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Effect of genotype, explant and culture media on direct plant regeneration in eggplant

Mohinder Kaur*, Ajmer S. Dhatt, J.S. Sandhu and S.S. Gosal****

Department of Vegetable Science, Punjab Agricultural University, Ludhiana 141004

ABSTRACT

Direct plant regeneration of three eggplant genotypes, *viz.*, BL-5, BR-14 and BSR-23 was studied with hypocotyl, cotyledon and leaf explants on Murashige and Skoog (MS) medium fortified with different concentrations BAP and Kin. The interaction of genotype, medium and explant gave 80.36% regeneration from cotyledons of BL-5 on MS medium fortified with 3.0 mgl^{-1} BAP and 75.19% for cotyledons along with 3.0 mgl^{-1} BAP and 1.0 mgl^{-1} Kin. However, the same genotype induced 21.76 buds per cotyledon on MS medium supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kin. In general, maximum shoot elongation (%) was recorded in BSR-23 (58.53%) followed by BL-5 (56.16%), whereas, maximum rooting (%) was induced in BL-5 (57.50%), followed by BSR-23 (57.14%). The rooted plantlets were hardened on wet filter paper with 0.2% carbendazim solution for 20 days, transferred to polythene bags in greenhouse and thereafter, at 4-5 leaf stage to the earthen pots for growth and flowering.

Key words: Eggplant, hypocotyl, cotyledon, *in vitro*, regeneration, leaf.

INTRODUCTION

Eggplant (*Solanum melongena* L., 2n = 2x = 24) is a widely adaptive and highly productive vegetable of tropical and subtropical region. However, its productivity and quality is severely impaired by eggplant shoot and fruit borer (*Leucinodes orbonalis* Guenee). It can cause up to 50% damage, even under chemical sprayed conditions (Kaur *et al*., 6). The conventional breeding approaches gained limited success due to non-availability of resistant source in the cultivated germplasm. Therefore, standardization of protocol is the pre-requisite for the use of biotechnology. The direct organogenesis is the formation of plantlets directly on explants on the culture media. Explant and growth regulators influence the *in vitro* regeneration through organogenesis in eggplant (Magioli and Mansur, 7). The requirement for exogenous auxin and cytokinin in the process of bud differentiation varies with the tissue system and apparently depends upon the endogenous level of hormones (Razdan, 11). Generally, high cytokinin to auxin ratio leads to shoot formation and intermediate callus production (Sarker *et al*., 13; Slater *et al*., 15). Variable response of the genotype, explant and media compositions for regeneration has also been substantiated (Sarker *et al*., 13; Sammaiah *et al*., 12). Although, many researchers reported somatic embryogenesis in eggplant, but limited information is available for direct plant regeneration. Therefore, present investigation was the first step of genetic transformation, wherein; direct plant regeneration in three genotypes was experimented using different explants.

MATERIALS AND METHODS

The investigation on *in vitro* direct regeneration in three eggplant genotypes, *viz.*, BL-5, BR-14 and BSR-23 was carried out in tissue culture laboratories of School of Agricultural Biotechnology, PAU, Ludhiana. Seeds of each genotype were first washed with Teepol^{TM} to remove dirt and light weight seeds. Only bold seeds were taken and disinfected with 50% commercial bleach (sodium hypochlorite 4%, sodium hydroxide 1%, amine oxide 1%) for 20 min. and washed till the foam formation stopped. Disinfected seeds were cultured on half-strength MS medium (Murashige and Skoog, 10) solidified with 0.8% agar-agar and incubated at 25 ± 2°C in dark for germination. Cotyledon and hypocotyl explants were excised from 15-day-old *in vitro* grown eggplant seedlings of each genotype, whereas, leaf explants were excised from 25-day-old seedlings, aseptically and cultured on MS medium fortified with different concentrations of cytokinins (2.0-3.0 mgl^{-1} BAP (6-benzyl aminopurine) with or without 1.0 mgl^{-1} kin (kinetin) for shoot regeneration and incubated on 16 h light/ 8 h dark cycles at 25 ± 2°C. Observations on plant regeneration and number of buds explant^{-1} were taken 20 after days of culture. The number of buds explant^{-1} was calculated from the average

*Corresponding author's E-mail: mkaur97@rediffmail.com

**School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana

of number of buds from ten regenerating explants. The regenerated buds were then elongated on half-strength MS medium containing 0.3 mgl^{-1} BAP and double-strength agar-agar. The shoot elongation (%) was calculated after 15 days from the number of buds elongated into shoots over total number of buds cultured for elongation. After elongation, 2-3 cm plantlets were excised aseptically and transferred onto already standardized MS medium for root induction. The rooting (%) was calculated after 15-20 days from number of plantlets rooted over the total number cultured. The rooted plants were then hardened on filter paper moistened with 0.2% carbendazim solution for 20 days and filter paper was replaced daily. Hardened plants were planted to polythene bags and kept in green-house for further growth at 25 ± 1°C. Then, plants with 4-5 expanded leaves were transferred to earthen pots for growth, flowering and fruiting. At least three repeats were maintained for each treatment and data was recorded. Statistical analysis was done in CRD (factorial) design using CPCS-1 software package developed at Punjab Agricultural University (Cheema and Singh, 1). Least square differences at 5 percent level of significance were calculated and interpreted accordingly.

RESULTS AND DISCUSSION

The direct plant regeneration and number of buds $explant^{-1}$ showed significant difference for genotype × medium interaction (Fig. 1A & B). The highest regeneration potential (48.26 and 48.13%) was observed in BL-5, when it was cultured on MS medium supplemented with 3.0 mgl^{-1} BAP + 1.0 mgl^{-1} kin and 3.0 mgl^{-1} BAP alone. The highest number of buds per explant (10.74) was also observed in BL-5 on MS medium supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin, followed by 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} kin (6.18), whereas, BR-14 and BSR 23 regenerated maximum of 5.76 and 4.62 buds on 2.0 and 2.5 mgl^{-1} BAP, respectively. Three eggplant genotypes showed differential response for direct plant regeneration on medium with different concentrations of the cytokinins (BAP and kin). It might be due to inherent differences of hormonal level among genotypes (Shivraj and Srinath, 14). Genotype × explant interaction also had considerable effect on direct plant regeneration and number of buds $explant^{-1}$ (Fig. 1C & D). Maximum regeneration was observed in cotyledon (64.37%), followed by leaf (44.23%) in BL-5, whereas, it was least in hypocotyl of BSR-23 (0.46%). However, cotyledon of BL-5 and BR-14 induced 10.80 and 5.86 buds per explant, respectively. Hypocotyl was very poor for bud formation in all the genotypes. Medium and explant interactions were also significant (Fig. 1E & F). Performance of cotyledon was statistically highest on MS medium supplemented with 2.5 mgl^{-1} BAP (50.36%) followed by 3.0 mgl^{-1} BAP (47.96%) and 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin (40.51%). The maximum regeneration in leaf (38.06%) was observed on 3.0 mgl^{-1} BAP and in hypocotyl (8.97%) on 2.5 mgl^{-1} BAP +1.0 mgl^{-1} kin medium composition. Cotyledon was also the best with 10.26 buds $explant^{-1}$ on MS medium added with 2.5 mgl^{-1} + 1.0 mgl^{-1} kin, followed by 2.5 mgl^{-1} BAP (7.92) and 2.0 mgl^{-1} BAP (6.50). The maximum extent of leaf and hypocotyl for bud formation was 4.25 and 1.51 on 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin and 2.5 mgl^{-1} BAP, respectively. The differential response of the three explants on a particular medium might be due to dissimilar the level of hormones in the explants. The differences for regeneration of hypocotyl (H), cotyledon (C) and leaf (L) was seen visually in Fig. 2A. It was observed that cotyledon expanded to almost double size on optimum concentration of hormones in a week (Fig. 2B) and then developed small buds, which elongated further into the shoots. The lower concentrations caused callusing (Fig. 2C). Similar results have also been reported by Kanna and Mayabalan (4), and Kaur *et al.* (5).

Three-way interaction of genotype × explant × medium (Table 1) reveals that cotyledon of BL-5 on MS medium fortified with 3.0 mgl^{-1} BAP resulted in 80.36% regeneration. It was followed by 3.0 mgl^{-1} BAP + 1.0 mgl^{-1} kin (75.19%), 2.5 mgl^{-1} BAP (74.75%) and 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin (66.47%) with same explant and genotype. Whereas, cotyledon of BR-14 and BSR-23 was able to give maximum 51.39 and 36.78% regeneration on 2.5 and 2.0 mgl^{-1} BAP supplemented media, respectively. There was no regeneration in all the explants of BR-14 and BSR-23 on MS supplemented with 3.0 mgl^{-1} BAP and 1.0 mgl^{-1} kin. It was also observed that hypocotyl had very poor response to regeneration irrespective of genotypes. However, response of leaf explant was much better than that of hypocotyl in all the genotypes and media combinations. Genotype, BL-5 induced 21.76 buds per cotyledon on MS fortified with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin, followed by 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} kin (13.23). Cotyledon formed highest number of buds in BR-14 (10.56) and BSR 23 (7.76) on 2.0 mgl^{-1} BAP and 2.5 mgl^{-1} BAP, respectively. Leaf and hypocotyl explants induced maximum of 7.63 and 2.83 buds on MS fortified with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin.

In general (Table 1), regeneration potential was found higher in BL-5 (39.24%) compared to other genotypes. BL-5 generated highest number of buds $explant^{-1}$ (5.09), followed by BR-14 (3.11) and BSR-23 (1.57). Genotype has been considered as the most important factor for the regeneration (Mutsuoka

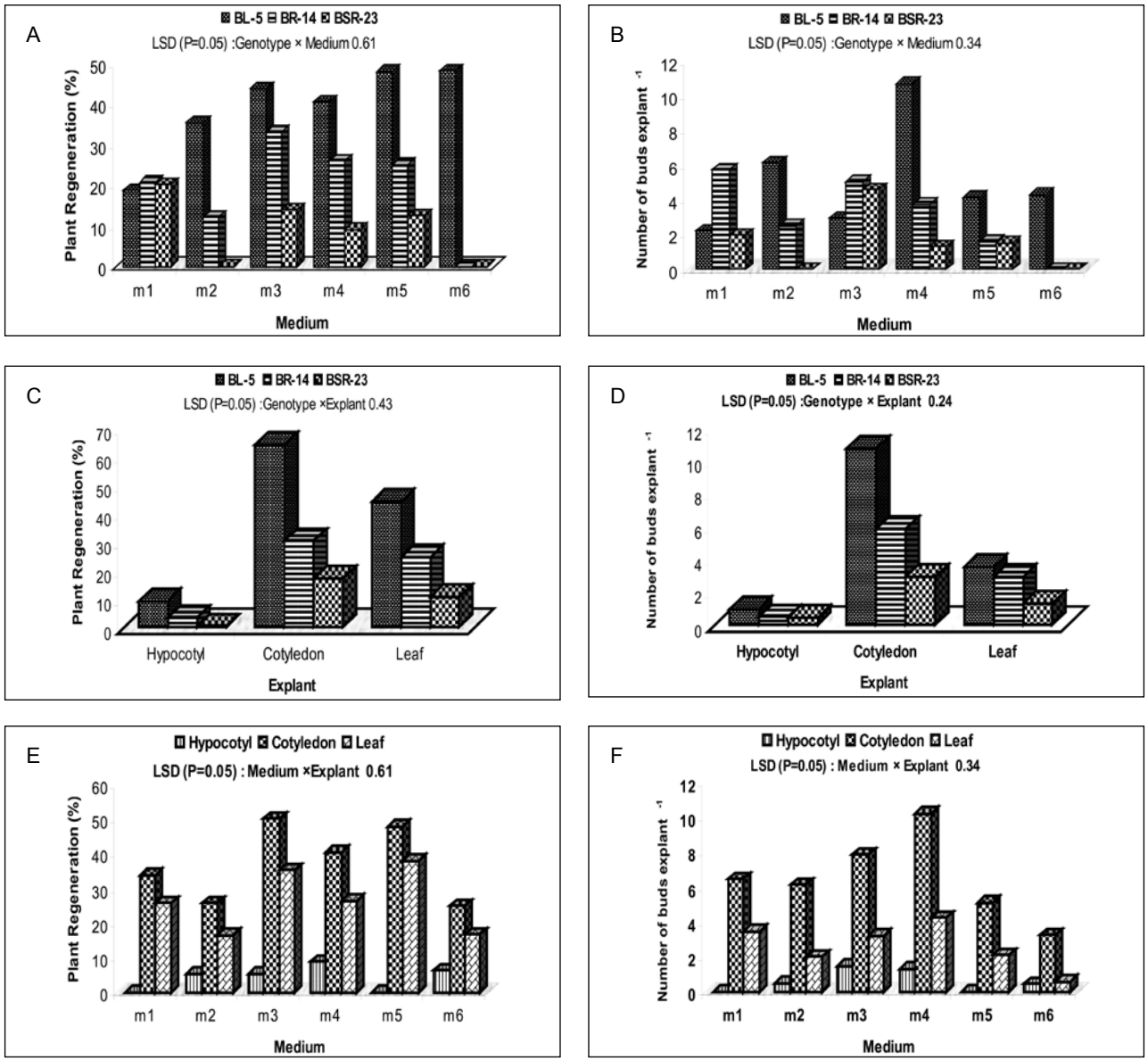

Fig. 1. A) Genotype and medium effects on direct plant regeneration, and B) Genotype and medium effects on number of buds explant^{-1} in eggplant, C) Genotype and explant effects on direct plant regeneration, and D) Genotype and explant effects on number of buds explant^{-1} in eggplant, E) Medium and explant effects on direct plant regeneration and F) Medium and explant effects on number of buds explant^{-1} in eggplant. m1: (2.0 mgl^{-1} BAP), m2: (2.0 mgl^{-1} BAP +1.0 mgl^{-1} kin), m3: (2.5 mgl^{-1} BAP), m4: (2.5 mgl^{-1} BAP +1.0 mgl^{-1} kin), m5: (3.0 mgl^{-1} BAP), m6: (3.0 mgl^{-1} BAP +1.0 mgl^{-1} kin).

and Hinata, 8). It was apparent from the experiment that BL-5 gave the maximum response, followed by BR-14 and BSR-23. The genotypic differences for direct plant regeneration in eggplant have also been reported (Sarker *et al*., 13). Overall effect of medium on direct plant regeneration in eggplant was significant and maximum regeneration (30.38%) was observed on MS medium supplemented with 2.5 mgl^{-1} BAP only, which was statistically higher than the other compositions. However, MS medium supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kin was the best with 5.28 buds explant^{-1}, followed by 2.5 mgl^{-1} BAP (4.21) and 2.0 mgl^{-1} BAP (3.32). The direct regeneration potential depends upon the proportion of endogenous auxins and cytokinins. The optimum ratio of cytokinin to auxin is required for shoot regeneration, as it was corroborated by Slater *et al*. (15). However, auxin and cytokinin (Sarker *et al*., 13; Kaur *et al*., 5) requirement

Table 1. Effect of genotype, medium and explant on direct plant regeneration and number of buds explant^{-1} in eggplant.

Genotype		Direct plant regeneration (%)					No. of buds explant^{-1}				
MS medium	Explant	BL-5	BR-14	BSR-23	Medium mean	Explant mean	BL-5	BR-14	BSR-23	Medium mean	Explant mean
2.0 mgl^{-1}BAP	Hypocotyl	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	19.95 (22.05)	Hypocotyl 4.35 (6.81)	0.00	0.00	0.00	3.32	Hypocotyl 0.63
	Cotyledon	31.79 (34.30)	33.44 (35.31)	36.78 (37.31)			4.66	10.56	4.26		
	Leaf	24.57 (29.70)	29.20 (32.69)	23.81 (29.19)			1.96	6.73	1.70		
2.0 mgl^{-1}BAP + 1.0 mgl^{-1}kin	Hypocotyl	16.10 (23.64)	0.00 (0.00)	0.00 (0.00)	15.93 (17.62)		1.50	0.00	0.00	2.89	
	Cotyledon	57.92 (49.38)	20.22 (26.69)	0.00 (0.00)			13.23	5.26	0.00		
	Leaf	33.14 (35.13)	16.29 (23.77)	0.00 (0.00)			3.83	2.23	0.00		
2.5 mgl^{-1}BAP	Hypocotyl	0.00 (0.00)	13.31 (21.38)	2.77 (9.56)	30.38 (30.44)	Cotyledon 37.31 (34.85)	0.00	1.93	2.60	4.21	Cotyledon 6.54
	Cotyledon	74.75 (59.82)	51.39 (45.77)	24.94 (29.94)			6.63	9.36	7.76		
	Leaf	57.13 (49.07)	34.70 (36.07)	14.48 (22.35)			2.30	3.83	3.50		
2.5 mgl^{-1}BAP + 1.0 mgl^{-1}kin	Hypocotyl	19.37 (26.08)	7.54 (15.93)	0.00 (0.00)	25.29 (27.74)		2.83	1.13	0.00	5.28	
	Cotyledon	66.47 (54.60)	39.24 (38.76)	15.83 (23.43)			21.76	6.36	2.66		
	Leaf	36.22 (36.98)	31.41 (34.07)	11.54 (19.85)			7.63	3.80	1.33		
3.0 mgl^{-1}BAP	Hypocotyl	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	28.67 (27.09)	Leaf 26.50 (28.10)	0.00	0.00	0.00	2.41	Leaf 2.60
	Cotyledon	80.36 (63.68)	38.77 (38.49)	24.74 (29.81)			8.76	3.60	3.10		
	Leaf	64.04 (53.13)	36.81 (37.33)	13.33 (21.40)			3.70	1.16	1.43		
3.0 mgl^{-1}BAP + 1.0 mgl^{-1}kin	Hypocotyl	19.30 (26.04)	0.00 (0.00)	0.00 (0.00)	16.08 (14.58)		1.40	0.00	0.00	1.43	
	Cotyledon	75.19 (60.10)	0.00 (0.00)	0.00 (0.00)			9.76	0.00	0.00		
	Leaf	50.29 (45.15)	0.00 (0.00)	0.00 (0.00)			1.76	0.00	0.00		
Genotype mean		39.24 (35.93)	19.57 (21.46)	9.34 (12.38)			5.09	3.11	1.57		
LSD (P = 0.05)		Genotype = 0.25; Medium = 0.35; Explant = 0.25; Genotype × Medium = 0.61; Genotype × Explant = 0.43; Medium × Explant = 0.61; Genotype × Medium × Explant = 1.06					Genotype = 0.14; Medium = 0.20; Explant = 0.14; Genotype × Medium = 0.34; Genotype × Explant = 0.24; Medium × Explant = 0.34; Genotype × Medium × Explant = 0.60				

*Figures in parenthesis indicate Arc Sin percentage transformation

in the medium depends upon the genotype for shoot bud regeneration in eggplant. Therefore, for shoot organogenesis uptake of BA and NAA from the medium is an essential requirement for increase of endogenous cytokinin and IAA levels (Mercier *et al*., 9). Among explants, cotyledon demonstrated best response for regeneration and number of buds formation. The differences among explants for direct regeneration have also been reported (Sarker *et al*., 13; Kaur *et al*., 5).

The elongation of buds was experimented on half-strength MS medium fortified with 0.3 mgl^{-1} BAP and double agar (Fig. 3). Maximum shoot elongation (%) was observed in BSR-23 (58.53), followed by BL-5 (56.16) and BR-14 (35.84). It was observed that elongation of regenerated buds was in negative

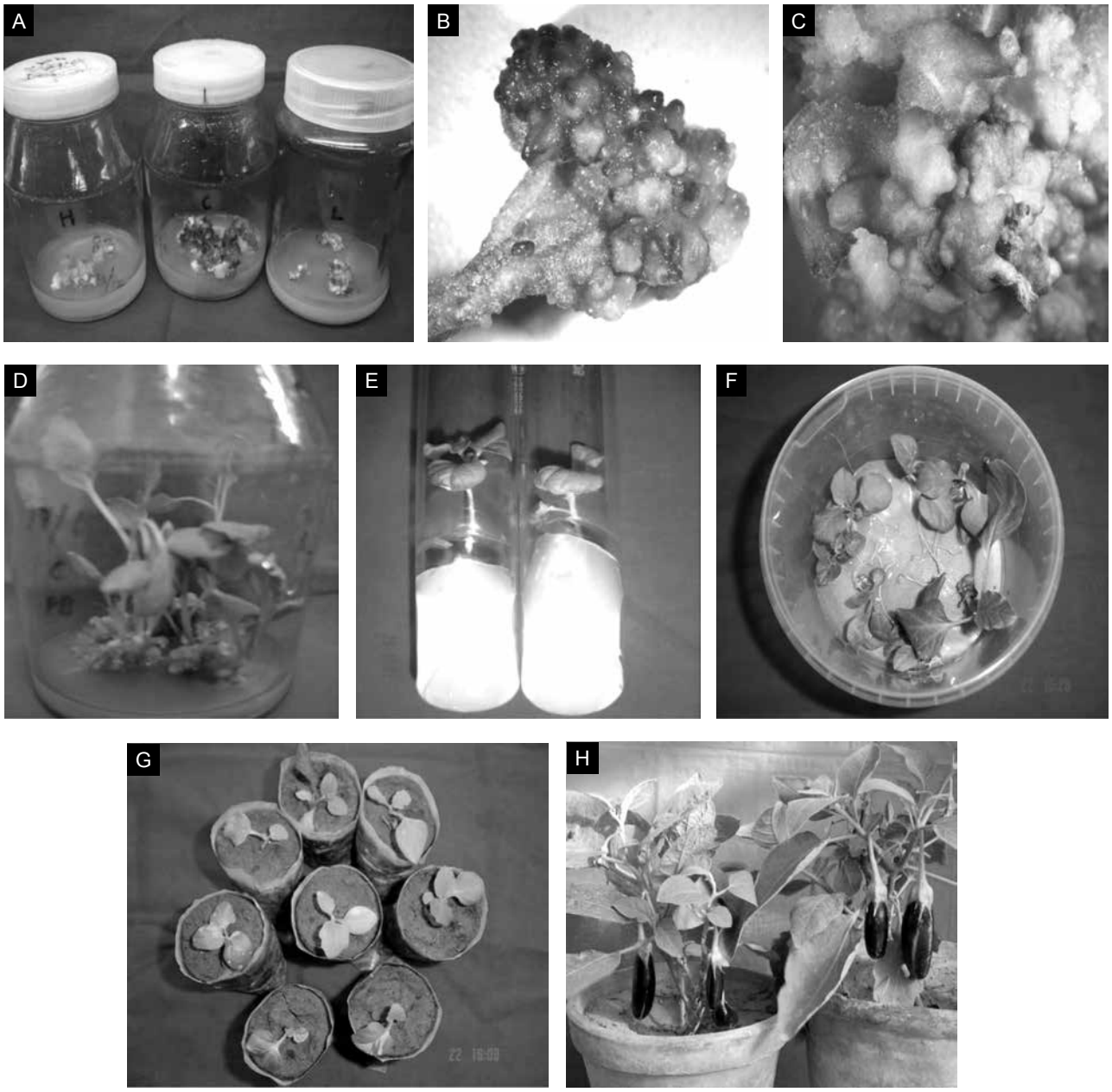

Fig. 2. Direct plant regeneration in eggplant. (A) Different explants showing regeneration, (B) stereoscopic view of regenerating cotyledon explant, (C) Plant regeneration at lower 2.0 mgl^{-1} BAP level, (D) Elongated plantlets half-strength MS medium containing 0.3 mgl^{-1} BAP and double agar-agar, (E) rooted plantlets on MS medium, (F) Hardening of plantlets, (G) Establishment in polythene bags, and (H) Fruit bearing eggplant.

association with callus proliferation. Genotypes with proliferated and watery callus were found less responsive to plantlet elongation and *vice versa*. The higher proliferation of callus covered the regenerated buds at faster rate. As it was observed in BR-14, where regenerated buds reverted to callus and hampered the elongation into shoots. The genotypic differences for shoot elongation can be due to their innate potential for inducing number of buds without callus proliferation. Further, the hormonal combination of different media makes optimal ratio and helps in shoot elongation. The shoot elongation was obtained on half-strength MS medium containing 0.3 mgl^{-1} BAP and double strength agar-agar (Fig. 2D). However, the shoot buds upon sub-culture onto MS basal medium elongated into healthy shoots after organogenesis (Franklin *et al*., 3; Sarker *et al*., 13).

The rooting of plantlets induced on MS basal medium has been depicted in Fig. 3. Among three genotypes, maximum rooting (57.50%) was induced in BL-5 followed by BSR-23 (57.14%), while it was least (43.75%) in BR-14. The rooting response in a particular genotype was also related to its ability for callus proliferation. Genotypes inducing excessive watery callus were found less responsive to rooting. The roots differentiation from the cut ends of plantlets was higher in genotypes with less callus proliferation, as was observed in BR-14. In general, lower auxin concentrations were good for rhizogenesis in most of the crop plants (Fobert and Webb, 2). The diverse rooting behavior of genotypes might be due to their inbuilt potential. In eggplant, inherent auxin level seems to be high and its application for rooting revert the tissue towards callus. Thereby, root induction was observed on hormone-free MS medium (Fig. 2E). It was also demonstrated by many researchers (Kaur *et al*., 5). Rooting was also induced on quarter-strength hormone-free MS medium (Sammaiah *et al*., 12). The rooted plantlets were hardened on filter paper moistened with 0.2% carbendazim solution for 20 days (Fig. 2F). Then, the plantlets were transferred on to polythene bags in greenhouse at 25 ± 1°C. Plants attained 4-5 leaves stage in polythene bags (Fig. 2G) were transferred to earthen pots for further growth, flowering and fruiting (Fig. 3H).

Therefore, it can be concluded from investigation that cotyledon excised from BL-5 has the highest regeneration potential on MS medium fortified with 3.0 mgl^{-1} BAP, which also induced the highest number of buds $explant^{-1}$ on MS medium supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kin. The rooted plantlets were successfully established into the earthen pots where they grew, flowered, and set fruits. The protocol standardized can be exploited for further studies related to genetic transformation.

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REFERENCES

1. Cheema, H.S. and Singh, B. 1990. A user's manual to CPCS-1. *A Computer Programme Package for the Analysis of Commonly used Experimental Designs*, PAU, Ludhiana, pp. 1.

2. Fobert, P.R. and Webb, D.T. 1988. Effect

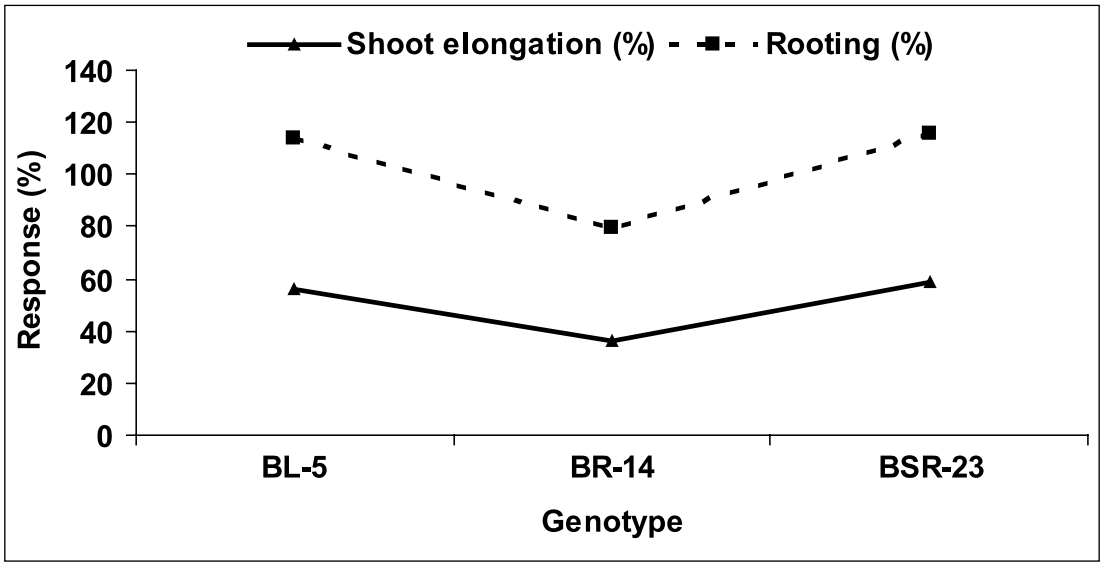

Fig. 3. Shoot elongation and rooting response in eggplant genotypes.

of polyamines, polyamine precursors, and polyamine biosythethic inhibitors on somatic embryogenesis from eggplant (*Solanum melongena* L.) cotyledons. *Canadian J. Bot*. **66**: 1734-42.

3. Franklin, G., Sheeba, C.J. and Sita, G.L. 2004. Regeneration of eggplant from root explants. *In Vitro Cell Dev. Biol. Plant.* **40**: 188-91.

4. Kanna, S.V. and Jayabalan, N. 2010. Influence of N6-(2-isopentenyl) adenine on *in vitro* shoot proliferation in *Solanum melongena* L. *Int. J. Acad. Res*. **2**: 98-100.

5. Kaur, M., Dhatt, A.S., Sandhu, J.S. and Gosal, S.S. 2011. *In vitro* plant regeneration in brinjal from cultured seedling explants. *Indian J. Hort*. **68**: 61-65.

6. Kaur, S., Bal, S.S., Singh, G., Sidhu, A.S. and Dhillon, T.S. 2004. Management of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee through nethouse cultivation. *Acta Hort.* **659**: 345-50.

7. Magioli, C. and Mansur, E. 2005. Eggplant (*Solanum melongena* L.) tissue culture, genetic transformation and use as an alternative model plant. *Acta Bot. Bras*. **19**: 139-48.

8. Matsuoka, H. and Hinata, K. 1979. NAA-induced organogenesis and embryogenesis in hypocotyls callus of *Solanum melongena* L. *J. Exp. Bot.* **30**: 363-70.

9. Mercier, H., Souza, B.M., Kraus, J.E., Hamasaki, R.M. and Sotta, B. 2003. Endogenous auxin and cytokinin contents associated with shoot formation in leaves of pineapple cultured *in vitro*. *Brazil J. Plant Physiol.* **15**: 107-12.

10. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiol*. **15**: 473-97.

11. Razdan, M.K. 2000. *An Introduction to Plant Tissue Culture*, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 81-87.

12. Sammaiah, D., Shekar, C., Goud, M.J.P. and Reddy, K.J. 2011. *In vitro* callus induction and organogenesis studies under pesticidal stress in eggplant (*Solanum melongena* L.). *Ann. Biol. Res*. **2**: 116-21.

13. Sarker, R.H., Sabina, Y. and Hoque, M.I. 2006. Multiple shoot formation in eggplant (*Solanum melongena* L.). *Plant Tissue Cult. Biotech*. **16**: 53-61.

14. Shivraj, G. and Srinath, R. 2011. Rapid and efficient plant regeneration of eggplant (*Solanum melongena* L.) from cotyledonary leaf explants. *Indian J. Biotech*. **10**: 125-29.

15. Slater, A., Scott, N. and Fowler, M. 2003. *Plant Biotechnology: The Genetic Manipulation of Plants*. Oxford University Press Inc, New York, 42 p.

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