Mean | BL 5 | BR 14 | BSR 23 | MS Media Mean | Explant Mean |
| 1.5 mg/l IBA + 1.0 mg/l BAP | Hypocotyl | 100.00 (89.96)* | 100.00 (89.96) | 100.00 (89.96) | 81.77 (69.58) | Hypocotyl: 100.00 (89.96) | 0.00 (0.00) | 3.88 (11.35) | 0.00 (0.00) | 44.51 (37.89) | Hypocotyl: 14.29 (18.51) |
| | Cotyledon | 89.02 (70.64) | 82.31 (65.10) | 53.83 (47.18) | | | 87.35 (69.19) | 78.49 (62.37) | 50.98 (45.54) | | |
| | Leaf | 88.20 (69.88) | 74.97 (59.95) | 47.59 (43.60) | | | 70.51 (57.09) | 66.62 (54.69) | 42.80 (40.84) | | |
| 1.0 mg/l IBA+ 1.0 mg/l BAP | Hypocotyl | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 75.85 (65.56) | Cotyledon: 64.55 (54.07) | 6.68 (14.97) | 33.37 (35.26) | 5.03 (12.93) | 45.68 (41.62) | Cotyledon: 63.62 (53.43) |
| | Cotyledon | 81.06 (64.17) | 71.28 (57.58) | 43.95 (41.50) | | | 81.06 (64.17) | 71.28 (57.58) | 43.95 (41.50) | | |
| | Leaf | 77.01 (61.33) | 68.63 (55.92) | 40.77 (39.66) | | | 66.17 (54.37) | 65.68 (54.11) | 40.77 (39.66) | | |
| 0.5 mg/l IBA + 1.0 mg/l BAP | Hypocotyl | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 67.57 (60.48) | Leaf: 60.64 (51.59) | 17.13 (24.43) | 38.34 (38.24) | 24.21 (29.46) | 42.56 (40.41) | Leaf: 54.84 (47.99) |
| | Cotyledon | 69.52 (56.47) | 57.58 (49.34) | 32.42 (34.69) | | | 69.52 (56.47) | 57.58 (49.34) | 32.42 (34.69) | | |
| | Leaf | 65.09 (53.76) | 54.16 (47.36) | 29.38 (32.81) | | | 60.33 (50.94) | 54.16 (47.36) | 29.38 (32.81) | | |
| Genotype Mean | | 85.54 (71.79) | 78.77 (67.24) | 60.88 (56.59) | | | 50.42 (43.51) | 52.15 (45.59) | 29.95 (30.82) | | |
| LSD ($P=0.05$) | | Genotype: 0.35; Media: 0.37; Explant: 0.35; Genotype \times media: 0.63; Genotype \times explant: 0.61; Media \times explant: 0.63; Genotype \times media \times Explant: 1.05 | | | | | Genotype: 0.48; Media: 0.50; Explant: 0.48; Genotype \times media: 0.83; Genotype \times explant: 0.81; Media \times explant: 0.83 Genotype \times Media \times explant: 1.41 | | | | |

* Figures in parentheses indicate arc sine transformation of values

by leaf (54.84%) and hypocotyl (14.29%) explants. Also, the differentiation of somatic embryos in callus depends upon the proportion of inbuilt auxins and cytokinins in the plant tissue as reported by Alicchio *et al.* (1982). Hypocotyl with quite less embryogenesis and voluminous callus seems to have higher auxin level than cytokinins, whereas, high somatic embryogenesis in cotyledon and leaf visible in Fig 2 indicates a balanced proportion. In different studies, explants, like hypocotyl (Sharma and Rajam 1995), cotyledon (Fari *et al.* 1995, Tarre *et al.* 2004, Huda *et al.* 2007), leaf (Macchia *et al.* 1983, Rao and Singh 1991) and root (Franklin *et al.* 2004) have been used for induction of somatic embryogenesis in eggplant.

Plant Regeneration

Regeneration is the ability of the callus to convert somatic embryos into plantlets and embryogenic callus of best combination of IBA and BAP (MS medium fortified with 1.5 mg/l IBA + 1.0 mg/l BAP) was used for this purpose. The embryogenesis and regeneration depend upon the concentration of auxins and cytokinins. Cytokinin-auxin interaction either promotes or inhibits the development of shoots and roots and this depends upon the ratio of hormones in the medium (Kamat and Rao, 1978). Generally, high cytokinin to auxin ratio favours shoot formation (Slater *et al.* 2003). Lower concentrations of BAP (0.5–2.0 mg/l) did not promote regeneration. Therefore, the regeneration of somatic callus in present study was experimented on medium with high concentration of cytokinins. Embryogenic callus cultured on MS medium supplemented with 2.5 mg/l BAP + 1.0 mg/l kin + 0.2% activated charcoal showed significant differences for regeneration in genotypes and explants (Table 2), It was highest with cotyledon explant of BSR 23 (64.86%), followed by same explant of BR 14 (57.33%) and BL 5 (52.83%), whereas, leaf explant showed 50.65, 43.39 and 40.46% in the respective genotypes. The hypocotyl was found very poor in response to regeneration, where, callus of BL 5 was not able to convert into a single plantlet. Genotype-explant interaction had highly significant effects on regeneration of somatic embryos with genotype exerting maximum effect on both these processes (Sharma and Rajam 1995, Dobariya and Kachhadiya 2004).

In general BSR 23 with compact, nodular and embryogenic callus had good potential for regeneration. Although, BL 5 and BR 14 had high somatic embryogenesis (%) but somatic embryos per explant were less and are less responsive to regeneration. Here, excessive proliferation of callus inhibited its differentiation into buds and their further elongation into plantlets. The genotypic differences for regeneration have also been observed (Matsuoka and Hinata, 1979; Alicchio *et al.* 1982, Afele *et al.* 1996, Huda *et al.* 2007). On an average, cotyledon performed best (58.34%), followed by leaf (44.83%), while hypocotyl (5.78) had very poor response to regeneration. Similarly, compact, nodular

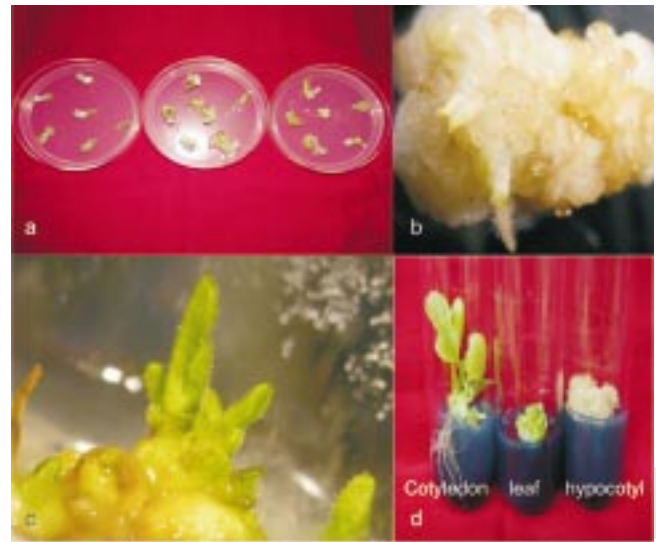


Fig 2 *In vitro* somatic embryogenesis and regeneration in eggplant: A. Callus induction in hypocotyl leaf and cotyledon explants, B. embryogenic callus induction with initiation of regeneration, C. plant regeneration in cotyledonary callus, D. organogenic differences in cotyledon, leaf and hypocotyl explants

and embryogenic callus from cotyledon had highest regeneration potential (Fig 2) among explants. The explants also had different regeneration potential from embryogenic calli induced on the callus induction medium (Fig 2). The different regenerative potential exhibited by the calli isolated from different organs depends upon different endogenous growth regulators may have important role in regenerative ability (Alicchio *et al.* 1982). The differential response of cotyledon and leaf explant from the embryogenic calli for regeneration has also been reported (Magioli *et al.* 1998, Huda *et al.* 2007). 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 regeneration and low concentrations of BAP

Table 2 Effect of genotype and explant on calli organogenesis (%) in eggplant

| Genotype | Explant | | | Genotype Mean |
|--------------------------|--------------------|-------------------|-----------------------------|------------------|
| | Hypocotyl | Cotyledon | Leaf | |
| BL 5 | 0.00 (0.00)* | 52.83 (46.60) | 40.46 (39.48) | 31.09 (28.69) |
| BR 14 | 7.09 (15.42) | 57.33 (49.19) | 43.39 (41.18) | 35.93 (35.26) |
| BSR 23 | 10.27 (18.68) | 64.86 (53.62) | 50.65 (45.35) | 41.92 (39.22) |
| Explant Mean | 5.78 (11.36) | 58.34 (49.80) | 44.83 (42.00) | |
| LSD (<i>P</i> =0.05) | Genotype: 0.64; | Explant: 0.65; | Genotype × explant: 1.11 | |

*Figures in parentheses indicate arc sine transformation of values

induced somatic embryogenesis in addition to organogenesis (Reynolds, 1986). Similar results with high concentrations of BAP have also been observed in this study (Fig 2). The regenerated plantlets also developed roots on the regeneration medium simultaneously as shown in Fig 2, which were acclimatized and transferred to polythene bags for further growth.

From the present investigation, it can be concluded that the cotyledon of BL 5 is best explant for somatic embryogenesis (%) on MS supplemented with 1.5 mg/l IBA + 1.0 mg/l BAP, but the cotyledon of BSR 23 induced embryogenic and good quality callus having greatest efficiency for regeneration on MS medium supplemented with 2.5 mg/l BAP + 1.0 mg/l kin + 0.2% activated charcoal. Thus, the cotyledon explant of BSR 23 can be exploited in further studies.

REFERENCES

- Afele J C, Tabei Y, Yamada T, Momiyama T, Takaiwa F, Kayano T, Nishimura S, Nishio T. 1996. Identification of mRNAs differentially expressed between embryogenic and non-embryogenic cultivars of eggplant during somatic embryogenesis. *JARQ* **30**: 175–9.
- Ali M L, Okubo H, Fujieda K. 1991. *In vitro* multiplication of intra and interspecific *Solanum* hybrids through somatic embryogenesis and adventitious organogenesis. *Journal of Japanese Society for Horticultural Sciences* **60**: 601–12.
- Alicchio R, Grosso E D, Boschieri E. 1982. Tissue culture and plant regeneration from different explants in six cultivars of *Solanum melongena*. *Experimentia* **38**: 449–50.
- Centeno M L, Rodriguez A, Feito I, Fernandez B. 1996. Relationship between endogenous auxin and cytokinin levels and morphogenic responses in *Actinidia deliciosa* tissue cultures. *Plant Cell Reports* **16**: 58–62.
- Dobariya K L, Kachhadiya J R. 2004. Role of genotype, explant, and culture medium on *in vitro* morphogenesis in eggplant (*Solanum melongena* L.). *The Orissa Journal of Horticulture* **32**: 52–54.
- Fari M, Nagy I, Csanyi M, Mityko J, Anderasfalvy A. 1995. *Agrobacterium*-mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv. Kecskemeti Lila) *Plant Cell Reports* **15**: 82–6.
- Fobert P R, Webb D T. 1988. Effect of polyamines, polyamine precursors, and polyamine biosynthetic inhibitors on somatic embryogenesis from eggplant (*Solanum melongena* L.) cotyledons. *Canadian Journal of Botany* **66**: 1734–42.
- Franklin G, Sheeba C J, Sita G L. 2004. Regeneration of eggplant from root explants. *In Vitro Cellular and Developmental Biology Plant* **40**: 188–91.
- Huda A K M N, Bari M A, Rahman M, Nahar N. 2007. Somatic embryogenesis of two varieties of eggplant (*Solanum melongena* L.). *Research Journal of Botany* **2**: 195–201.
- Kamat M G, Rao P S. 1978. Vegetative multiplication of eggplants (*Solanum melongena* L.) using tissue culture techniques. *Plant Science Letter* **13**: 57–65.
- Kantharajah A S, Golegaonkar P G. 2004. Somatic embryogenesis in eggplant. *Scientia Horticulturae* **99**: 107–17.
- Macchia F, Scaramuzzi F, Porcelli S. 1983. Organogenesis and propagation of F1 hybrid of *Solanum melongena* L. from vegetative segments. *Acta Horticulturae* **131**: 117–24.
- Magioli C, Mansur E. 2005. Eggplant (*Solanum melongena* L.) tissue culture, genetic transformation and use as an alternative model plant. *Acta Botanica Brasilia* **19**: 139–48.
- Magioli C, Rocha A P M, de Oliveira D E, Mansur E. 1998. Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. *Plant Cell Reports* **17**: 661–3.
- Magioli C, Barroco R M, Rocha C A B, Tarre E, Fernandes L D S, Mansur E, Engler G, Margis-Pinheiro M, Sachetto-Martins G. 2001. Somatic embryo formation in Arabidopsis and eggplant is associated with the expression of glycine rich protein gene (*Atgrp-5*). *Plant Science* **161**: 559–67.
- Matsuoka H, Hinata K. 1979. NAA-induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena* L. *Journal of Experimental Botany* **30**: 363–70.
- Momiyama T, Afele J C, Saito T, Kayano T, Tabei Y, Takaiwa F, Takayanagi K, Nishimura S. 1995. Differential display identifies developmentally regulated genes during somatic embryogenesis in eggplant (*Solanum melongena* L.). *Biochemical and Biophysical Research Communication* **213**: 376–82.
- Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology* **15**: 473–97.
- Rao P V L, Singh B. (1991) Plantlet regeneration from encapsulated somatic embryos of hybrid *Solanum melongena* L. *Plant Cell Reports* **10**: 7–11.
- Razdan M K. 2000. *An Introduction to Plant Tissue Culture*. Oxford & IBH Publishing Co. Pvt Ltd, New Delhi
- Reynolds T L. 1986. Somatic embryogenesis and organogenesis from callus cultures of *Solanum carolinense*. *American Journal of Botany* **73**: 914–18.
- Sharma P, Rajam M V. 1995. Genotype, explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). *Journal of Experimental Botany* **46**: 135–41.
- Slater A, Scott N, Fowler M. 2003. *Plant Biotechnology: The Genetic Manipulation of Plants*, pp 42. Oxford University Press Inc, New York.
- Tarre E, Magioli C, Margis P M, Sachetto M G, Mansur E, Santiago Fernandes L D R. 2004. *In vitro* somatic embryogenesis and adventitious root initiation has common origin in eggplant (*Solanum melongena* L.). *Revista Brasilia Botanica* **27**: 79–84.
- Ulvskov P, Nielson T H, Sieden P Mareussen, I. 1992. Cytokinins and leaf development in Sweet pepper (*Capsicum annum* L.) I. Spatial distribution of endogenous cytokinins in relation to leaf growth. *Planta* **188**: 70–7.
- Yadav J S, Rajam M V. 1997. Spatial distribution in free and conjugated polyamines in leaves of *Solanum melongena* L. associated with differential morphogenic capacity: efficient somatic embryogenesis by putrescine. *Journal of Experimental Botany* **48**: 1537–45.
- Yadav J S, Rajam M V. 1998. Temporal regulation of somatic embryogenesis by adjusting cellular polyamine content in eggplant. *Plant Physiology* **116**: 617–25.