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In vitro propagation of liquorice (*Glycyrrhiza glabra* L.) through multiple shoot formation[1]

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Abstract

In vitro plant regeneration of liquorice (*Glycyrrhiza glabra*) through multiple shoot formation from nodal explants is reported. Addition of kinetin (0.05 mgl^{-1}) and indole-3-acetic acid (1.0 mgl^{-1}) to Nitsch medium resulted in rapid elongation of axillary buds. Proliferation of multiple shoots was more effective in Murashige and Skoog medium containing benzylaminopurine (2 mgl^{-1}) and indole -3-acetic acid (1 mgl^{-1}). *In vitro* regenerated shoots were rooted on Murashige and Skoog medium supplemented with 1 mg^{-1} indole-3-acetic acid and after acclamatization in the glasshouse were transferred to field with 95% survival.

Key words : *Glycyrrhiza glabra,* liquorice, micropropagation.

Abbreviations

BAP : 6-Benzylaminopurine
2,4-D : 2,4-Dichlorophenoxyacetic acid
IAA : Indole-3-acetic acid
Kn : Kinetin (6-Furfurylaminopurine)
MS : Murashige & Skoog medium
NAA : α-Naphthaleneacetic acid
NB : Nitsch medium

Introduction

Dried roots of liquorice (*Glycyrrhiza glabra* L.; Family: Fabaceae) are the principal source of glycerrhizin-a natural sweetening agent (Morris 1976) widely used as a flavouring agent in tobacco and confectionary industries in addition to its use in pharmaceutical industry (Li & Yeh 1960; Takegi *et al.* 1963; Pompei *et al.* 1979, 1980; Fukui *et al.* 1988; Hatano *et al.* 1988). Poor flowering and poor seed viability are the major limiting factors for its commercial cultivation in India. Plant tissue culture offers the

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possibility of rapid clonal propagation and conservation of germplasm of this species. The present investigation deals with standardization of culture conditions conducive for rapid multiplication of *G. glabra* via multiple shoot regeneration from nodal segments and establishment of *in vitro* regenerated plants in the field.

Material and methods

G. glabra plants maintained in the farm of the Institute served as source plants. Explants consisting of 2-3 cm long stem segments with a node, axillary bud and pētiolar base from the source plants were washed with distilled water containing Tween-20, surface sterilized with mercuric chloride (0.1%) for 1 min and rinsed several times with sterilised double distilled water before implanting vertically onto the nutrient medium. The basal media of MS (Murashige & Skoog 1962) and NB (Nitsch 1969) were supplemented with sucrose 3% and 2%, respectively, meso-inositol 100 mgl^{-1} and agar 0.8%. Auxins (IAA and NAA, 0.05-5.00 mgl^{-1}) and cytokinins (Kn and BAP, 0.05-5.00 mgl^{-1}) were incorporated in the media in combinations as indicated in Table 1. The pH values of all the media combinations were adjusted to 5.8 before autoclaving. The cultures were maintained under 16 h light regime (3000 lux) at 25 ± 3° C and 60-70 % relative humidity. Each treatment consisted of 24 replicates and the experiment was repeated twice. The duration of each culture passage varied between 6-8 weeks. Rooted plants were transferred to earthen pots containing a mixture of sand, soil and organic manure (1:1:1). These plants were irrigated with nutrient broth (Hoagland & Arnon

Table 1. Morphogenetic responses of nodal explants of *Glycyrrhiza glabra* cultured on NB or MS medium supplemented with auxin (IAA or NAA) and cytokinin (Kn or BAP) alone or in combination after 4 weeks

Culture medium	Response of nodal explants
MS basal or NB basal	Slight elongation of axillary bud and occasional callus formation from the proximal cut end in MS alone
MS + IAA or NAA (0.05-5.00 mgl^{-1}) or NB + IAA or NAA (0.05-5.00 mgl^{-1})	Axillary bud growth at lower levels and callusing followed by root formation at higher levels
MS + Kn or BAP (0.05-5.00 mgl^{-1}) or NB + Kn or BAP (0.05-5.00 mgl^{-1})	Generally no response; occasionally callusing at lower levels of Kn only
MS + Kn or BAP (0.05-1.00 mgl^{-1}) + IAA (1 mgl^{-1}) or NB + Kn or BAP (0.05-1.00 mgl^{-1}) + IAA (1 mgl^{-1})	Rapid elongation of axillary shoot
MS + BAP (2-3 mgl^{-1}) + IAA (1 mgl^{-1}) or NB + BAP (2-3 mgl^{-1}) + IAA (1 mgl^{-1})	Regeneration of adventitious shoots without any intervening callus
MS or NB + BAP (0.50-5.00 mgl^{-1}) + IAA (> 1 mgl^{-1})	Callus formation from proximal cut end
MS or NB + IAA (1 mgl^{-1})	Root induction in excised nodal segments

1950) and hardened for 4-6 weeks in the glasshouse. These plantlets were then transplanted to the field.

Results and discussion

The morphogenetic response of nodal explants cultured on NB or MS medium supplemented with IAA or NAA (0.05-5.00 mgl^{-1}) and Kn or BAP (0.05-5.00 mgl^{-1}) are summarized in Table 1. Nodal explants cultured on hormone-free MS and NB media showed slight elongation of axillary buds with swelling of the node within a week of incubation. The petioles turned brown and generally abscised during this period while nodal portions remained green even after 4 weeks of incubation. Callus initiation from the proximal cut end of the explants was observed on MS medium only. Lower levels (<0.5 mgl^{-1}) of IAA or NAA induced axillary bud elongation (Fig. 1A) while higher levels (>1.0 mgl^{-1}) supported callus formation from the proximal cut end of the explant followed by root initiation. Kn or BAP supplemented alone to MS or NB media were ineffective at all levels tested for axillary shoot bud elongation. Occasionally callus formation from the explants was observed at lower levels (>0.5 mgl^{-1}) of Kn. A rapid elongation of axillary bud was observed on NB medium supplemented with 0.05-0.50 mgl^{-1}Kn or BAP and 1.00 mgl^{-1} IAA (Fig.1B). The axillary shoot attained a height of 15-20 cm (4-6 nodes) within 4 weeks of incubation. Thus, a maximum of only six node explants could be obtained from a single node cultured after 4 weeks of incubation. In the case of MS medium fortified with BAP (0.5-3.0 mgl^{-1} and IAA (1.0 mgl^{-1}), optimal response was observed with BAP (0.5 mgl^{-1}) and IAA (1.0 mgl^{-1}). However, MS medium fortified with higher levels of BAP (2-3 mgl^{-1}) and IAA (1mg^{-1}) supported regeneration of multiple adventitious shoots from the swollen nodal region without an intervening callus phase (Fig. 1C). About 6-8 adventitious shoots each having 2-4 nodes could be observed after 4 weeks of culture. Thus, in the present study, an average of 20 nodal explants could be obtained from a single node cultured on MS medium containing BAP (0.5 mgl^{-1}) and IAA (1.0 mgl^{-1}) after 4 weeks of culture compared to four axillary buds produced from one original shoot bud as earlier reported by Shah and Dalal (1980). Higher levels of IAA (>1.0 mgl^{-1}) in the presence of different levels of BAP (0.05-5.00 mgl^{-1}) tested resulted in callus formation from the proximal cut end of the explants. Interestingly, it was observed that incubating the mother explant on the same medium after excising the sprouted axillary shoot, new shoots continued to develop through axillary bud sprouting which were further divided and multiplied. The nodal explants containing axillary buds when excised and subcultured on hormone-free NB or MS basal media readily rooted. Rooting frequency could be increased significantly by supplementing IAA (0.05-5.00 mgl^{-1}) to both media. IAA at 1 mgl^{-1} level was optimal for root induction (Fig. 1D). The number of shoots producing roots gradually declined with a reciprocal increase of IAA in the medium. On the other hand, NAA even at low levels (<1mg^{-1}) exhibited inhibitory effect on rhizogenesis. Shah & Dalal (1980) also reported delayed rooting in *in vitro* regenerated shoots of liquorice in the presence of NAA. *In vitro* regenerated plantlets were successfully transferred to earthen pots in the glasshouse (RH 80-90%; temperature 30° C) and after a hardening period of 10-15 days were transplanted in the

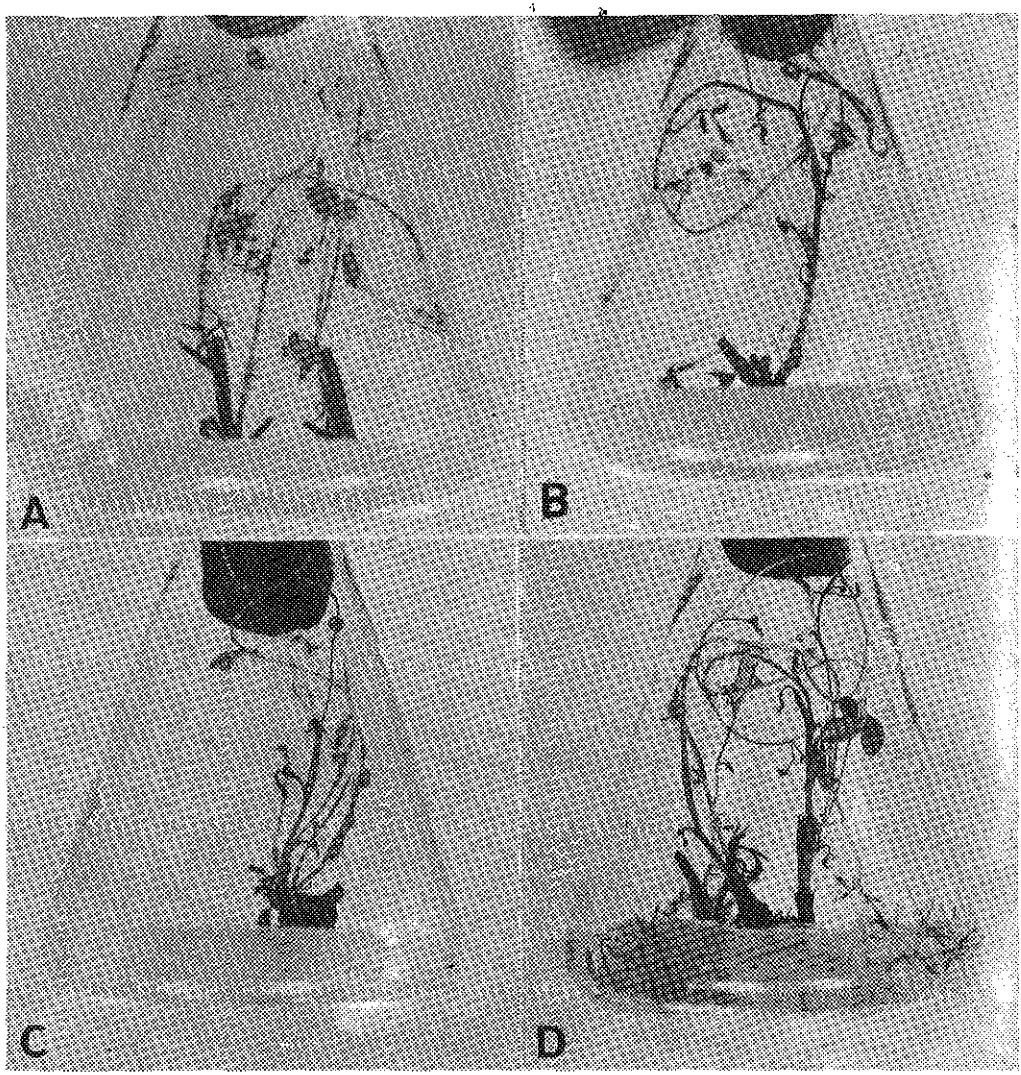

Fig. 1. *In vitro* propagation of *Glycyrrhiza glabra* A-Axillary shoot elongation in nodal segments cultured on MS medium supplemented with low levels of auxin. B-Axillary bud elongation on NB medium. C-Multiple shoots on MS medium fortified with 2 mgl^{-1} BAP and 1 mgl^{-1} IAA. D-Rhizogenesis in *in vitro* cultured shoots on NB medium containing 1 mgl^{-1} IAA.

field with a high survival rate (90-95%). Thus, the present study shows that propagation through adventitious shoots is also a viable strategy for the production of a large number of liquorice for commercial purpose. Through a rapid increase in number of shoots which can ultimately give rise to rooted plants can be achieved by induction of multiple shoot formation, incidence of genetically aberrant plants was not uncommon with this method; however, this requires confirmation for liquorice.

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