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STANDARDIZATION OF EFFICIENT INDIRECT PLANT REGENERATION PROTOCOL IN BRINJAL (*Solanum melongena* L.)

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ABSTRACT : The auxins viz.2,4-D, NAA alone or in combination to BAP (1.0mgl^{-1}) did not induce good quality callus. More than 1ppm IBA formed less and quite compact callus at cut ends with rooting only after two weeks. IBA (0.5-2.0 mgl^{-1}) + 1.0 mg/l BAP increased the mass of pale white, compact, nodular callus with embryogenesis in ten days that differentiated on the callusing media. With IBA and BAP, hypocotyl induced 100.00% callus on all the MS media combinations (1.5mgl^{-1} IBA + 1.0mgl^{-1} BAP, 1.0mgl^{-1}IBA +1.0 mgl^{-1}BAP and 0.5mgl^{-1}IBA + 1.0mgl^{-1}BAP), while cotyledons produced maximum callus on MS with 1.5mgl^{-1}IBA + 1.0mgl^{-1}BAP (97.03%). However, cotyledon induced 95.64% somatic embryogenesis on MS +1.5mgl^{-1} IBA + 1.0mgl^{-1} BAP followed by leaf (94.10%). Hypocotyl had no somatic embryogenesis on MS media fortified with conc. of 1.5 mgl^{-1}IBA + 1.0mgl^{-1}BAP, which could be increased with further decrease in IBA levels. Callus induced by NAA, 2,4-D and IBA auxins suppressed shoot bud initiation, while IBA +BAP induced callus diverted towards differentiation into shoots on higher levels of BAP+ kin. Furthermore, organogenesis was not observed in the callus induced from the hypocotyl, whereas it was maximum in cotyledon (55.02%) followed by leaf (44.57%) on MS medium supplemented with 2.5mgl^{-1}BAP + 1.0mgl^{-1}kin + 0.2% activated charcoal.

Keywords: *Brinjal, callus, somatic embryogenesis, explant, regeneration.*

Brinjal (*Solanum melongena* L., 2n=2x=24) is one of the most popular and principle vegetable crop grown in south Asia, especially in Indian subcontinent. There are various abiotic and biotic stresses which affect the productivity of brinjal. Among biotic stresses, shoot and fruit borer (*Leucinodes orbonalis* Guenee) is the most serious and causes up to 55.08% losses (Kaur *et al.,* 9). The combination of breeding methods with biotechnological tools such as tissue culture, somatic hybridization, genetic engineering and molecular biology has a immense scope to overcome such constraints because it also helps in the identification as well as introduction of gene (s) of interest. The innovative method of tissue culture and genetic transformation is being exploited for induction of insect resistance as well as for improvement in productivity of the existing cultivars. The successful application of in-vitro techniques depends upon ability to produce embryogenic callus and to regenerate plants from desired tissue (Gill *et al.,* 15) . Although brinjal is most amenable to cell, tissue and organ culture methods, the regeneration potential of crop varies with genotype, type of explant and composition of the culture medium (Huda *et al.,* 7). Therefore, keeping this in view, a present study was undertaken with the objectives of standardization of medium, explant and cultural conditions affecting indirect plant regeneration from cultured seedling explants of brinjal.

MATERIALS AND METHODS

The present investigation was carried out in tissue culture and transformation laboratories in School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana. Murashige and Skoog (12) medium was used for the development of regeneration protocol. Seeds of genotype BL-3 were first washed with teepol to remove dirt and light weight seeds that were not supposed to germinate. Only bold seeds were taken and treated with 50% commercial bleach for 20min and then washed till the foam formation stopped. Disinfected seeds were then cultured on half strength MS medium solidified with 0.8% agar for germination and incubated at 25±2°C in dark for 20 days. Cotyledons and hypocotyls were excised from 15-day-old in vitro grown seedlings, whereas leaves were excised from 30 day old seedlings aseptically and cultured on MS medium containing different concentrations of Auxins and Cytokinins for callus induction. All the cultures were incubated at 25±2°C in dark for callusing. The callus induced from different (IBA, NAA or 2,4-D alone or with BAP) medium was sub-cultured to different concentrations of BAP (0.5-3.0 mgl^{-1}) alone or with combination of 1.0 mgl^{-1} kin for further regeneration and incubated in 16/8 light/dark cycles at 4000 lux light intensity and 60%-70% relative humidity. MS medium supplemented with 2.5mgl^{-1} BAP + 1.0mgl^{-1} kin + 0.2% activated

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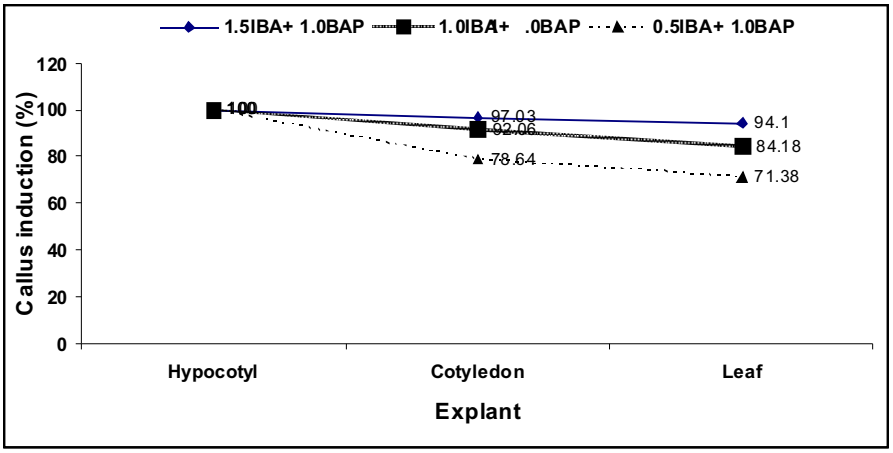
charcoal was finally used for evaluation of different explants calli for organogenesis. Statistical analysis was done in CRD factorial design using CPCS-1 soft ware package (Cheema and Singh, **3**). First and second factors used for the study were media and explant respectively. Three repeats were maintained for each treatment and data were recorded and compiled for each observation. Results were compared at 5 percent level of least square differences (LSD) and interpreted.

RESULTS AND DISCUSSION

Callus induction and somatic embryogenesis

Each explant cultured on MS medium supplemented with 0.5-10 mgl^{-1}NAA initiated green callus after a week that was quite watery and turned brown later on. When NAA (0.5-4.0mgl^{-1}) was combined with 1.0 mgl^{-1}BAP, a little callus from cotyledon and leaf showed somatic embryogenesis (Plate 1 A, B, C). Here again the callus was induced after a week of culturing. Hypocotyls were earliest in inducing callus followed by cotyledons and leaves, respectively. Matsuoka and Hinata (11) and Macchia *et al.* (9) reported similar results for callusing with NAA. Another auxin, 2, 4-D induced highly proliferating and watery callus within four days, which did not show embryogenesis and turned brown after ten days. When IBA alone was tried for callus induction, its concentration below 1ppm developed only roots at cut ends of the explants, while conc. greater than 1ppm formed a little callus at cut ends with rooting only after two weeks, which was quite compact. These results of different types of callus induction with IBA, NAA and 2, 4-D medium can be further supported with the findings of Anwar *et al.* (2).

Furthermore, different concentrations (0.5-2.0 mgl^{-1}) of IBA was combined with 1.0mg/l BAP increased the callus mass in ten days. Hypocotyls and leaves produced watery and proliferating callus on medium fortified with 2.0 mgl^{-1}IBA and 1.0 mgl^{-1}BAP. Quality callus was formed in less number of explants as the concentration of IBA reduced to 0.5 mg/l. Thus callusing (%) was less at this combination (0.5mgl^{-1}IBA+1.0mgl^{-1}BAP), but it was pale white, compact, nodular, and quite embryogenic which started differentiation on the callusing media itself. Macchia *et al*. (9) reported similar results for callus with MS supplemented with IBA (indole butyric acid) using leaf explant. As we know that 2, 4-D and NAA are strong auxins used for callus induction in different crop plants. But in brinjal, callus proliferation occurs even without the application of auxins to MS medium. It means that this crop has higher auxin level in the plant tissue itself. Thus, application of strong auxins enhances the callus proliferation as described by Fobert and Webb (5). However, IBA is a weak auxin and callus volume is comparatively less than other auxins. It forms callus as well as roots from the explant. Masuoka and Hinata, (11); Fobert and Webb (5) and Tarre *et al.* (14) also reported initiation of root primordial due to the decreased level of auxin. When cytokinin (BAP) was added to the medium along with IBA, it enhanced the somatic embryogenesis. It might be due to the reason that cytokinins are antagonistic to the auxins and directed the callus towards differentiation as stated by Huda *et al.* (7). So various combinations of IBA (0.5-1.5 mgl^{-1}) and BAP (1.0mgl^{-1}) were used for the evaluation of different explants and their organogenesis was studied with the best callus induced from 0.5mgl^{-1} IBA and1.0 mgl^{-1}BAP.



LSD (P=0.05): Medium 2.36, Explant 2.36, Medium× Explant 4.09

Fig. 1 : Effect of medium and explant on callus induction (%) in brinjal.

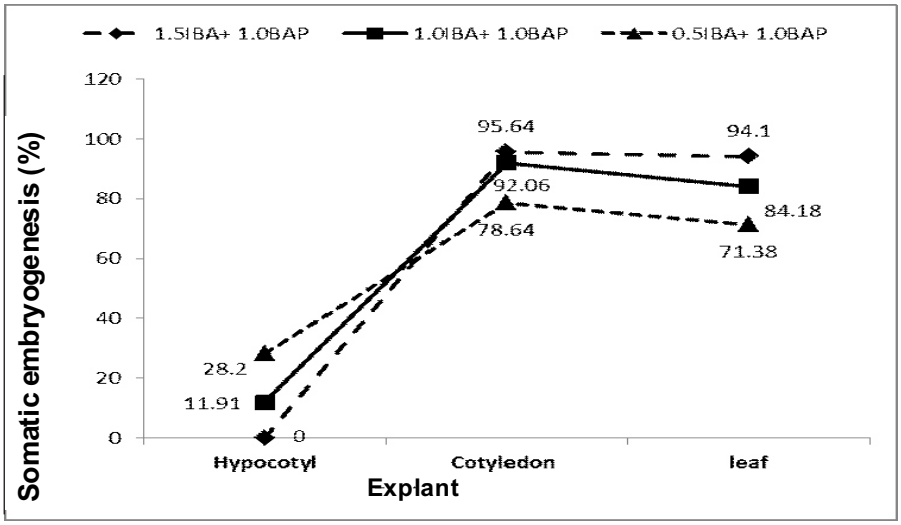
The interaction of media and explant (Fig. 1) inferred that hypocotyl induced 100.00% callus on all the MS media combinations (1.5mgl^{-1} IBA + 1.0 mgl^{-1} BAP, 1.0mgl^{-1}IBA +1.0 mgl^{-1}BAP and 0.5mgl^{-1}IBA + 1.0 mgl^{-1}BAP), while cotyledons produced maximum callus on MS with 1.5 mgl^{-1} IBA + 1.0 mgl^{-1}BAP (97.03%), followed by MS with 1.0 mgl^{-1}IBA + 1.0 mgl^{-1}BAP (92.06%) and MS with 0.5 mgl^{-1}IBA + 1.0 mgl^{-1}BAP (78.64%). Leaf explant also induced 94.10%, 84.18% and 71.38 on different media, respectively. The results for interactions of media and explant can be substantiated with the reports of Dobariya and Kachhadiya (4).

As discussed earlier, the callus induction ability of plant also depends upon the level of auxins in the plant tissue itself. Thus the differences in the callus induction potential of various brinjal explants might be due to the different level of auxins in their tissue. BL-3 induced quite less callus volume, but callus quality was too good. Among explants hypocotyls seems to have higher auxin level than leaf and cotyledon. Good quality callus in cotyledon indicates a balanced proportion of auxins and cytokinins in the tissue (Plate 1D). These differences can be supported by the findings of Alicchio *et al*. (1).

The interaction of medium and explant (Fig. 2) for somatic embryogenesis inferred that cotyledon induced 95.64% response on MS media having 1.5 mgl^{-1}IBA + 1.0 mgl^{-1} BAP followed by leaf (94.10%). Similarly, the leaf explant induced maximum somatic embryos on the same medium as mentioned in cotyledon. The interaction of all the factors detected no somatic embryogenesis (0.00%) capability in hypocotyl, when cultured on MS media fortified with conc. of 1.5 mgl^{-1}IBA + 1.0 mgl^{-1}BAP. Further, the somatic embryogenesis in hypocotyl increased with decrease in IBA levels.

Organogenesis

Organogenesis is the ability of the callus to convert into plantlets. When the callus from NAA or 2, 4-D alone was subjected to different concentrations of BAP (0.5-3.0 mgl^{-1}) or with combination of 1.0 mgl^{-1} kin, there was no organogenesis, whereas the callus from NAA in combination with BAP produced some green buds at higher BAP (2.5-3.0 mgl^{-1}) concentrations. Shoot bud suppression in callus induced on NAA and NOA was also reported earlier (Kamat and Rao, 8). The embryogenic callus of IBA and BAP (Plate 1D, E, F) further proliferated on lower conc. of BAP (0.5-2.0 mgl^{-1}), but showed some regeneration on 2.5 mgl^{-1}BAP + 1.0 mgl^{-1} kin along with callus proliferation and rooting again. This medium when supplemented with 0.2% activated charcoal suppressed the callus proliferation and increased its organogenesis (Plate 1G). So, MS medium supplemented with 2.5 mgl^{-1}BAP + 1.0 mgl^{-1}kin + 0.2% activated charcoal was finally used for further evaluation of different explants for organogenesis. Organogenesis was not observed in the callus induced from the hypocotyl (Plate 1G), whereas it was maximum in cotyledon (55.02%) followed by leaf (44.57%) on MS medium supplemented with 2.5 mgl^{-1}BAP + 1.0 mgl^{-1}kin + 0.2% activated charcoal (Fig 3). The results can be substantiated with the findings of Kamat and Rao (8), Reynolds (13) and Slater *et al.* (15) who reported that a high cytokinin to auxin ratio favours shoot formation. Compact nodular and embryogenic callus had good potential for organogenesis, whereas the explant producing watery



LSD (P=0.05): Medium 0.63, Explant 0.63, Medium× Explant 1.10

Fig. 2 : Effect of medium and explant on somatic embryogenesis (%) in brinjal.

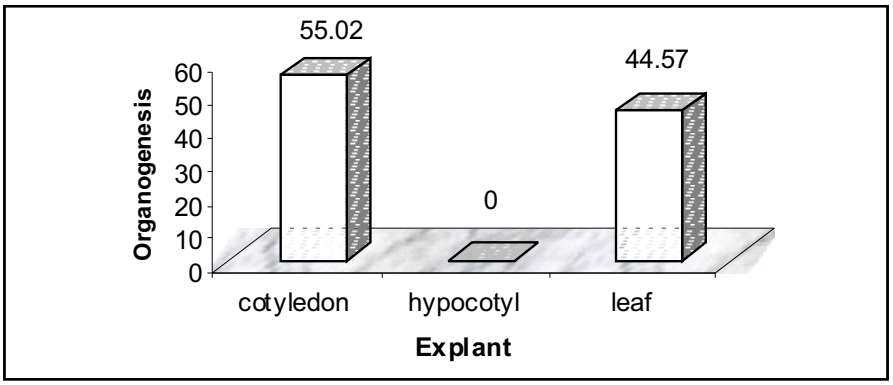


Fig. 3 : Organogeneiss (%) of different explants of brinjal on MS medium supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} kin + 0.2% activated charcoal.

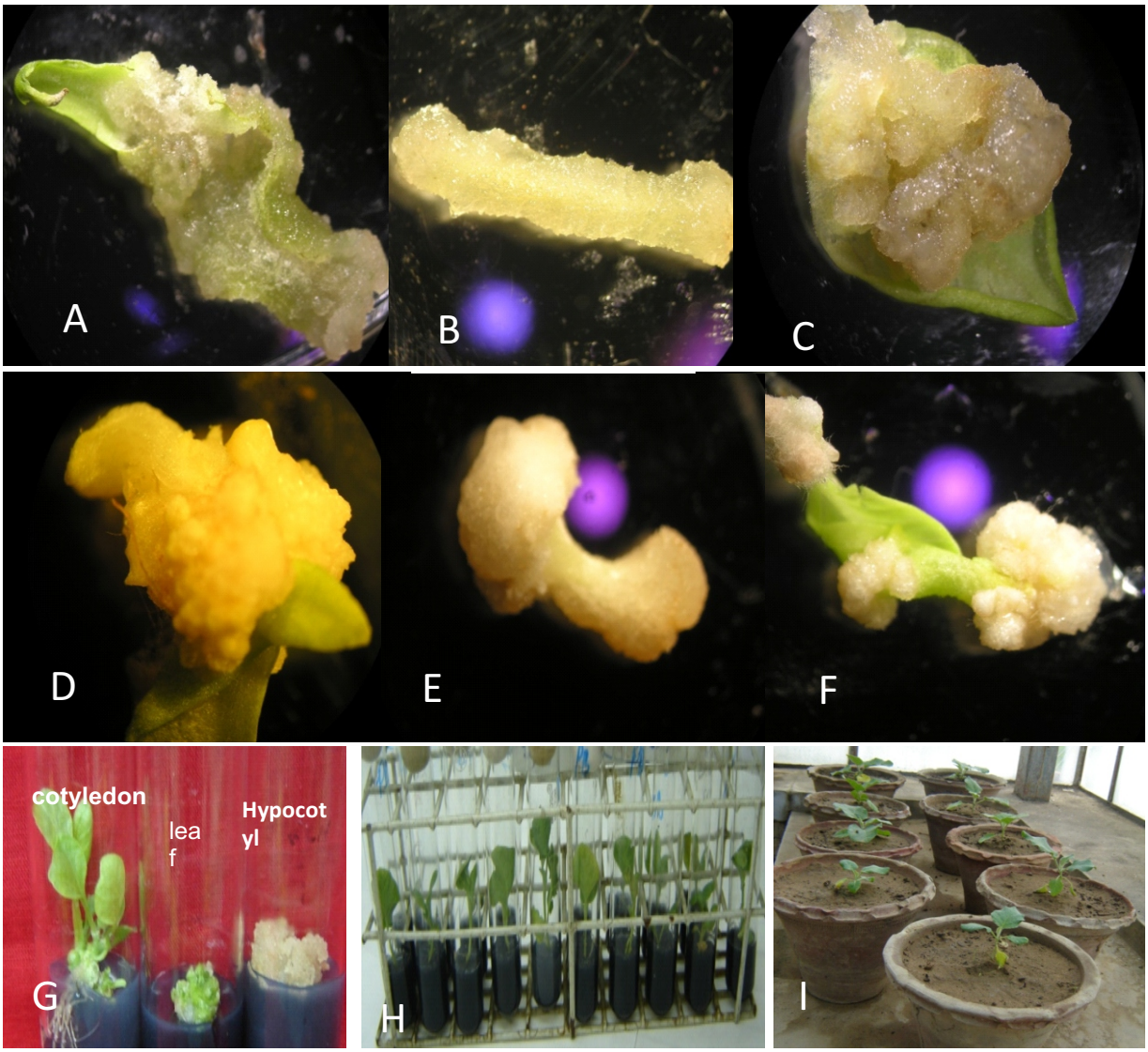


Plate 1 : Callus induction and somatic embryogenesis in brinjal : (A), (B), (C) Callus induction in cotylydon, hypocotyl and leaf respectively on NAA + BAP medium, (D), (E), (F) Callus induction in cotyledon, hypocotyl and leaf respectively on IBA + BAP medium, (G) Callus regeneration in different explants, (H) and (I) establishment of plantels.

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 elongated plantlets were

rooted, and established in pots after 14 days hardening (Plate 1H&I). Alicchio *et al* (1) also reported similar findings with different endogenous growth regulators.

CONCLUSION

Among the various auxins, IBA has good response for quality callus induction. The cotyledon and leaf induced best callus from MS+1.5 mgl^{-1} IBA +1.0 mgl^{-1}BAP. Contrarily, hypocotyl performs best on MS+0.5 mgl^{-1} IBA +1.0 mgl^{-1} BAP. MS medium supplemented with 2.5 mgl^{-1}BAP + 1.0 mgl^{-1}kin + 0.2% activated charcoal can be used for organogenesis. Plantlets can be rooted on MS only. This protocol would be helpful in evaluating other brinjal genotypes for response to tissue culture.

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