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In vitro plant regeneration in brinjal from cultured seedling explants

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ABSTRACT

***In vitro* plant regeneration of brinjal genotype BL-3 was tried using hypocotyl, cotyledon and leaf explants from *in vitro* raised seedlings on Murashige and Skoog medium fortified with 6-benzylamino purine (BAP) and kinetin (kin) combination (2.0-3.0 mgl^{-1} BAP with or without 1.0 mgl^{-1} kin). The cotyledon explant gave cent percent regeneration on MS medium fortified with 2.0 mgl^{-1} BAP, 2.5 mgl^{-1} BAP, or 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin, while the highest numbers of buds on 2.5 mgl^{-1} BAP (24.90), followed by 2.0 mgl^{-1} BAP (17.90). Leaf explant also induced cent percent regeneration on MS medium fortified with 2.0 mgl^{-1} BAP and maximum number of buds (9.53) regenerated with 2.5 mgl^{-1} BAP. Hypocotyl had the maximum regeneration (66.53%) and maximum buds (3.96) on MS with 2.5 mgl^{-1} BAP. Maximum bud elongation (58.73%) was obtained on ½ MS medium supplemented with 0.3 mgl^{-1}BAP + double agar. MS basal medium induced maximum rooting of 61.11% plantlets. The hardening with 0.2% bavistin solution enhanced the survival efficiency of plantlets to 81.81%. The plantlets were established in the polythene bags and then transferred to earthen pots in the glasshouse, where they grew, flowered and set fruits.**

Key words: Egg plant, *in vitro*, regeneration, hypocotyl, cotyledon.

INTRODUCTION

Brinjal (*Solanum melongena* L., 2n = 2x = 24) also known as egg plant, aubergine or Guinea squash, is a widely adaptive and highly productive vegetable of tropical and subtropical regions. For the improvement against biotic and abiotic stresses as well as quality improvement through genetic transformation, standardization of plant regeneration protocol is the prerequisite. The direct organogenesis is the formation of plantlets directly from explants on the culture media. The various factors like explant and growth regulators influenced the *in vitro* regeneration through organogenesis in eggplant (Magioli and Mansur, 8). The concentration and combination of exogenous auxin and cytokinin in the process of bud differentiation as well as the tissue system had variable response on plant regeneration in brinjal (Prakash *et al.,* 11). Generally, high cytokinin to auxin ratio leads to shoot formation and intermediate callus production (Sarker *et al.,* 13). Variable response of genotypes, explants and media for regeneration have also been substantiated by Sharma and Rajam (15), Jahan and Syed; Magioli *et al.* (7), Picoli (10), Dobariya and Kachhadiya (5), and Sarker *et al.* (13). As successful application of *in vitro* techniques for crop improvement rests upon reproducible plant regeneration protocol, the present investigation deals with an efficient method of direct plant regeneration from cultured seedling explants in brinjal.

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MATERIALS AND METHODS

The investigation for plant regeneration in brinjal was carried out during 2005-2008 in Tissue Culture laboratories of School of Agricultural Biotechnology, PAU, Ludhiana. Seeds of BL-3 were first washed with Teepol™ (Labolene). Then bold seeds were disinfected with 50 and 75% commercial bleach *i.e.*, 'Ala Bleach®' (sodium hypochlorite 4%, sodium hydroxide and amine oxide 1%) for 20 and 25 min. Disinfected seeds were then cultured on half-strength MS (Murashige and Skooge, 9) solid medium for germination and incubated at 25 ± 2°C in dark for 20 days. The seed germination (%) was calculated from the number of seeds germinated over total number of seeds cultured *in vitro*. Cotyledon, hypocotyl and leaf explants were excised aseptically from 15 to 20-day-old seedlings, cultured on MS medium fortified with different concentrations of BAP and kinetin (2.0-3.0 mgl^{-1} BAP with or without 1.0 mgl^{-1} kin) and regenerated at 25 ± 2°C for 16/8 h light and dark cycles. Plant regeneration (%) was calculated from the number of explants regenerated after 20 days over the total number of explants cultured for regeneration. The number of buds per explant was calculated from the average number of buds from 10 regenerating explants. The regenerated buds were then elongated on half-strength MS with different BAP concentrations. The shoot elongation (%) was calculated. The elongated plantlets were excised aseptically and transferred to different MS basal medium for root induction. The rooting (%) was calculated after 15-20 days from number of plantlets rooted over the total number

of plantlets cultured. The rooted plants were then hardened for different days on moistened cotton and filter paper with sterilized water as well as with 0.2% bavistin solution. Hardened plants were transplanted to polythene bags filled with mixture of sand, soil and FYM in 1:2:1 ratio and kept in greenhouse for further growth at 25°C. The, plants with 4-5 expanded leaves were grown to flowering and fruiting. At least three repeats were maintained for each treatment. Statistical analysis was done in CRD factorial design using CPCS-1 software package developed by Cheema and Singh (4). Least square differences at 5 percent level of significance were calculated and interpreted accordingly.

RESULTS AND DISCUSSION

The seeds disinfected with commercial bleach in the present investigation showed quite good germination. The interaction with time duration of treatment (Table 1) imply that 50% commercial bleach encouraged seed germination (85.80%), while increase in concentration limited it to 51.91%. Commercial bleach disinfection for 20 min. had significant effect on seed germination (74.58%) that declined to 63.13% on 25 min. treatment. The interaction of concentration and treatment duration indicate that the highest seed germination (%) was obtained from 20 min. disinfection with 50% commercial bleach. As per Sarker *et al.* (13), seed treated with 0.1% (w/v) mercuric chloride for 5-6 min. showed germination in brinjal. Seed germination with commercial bleach was quite good as compared to $HgCl_2$ and exhibited the normal growth and development of the seedlings (Fig. 1a). Commercial bleach contains 4% sodium hypochlorite (NaOCl), which acts as sterilizing agent.

The interaction of explant and medium composition for plant regeneration and number of buds explant^{-1} (Table 2) indicates that there was cent percent regeneration of cotyledon on MS medium fortified with 2.0 mgl^{-1} BAP, 2.5 mgl^{-1} BAP, 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin, while the highest number of buds were developed by cotyledon explant on 2.5 mgl^{-1} BAP (24.90). MS medium fortified with 2.0 mgl^{-1} BAP also induced 100 percent regeneration in leaf explant, whereas hypocotyl had highest regeneration (66.53%) potential with 2.5 mgl^{-1} BAP. MS medium fortified with 2.5 mgl^{-1} BAP induced maximum number of buds in leaf (9.53) and hypocotyl (3.96) explant also. Increase in BAP concentration above 2.5 mgl^{-1} BAP as well as addition of kin decreased the regeneration capability and number of buds on all the explants and lead to the browning of explants that could not elongate their buds into shoots. Leaf did not regenerate with 3.0 mgl^{-1} BAP. Also, higher concentrations of BAP could not initiate regeneration on hypocotyl explant. In general, MS medium supplemented with 2.0 mgl^{-1} BAP (87.07%) was the best combination for direct regeneration and 2.5 mgl^{-1} BAP for the highest number of buds (12.80). Different concentrations of BAP and kin had differential response for adventitious shoot formation. The direct regeneration potential depends upon the proportion of auxin and cytokinin. Also, the requirement for exogenous auxins and cytokinins in the process of bud differentiation varies with the tissue system and apparently depends on endogenous level of two hormones in the tissue (Sasan *et al.*, 14). In present study, callus induction was observed at lower concentrations of cytokinins and regeneration response increased with augmentation to an optimum level in a particular genotype. It may be due to higher concentration of auxins in the explant itself and was balanced to a desired level by addition of cytokinins in the culture medium for better plant regeneration. It was also observed that higher concentration of hormones caused browning of explants and hampered the regeneration and growth of buds. Therefore, optimum ratio of cytokinin to auxin is required for shoot regeneration. Hypocotyl, cotyledon and leaf explants also demonstrated differential response for direct plant regeneration on different media concentrations of the cytokinins (BAP and kin). It can be due to inherent differences in the level of expression in the explants on a particular medium. As a whole, cotyledon was the best explant with 69.16% regeneration and 11.48 buds, followed by leaf (45.65%, 4.47) and hypocotyl (24.18%, 1.29) explants. The differences for regeneration hypocotyl, cotyledon and leaf explants on can be seen visually in Fig. 1b, c, d respectively. It was observed

Table 1. Effect of commercial bleach on seed germination.

Bleach conc. (%)	Time duration (min.)		Mean germination
	25	20	
75	44.63 (41.90)*	59.19 (50.27)	51.91 (46.08)
50	81.64 (64.60)	89.97 (71.52)	85.80 (71.42)
Mean germination (%)	63.13 (53.25)	74.58 (60.90)	
LSD (P = 0.05)	Conc.= 0.92; Time duration = 0.92; Conc. × time duration = NS		

*Figures in parenthesis indicate arc sine transformation of values.

that cotyledon expanded to almost double size in a week and then developed small buds, which elongated further into the shoots (Fig. 1e). The differences among explants for direct regeneration have also been reported (Bora *et al.*, 2; Prakash *et al.*, 11; Sharma and Rajam 15; Sarker *et al.*, 13; Magioli *et al.*, 7; Taha and Tizan, 16). Even the difference within different portions of hypocotyl for morphogenetic potential has also been detected (Sharma and Rajam, 15). The formation of shoot buds was characterized by the appearance of shoot apex with the developing leaf primordial (Sarker *et al.*, 13).

Elongation of buds into plantlets was experimented with four medium compositions trong Maximum bud elongation (58.73%) resulted from half-strength ½ MS medium supplemented with 0.3 mgl^{-1} BAP and double agar. The least effect was seen when with 0.5 mgl^{-1} BAP and the addition of double agar increased the elongation (26.31%) of buds. However, it was 28.88% on hormoreifree MS (Fig. 2). There was excessive callus proliferation with 0.5 mgl^{-1} BAP, which converted most of buds into callus and did not let them elongate into plantlets. The addition of double agar reduced this proliferation. Decrease in BAP concentration also lowered the callus induction and increased the bud elongation as visible in Fig. 1e. Most of the scientists like Sarkar *et al.* (13) and Borgato *et al.* (3) reported that the shoot buds upon subculture to MS basal medium elongated into healthy shoots after

Fig. 1. *In vitro* regeneration in brinjal: (a) germinated seedlings, (b) regenerated hypocotyl, (c) regenerated cotyledon and , (d) regenerated leaf, (e) elongated plantlets from cotyledonary buds, (f) rooted plantlets, (g) hardened plantlets, (h) establishment in polybags, and (i) well established plants with fruits in greenhouse.

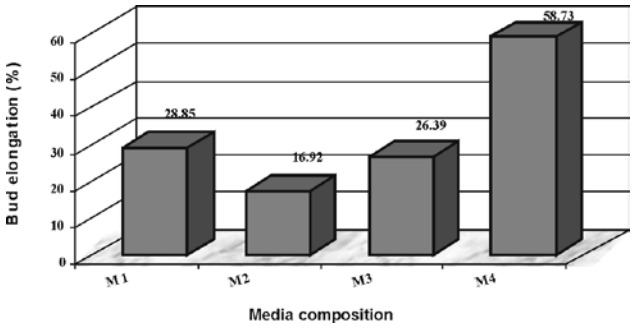

Fig. 2. Shoot elongation on different MS media compositions. M_1–1/2 MS, M_2 – 1/2MS + 0.5 mgl^{-1} BAP, M_3 – 1/2MS + 0.5 mgl^{-1} BAP + double agar, M_4 – ½ MS + 0.3 mgl^{-1} BAP + double agar.

organogenesis. Small shoots were elongated on MS medium containing zeatin and augmentin by Billings *et al.* (1).

Among different media compositions (Fig. 3), the maximum rooting of plantlets (61.11%) was observed on MS basal medium followed by MS liquid (54.83%). The addition of 0.5 mgl^{-1}IBA reduced the rooting to 4% only. The lowered concentration of IBA (0.1 mgl^{-1}) increased it to some extent, whereas only 14.70% rooting was observed on half-strength MS medium. IBA (weak auxin) is generally used for root induction in most of the plants, induced callusing at cut ends of brinjal plantlets that inhibited the differentiation of roots. This might be due to the reason that it has high auxin level in the plant tissue itself, which is increased further. Thus, MS basal medium best was root induction (Fig. 1f). Here, in brinjal, the inherent level of auxins seems to be high and its application for rooting revert the tissue towards callus. The root formation in brinjal was reported in half-stength MS medium (Taha and Tizan; 16 Sarker *et al.,* 13), ½ MS medium

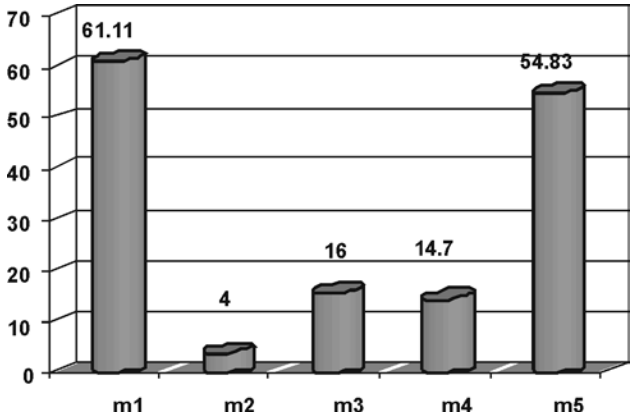

Fig. 3. Root induction on different MS media compositions. M_1 - MS, M_2 - MS + 0.5 mgl^{-1} IBA, M_3 - MS + 0.1 mgl^{-1} IBA, M_4 - ½ MS, M_5 - MS liquid.

Table 2. Effect of medium composition and explants on direct plant regeneration and number of buds per explant in brinjal.

Treatment	Plant regeneration				No. of buds per explant			
	Hypocotyl	Cotyledon	Leaf	Mean	Hypocotyl	Cotyledon	Leaf	Mean
2.0 mgl^{-1} BAP	61.23 (51.47)	100.00 (89.96)	100.00 (89.96)	87.07 (77.13)	3.03	17.90	8.06	9.66
2.0 mgl^{-1} BAP + 1.0 mgl^{-1} kin	0.00 (0.00)	35.56 (36.59)	9.66 (18.07)	15.07 (18.22)	0.00	7.03	2.93	3.32
2.5 mgl^{-1} BAP	66.53 (54.63)	100.00 (89.96)	73.13 (58.75)	79.88 (67.78)	3.96	24.90	9.53	12.80
2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin	41.50 (40.08)	100.00 (89.96)	58.33 (49.77)	66.61 (59.94)	2.06	10.16	4.83	5.68
3.0 mgl^{-1} BAP	0.00 (0.00)	75.80 (60.50)	53.73 (47.12)	43.17 (35.87)	0.00	11.93	3.93	5.28
3.0 mgl^{-1} BAP + 1.0 mgl^{-1} kin	0.00 (0.00)	52.63 (46.49)	24.70 (29.78)	25.77 (25.42)	0.00	4.23	2.03	2.08
3.5 mgl^{-1} BAP	0.00 (0.00)	20.13 (26.64)	0.00 (0.00)	6.71 (8.88)	0.00	4.20	0.00	1.40
Explant mean	24.18 (20.88)	69.16 (62.87)	45.65 (41.92)		1.29	11.48	4.47	
LSD (P = 0.05)	Medium = 0.59; Explant = 0.38; Medium × explant = 1.02				Medium = 0.23; Explant = 0.15; Medium × explant = 0.39			

*Figures in parenthesis indicate arc sine transformation of values.

supplemented with 0.6 μm IAA (Magioli *et al*., 7), ¼ MS medium (Dobariya and Kachhadiya, 5) and MS medium containing 1.0 mgl^{-1} 3-indole butyric acid (Borgato *et al.,* 3).

Hardening of rooted plantlets in wet cotton resulted in softening and killing of plants. That may be due to the excess of water supplied by wet cotton during hardening. Hardening of rooted plantlets on wet filter paper increased the survival (Fig. 1g). The plantlets were transferred to polythene bags (Fig. 1h) after 7, 10, 15 and 20 days of hardening and kept in greenhouse at 25 ± 1°C. No plant survived after 7 and 10 days of hardening. However, survival increased to 40% (4 out of 10) and 65% (13 out of 20) when plantlets hardened for 15 and 20 days, respectively. The addition of 0.2% bavistin to the tap water further enhanced the survival efficiency of plantlets to 81.81% (8 out of 11). The plants with 4-5 healthy leaves were transferred to the earthen pots in the glasshouse, where they grown up, flowered and set fruits (Fig. 1i). The *in vitro* regenerated plantlets are very sensitive and prone to the attack of microorganisms, when subjected to external environment. Treatment of bavistin checked the fungal infection and longer duration of hardening made plantlets acclimatized to the external conditions. Most species grown *in vitro* require acclimatization process in order to ensure that sufficient number of plants survive and grow on transferring to the soil (Hazarika 6). Taha and Tizan (16) achieved 80% acclimatization in field-transferred plantlets. Successful acclimatization and transfer of plants to the soil were stated by different workers (Salih and Al-Mallah, 12; Sarker *et al.,* 13; . Dobariya and Kachhadiya (5) also could established rooted shoots in polythene bags filled with a potting mixture of sand, soil and FYM in 1:2:1 ratio.

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