

TISSUE CULTURE RESPONSE AND MORPHOGENESIS IN SEEDLING EXPLANTS OF SOYBEAN [*GLYCINE MAX* (L.) MERRILL]

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In vitro morphogenic response of seedling explants of soybean was developed for callus induction, multiple shoot formation and subsequent plant regeneration. Hypocotyl, cotyledon, cotyledonary node and epicotyl explants excised from aseptically grown seedlings were cultured on MS based medium contained different levels of auxins (2,4-D/NAA/IAA/IBA) and cytokinins (Kn/BAP). Callus could be induced from any explants using a wide range of 2,4-D and BAP alone or combinations of 2,4-D + Kn/BAP. Rhizogenesis was observed directly or indirectly from the callus on media with different levels of NAA/IAA/IBA/Kn or combinations of NAA/IAA/IBA + Kn. Proliferation of axillary shoots occurred in cultured nodes on lower levels of NAA/Kn/BAP while more number of multiple shoot (6-10 in number) formation was observed on medium with BAP alone or IAA/IBA + BAP. The best response of multiple shoot formation was found on IBA + BAP. Shoot multiplication was increased upon subculturing on medium with BAP alone or IAA/IBA + BAP. Shoots were elongated and rooted on MS medium supplemented with IBA (1.0 mg/l). The elongated shoots were rooted and transferred to soil where they grew normal and set seeds.

Keywords : Callus induction; Plant regeneration; Shoot multiplication; Soybean; Tissue culture.

Introduction

The significant values of grain legumes in terms of food and fodder, for their role in the biological exchange of nitrogen and as raw material for industries have stimulated increased research of these crops. The importance of grain legumes hence attracts the attention of scientists all over the world to use biotechnology for genetic improvement of legumes particularly of soybean which is amongst the most valuable. In India, soybean is recognised as a food crop to bridge the gap between national need and availability of high protein as well as oil contents. In the last three decades, substantial progress has been made in tissue culture of grain legumes but *Glycine max* reported most recalcitrant *in vitro*^{1,2}. Recent reports in *G. max* also showed difficulties where plant regeneration highly differs from genotype to genotype²⁻⁴ and depends on the choice of a specific explant⁵⁻⁷. The present study was undertaken to see the morphogenetic competence of seedling explants of *G. max* in tissue cultures.

Material and Methods

Seeds of soybean [*Glycine max* (L.) Merrill cv. MACS-

13] were obtained from Agricultural Research Station, Borkhera, Kota (India). Seeds were surface sterilized with 0.1% HgCl₂ solution for 5-7 min, rinsed 4-5 times and germinated on semi-solid half-strength hormone-free MS medium⁸. After two weeks of germination, cotyledon (entire), and 1-2 cm long each of hypocotyl, cotyledonary node and epicotyle explants excised and cultured on medium containing MS mineral salts, B₅ vitamins⁹, 3% sucrose (hereafter referred as MSB medium), various auxins (2,4-D/NAA/IAA/IBA) and cytokinins (Kn/BAP) at different concentrations used either alone or in combinations. The pH of the medium was adjusted at 5.8, solidified with 0.8% agar and sterilized by autoclaving. All cultures were incubated in a growth chamber at 26 ± 2°C temperature under 16h photoperiod (1600 lux). The observations were taken every week and final morphogenetic data were recorded after 6 weeks. The cultures were maintained by regular subculturing onto fresh medium in every 3 week intervals. The MS medium supplemented with IBA (1.0 mg/l) was used for rooting of the differentiated shoots. A total of 25-30 each type of

explants were used for culture and each regeneration experiment was repeated twice.

Results and Discussion

Initially, all explants were cultured on MSB medium devoid of growth regulators where no morphogenetic response was observed from any of the explants. Swelling at the cut ends and margins were observed in some explants after 2-3 weeks of incubation but after 5-6 weeks, they turned yellowish brown and finally died. The morphogenetic responses viz. callusing, rhizogenesis and shoot multiplication were initiated from the explants when cultured on MSB medium supplemented with auxins and cytokinins at various concentrations (0.5, 1.0, 3.0, 5.0 or 10.0 mg/l) and their combinations in which auxins at 0.5, 1.0, 3.0 mg/l and cytokinins at 0.5, 1.0, 3.0 mg/l were used.

Effect of auxins

Hypocotyl - Rhizogenesis was observed from one end of the explants directly on MSB medium with low levels of NAA/IBA (0.5 and 1.0 mg/l) and higher levels of IAA (5.0 and 10.0 mg/l). All the levels of 2,4-D and higher doses of NAA (3.0-10.0 mg/l) supplemented media induced callus which was maximum on higher levels of 2,4-D (Fig. 1). Callus was cream and light green in colour and friable to semi-compact in texture.

Cotyledon - Light green and semi-compact callusing was observed on all the levels of 2,4-D tested. Lower levels of NAA and high levels of IAA/IBA evoked two to many, 3-15 cm long, thick roots directly from the cut ends of the explants (Fig. 2). Rhizogenic callus was observed on medium with higher levels of NAA (3.0-10.0 mg/l).

Cotyledonary node - Cultured nodes showed proliferation of 2-5 axillary shoots along with little callus formation on lower levels of NAA (0.5 and 1.0 mg/l) whereas increasing the levels (3.0-10.0 mg/l) favoured friable and creamish callus. When 2,4-D was added in the medium, poor to good amount of callus developed which was also creamish and friable. Medium with almost all the levels of IAA/IBA induced light green and compact rhizogenic callus.

Epicotyl - Epicotyl segments showed creamish pale yellow and friable callusing in response to various levels of auxins tested. The amount of callus was good on higher levels of NAA (10.0 mg/l). Medium with IAA/IBA at lower level (0.5 mg/l) induced rhizogenic callus in poor amount.

Effect of cytokinins

When cytokinins (Kn/BAP) were added in the medium, the hypocotyl and epicotyl segments formed callus on all the levels used. The cotyledon explants formed callus on higher levels of Kn (3.0-10.0 mg/l) and on all the levels of

BAP tested whereas rhizogenic callus formation was found on low levels of Kn (0.5 and 1.0 mg/l). Formation of axillary shoots (2-5 in number) with poor callusing developed from nodes on lower levels of Kn. Multiple shoots (4-10 in number) induced on 3.0 mg/l of BAP (Fig. 3) and callus induced on all the other levels tested. In all cases, medium with Kn induced pale-yellow and semi-compact callus while BAP induced green, compact and nodulated callus from all the explants. Greening of the callus intensified with increasing the levels of BAP. BAP at 1.0-5.0 mg/l showed highest amount of callus from hypocotyl and epicotyl segments.

Effect of combinations of auxins and cytokinins

2,4-D + Kn/BAP - Explants cultured on medium with various combinations of 2,4-D + Kn/BAP showed callusing except cotyledons and epicotyls which induced root callus on some combinations. Combinations of 2,4-D + Kn induced creamish and friable to semi-compact callus whereas 2,4-D + BAP induced yellowish green and compact callus.

NAA + Kn/BAP - Cultured explants produced many roots with intervening callus on medium with NAA + Kn (Fig. 4). This type of response was maximum in epicotyl segments than cotyledonary node, cotyledon and minimum in hypocotyl explants. Some elongated axillary shoots also developed with root callus in the cultured nodes on some combinations of NAA + Kn. The presence of NAA + BAP in the media showed vigorous amount of callusing.

IAA/IBA + Kn/BAP - When medium was fortified with IAA/IBA + Kn, origin of pale-yellow and semi-compact callus with roots noticed from the cultured hypocotyls, cotyledons, and nodes. Some elongated axillary shoots were also developed from the nodes. Treated epicotyls induced yellowish-green and compact callus on all the combinations of IAA/IBA + Kn tested. Multiple shoot (2-10 in number) formation was observed from the nodes on IAA/IBA + BAP. Shoot multiplication ability was highest on IBA + BAP supplemented media.

Effect of subculturing

The growth of callus with or without shoots was increased upon serial subcultures when transferred to MSB medium with 2,4-D/BAP (3.0 mg/l) alone or 2,4-D/NAA/IAA/IBA (1.0 mg/l) + Kn/BAP (3.0 mg/l). In each passage, callus became creamish and friable on media contained 2,4-D (3.0 mg/l) and/or IAA/IBA (1.0 mg/l) + Kn (3.0 mg/l). Whereas on BAP (3.0 mg/l) and/or IAA/IBA (1.0 mg/l) + BAP (3.0 mg/l), callus became more greenish in color, compact and nodulated in texture. In the presence of

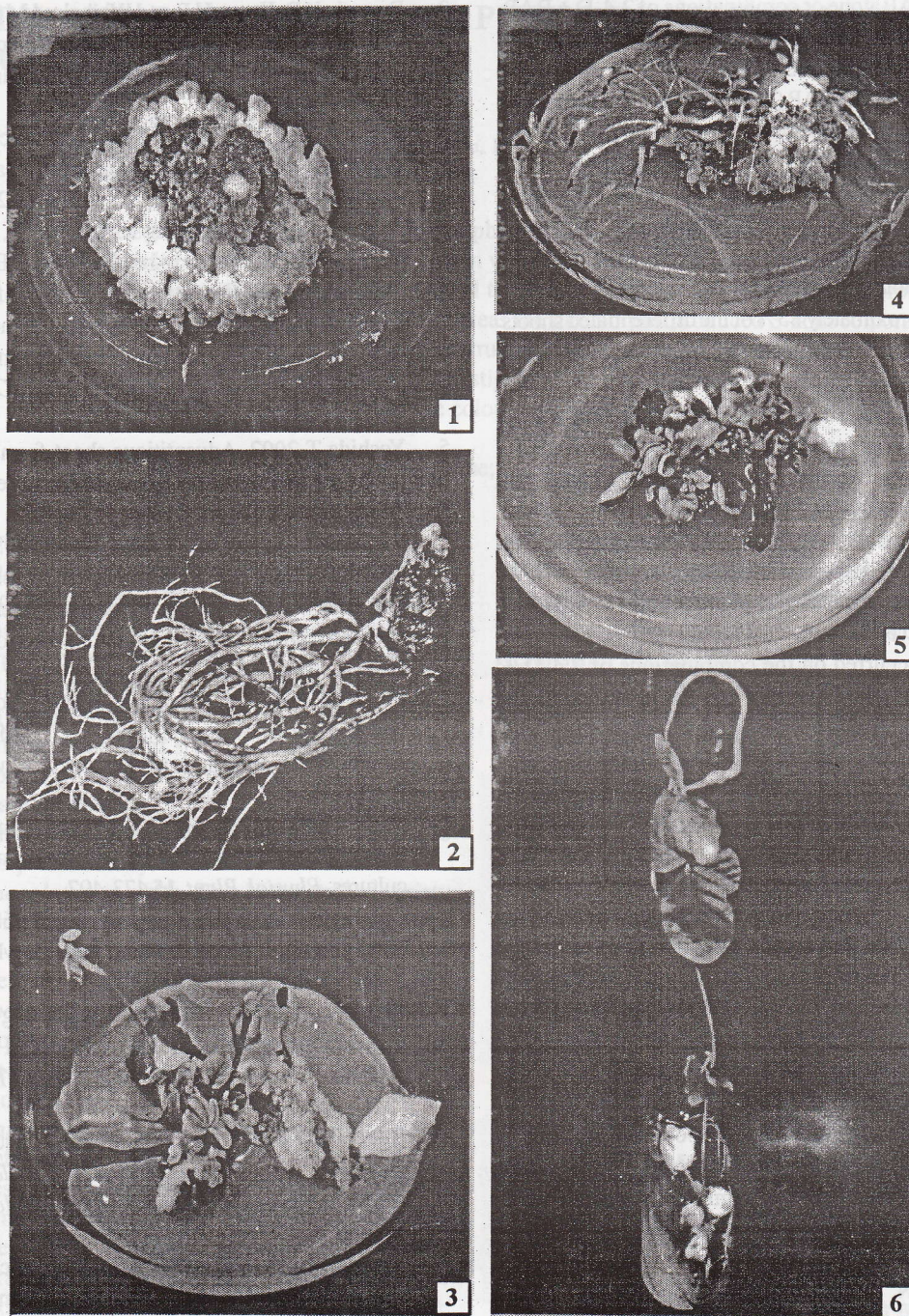


Fig. 1. Callus from hypocotyl segment on MSB medium with 2, 4-D (3.0 mg/l); **Fig. 2.** Rhizogenesis from cotyledon on IAA (5.0 mg/l); **Fig. 3.** Multiple shoots alongwith callus from cotyledonary nodes on BAP (1.0 mg/l); **Fig. 4.** Rhizogenic callus from epicotyls on NAA (1.0 mg/l) + Kn (1.0 mg/l); **Fig. 5.** Several shoot buds from cotyledonary nodes on IBA (0.5 mg/l) + BAP (3.0 mg/l) after the 2nd passage of subculturing; **Fig. 6.** Rooted shoot on MS medium with IBA (1.0 mg/l).

2,4-D and/or BAP alone or combinations of 2,4-D + BAP, vigorous growth of the callus was recorded. The number of shoots increased was observed on BAP (3.0 mg/l) alone or IAA/IBA (0.5 mg/l) + BAP (3.0 mg/l). Best multiple shoot forming response was observed on medium with IBA (0.5 mg/l) + BAP (3.0 mg/l) where 10-15 shoots developed after the 2nd passage (Fig. 5). The number of shoots increased was found upto the 4th passage where upto 20 shoots developed from a single node. In each passage, early formed shoots elongated upto 3 cm long. Complete plantlet formation in approximately 85% of the differentiated shoots was observed on MS medium supplemented with IBA (1.0 mg/l) (Fig.6).

In most of the leguminous species, callus can be produced from any parts on a variety of tissue culture media¹. In our experiments, 2,4-D incorporated singly in the medium proved to be the most effective amongst the auxins tested for callus induction in all the explants. Similar observations were also made in other legumes¹⁰. BAP amongst cytokinins was found to be the best responsive for callusing as well as multiple shoot formation. Excellent callus growth occurred on the combinations of 2,4-D + BAP. The combinations of IBA + BAP were most suitable for plant regeneration via shoot multiplication as well as shoot organogenesis with or without callusing as also reported in other species of *Glycine*¹⁰⁻¹² and recently in *G. max*⁷. The callus induced from auxin supplemented media was creamish and friable whereas cytokinins generally induced green compact callus. No shoot organogenesis was observed from any of the callus type. In various explants, rhizogenesis either directly or via callus on media with NAA/IAA/IBA added singly or in combination with Kn is quite common in reported literature. In our experiments, the amount of both type of callus (pale-yellow, friable and green, compact) and the intensity of shoot multiplication increased during subculturing as also reported earlier in a perennial wild species of soybean³. The present system of *in vitro* morphogenesis from various explants might be useful to improve the recalcitrant Indian commercial cultivars of soybean.

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