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CALLUS CULTURE AND REGENERATION POTENTIAL IN SOYBEAN [*GLYCINE MAX* (L.) MERRILL]

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In order to see the regeneration potential in soybean callus, the callus raised from hypocotyl segments was transferred to a variety of media formulations. These media formulations were contained MS basal medium with B_5 vitamins and supplemented with different levels of auxins (2,4-D, NAA, IAA, IBA), cytokinins (Kn, BAP) added singly and in various combinations. The factors affecting callus growth and regeneration in callus cultures were examined. It was possible to distinguish between high or poor growth of callus on different media by visually observing the callus. Although, callus could be proliferated and maintained on a variety of media formulations but plant regeneration in callus was found difficult and limited up to certain media. Shoot morphogenesis in callus cultures was achieved on medium fortified with low levels of IBA in combination with higher levels of BAP. The regenerated plantlets transplanted to soil did not show any phenotypic variation.

Keywords: Callus culture; Organogenesis; Soybean, Tissue culture.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important protein and oil yielding grain legume crops in many countries[1]. Hence, there is a great interest to improve soybean crops through biotechnological approaches like tissue culture and genetic transformation[2-4]. However, the successful application of biotechnology in crop improvement is strongly depend on an efficient *in vitro* system of plant regeneration[4, 5].

A lot of research work is going on in the last two decades to develop regeneration systems in commercial cultivars of soybean especially with a view to facilitate genetic transformation[4-7], until recently efficient, reproducible and reliable plant regeneration studies have not become routine for a wide range of cultivars. However, several previous reports had determined that immature cells and tissues are the most suitable explants for plant regeneration both *via* shoot organogenesis and somatic embryogenesis[8-10]. As an alternate regeneration system in soybean, many authors have used readily available explants (parts of mature seeds and juvenile seedlings)[11-13] like mature embryos and/or cotyledons, embryonic axis, hypocotyl segments, cotyledonary nodes, epicotyl segments, primary leaf nodes, primary leaf, stem nodes and shoot tips. The use of seed and seedling parts as explants has remarkable advantages as compared with immature tissues as explants. Thus, as source of immature embryos, the need for growing donor material in greenhouse under controlled environment conditions requiring intensive care, labour, time, and space can be avoided. It was also very well assessed that plant regeneration in most of the important crops including soybean is still limited and especially restricted to a particular developmental stage of an explant (specific size, age and orientation)[13], type of media with hormonal regime, and few genotypes (so called model genotypes)[14-16]. However, some authors have emphasized the use of proper medium can overcome explants and genotype associated problems with regeneration in soybean[17-20]. Country - wise, an efficient and reproducible system of plant regeneration and transformability of commercial cultivars using wide range of explants is yet to be exploited. The present research was motivated to evaluate the plant regeneration capabilities in callus cultures that could be used for genetic transformation of Indian commercial cultivars of soybean.

Material and Methods

The seeds of soybean [*Glycine max* (L.) Merrill] cultivar 'MACS-13', obtained from the Agricultural Research Station, Ummedganj, Kota (Rajasthan), India, were surface sterilized and germinated as described[13]. Hypocotyl segments (0.5-1.5 cm in length) were prepared from *in vitro* germinated seedlings after 7-10 days of germination. The excised hypocotyl explants were cultured on MSB medium[13] supplemented with different levels of 2,4-D (3.0, 5.0, 7.0 and 10.0 mg/l) for callus induction. After 5-6

weeks of culture initiation, the callus was transferred to MSB medium supplemented with 3.0 mg/l 2,4-D for its proliferation and maintenance. The callus was further maintained on the same medium by regular subculturing on to fresh medium in every 3-4 weeks of interval. For further experiments to see the growth of callus and its morphogenetic potential, approximately 0.25 g (fresh weight) callus was transferred to different formulations of media that contained MSB having different levels of (1) auxins (0.5, 1.0, 3.0, 5.0 and 10.0 mg/l each of 2,4-D, NAA, IAA and IBA), (2) cytokinins (0.5, 1.0, 3.0, 5.0 and 10.0 mg/l each of Kn and BAP) and (3) combinations of auxins (0.5, 1.0 and 3.0 mg/l) with cytokinins (0.5, 1.0 and 3.0 mg/l). The final data for callus growth in terms of fresh weight and regeneration on various media were recorded after 4-5 weeks. The procedure adopted for media preparation, culture conditions and rooting of the shoots was the same as reported[13]. Well rooted and strengthened shoots were transferred to soil and the phenotype of potted plants was checked. Five-10 replicates of callus cultures were made for each medium formulation. The data analysis of different replicates was determined by their arithmetic mean and standard error for each treatment.

Results and Discussion

The cultured hypocotyl explants showed callus formation on all the levels of 2,4-D tested in 80-100% of the cultures. The best callus formation in terms of fresh weight (2-3 g callus/explant) was observed on media supplemented with 3.0 and 5.0 mg/l 2,4-D after 5-6 weeks of culture initiation. Upon subculturing, the higher levels of 2,4-D (7.0 and 10.0 mg/l) evoked more friable and watery callus while 2,4-D at levels 3.0 and 5.0 mg/l favoured friable to semicompact callus. Thus, for callus maintenance and proliferation, MSB medium with 3.0 mg/l 2,4-D was considered best. The callus on maintenance medium was remained creamy and light green in colour and friable to semicompact in texture in over all cultures. The callus maintained on this medium was used as stock callus and transferred to different formulations of MSB medium as described in Material and Methods to see its growth and morphogenetic competence *in vitro*. Visually, the growth of callus was graded as poor (up to 0.5 g), moderate (0.5-1 g), good (1-2 g) and excellent (more than 2 g).

Effect of auxins - Among the auxins tried, best callus growth in terms of fresh weight was observed on 2,4-D containing media. On increasing the levels of 2,4-D from 0.5 mg/l to 5.0 mg/l, the yield of callus increased. However, on further increasing the levels to 10.0 mg/l, the growth declined. Maximum growth was found on 3.0 mg/l 2,4-D (Fig.1).

On all the levels of NAA, the growth of callus was poor except at very high level (10.0 mg/l), where callus became brown at the end of 4 weeks. On all the levels of IAA, no callus growth was observed and all callus turned dark brown and finally died after 5 weeks of incubation. At 0.5 mg/l IBA callus growth declined while at 1.0 and 3.0 mg/l poor growth was observed, and rhizogenesis with thick and long roots (2-10 in number and 3-12 cm in length in different cultures) occurred on 5.0 and 10.0 mg/l IBA in almost 80-100% of the cultures.

Effect of cytokinins - Growth of callus was poor on low levels (0.5, 1.0 mg/l) of both Kn and BAP. The fresh weight of the callus incubated on media with BAP, increased with increasing the levels of BAP. Minimum growth was found on 0.5 mg/l and maximum on 10.0 mg/l of BAP (Fig. 2). Few roots in the callus were observed on 3.0 mg/l Kn. Callus proliferated on media with cytokinins turned green and light brown in colour and compact and nodular in texture.

Effect of combinations of auxins and cytokinins- 2,4-D + Kn: Poor growth was observed on low levels of 2,4-D (0.5 mg/l) + Kn (0.5 mg/l). On increasing levels of both 2,4-D and Kn the fresh weight of callus increased. Maximum growth was observed on 3.0 mg/l 2,4-D + 1.0 mg/l Kn (Fig. 3). Callus was creamy and friable on all combinations of 2,4-D + Kn.

2,4-D + BAP: All the combinations of 2,4-D + BAP evoked callus growth. Maximum fresh weight of callus was observed on 0.5 mg/l 2,4-D + 3.0 mg/l BAP (Fig.3). The callus turned nodular in texture in most of the cultures.

NAA + Kn: Poor growth was observed on low levels (0.5 mg/l) of NAA + Kn. Moderate to good growth was observed on other combinations of NAA + Kn as shown in Figure 4. Best growth was recorded on NAA (3.0 mg/l) + Kn (3.0 mg/l). Mostly, callus was green and compact in texture.

NAA + BAP: All the combinations of NAA + BAP evoked good to excellent growth of callus in fresh weight. Maximum growth was recorded on NAA (3.0 mg/l) + BAP (3.0 mg/l) (Fig. 4).

IAA + Kn: As shown in Figure 5, on various combinations tested, poor to good growth of callus was observed with maximum growth on IAA (3.0 mg/l) + Kn (3.0 mg/l).

IAA + BAP: On most of combinations of these hormones increased growth of callus was observed with maximum growth on IAA (1.0 mg/l) + BAP (3.0 mg/l) (Fig. 5).

IBA + Kn: Rhizogenesis with thick and white roots (1-6 cm long) was observed in callus on most of the

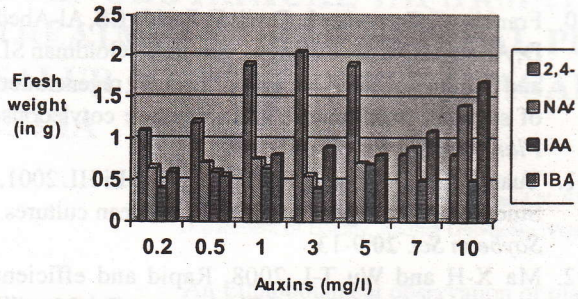

Fig.1. Effect of auxins on fresh weight of hypocotyl callus of *G. max*

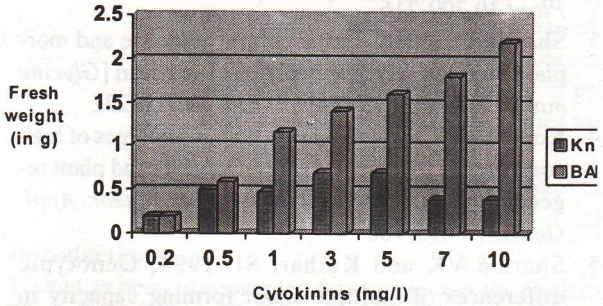

Fig.2. Effect of cytokinins on fresh weight of hypocotyl callus of *G. max*

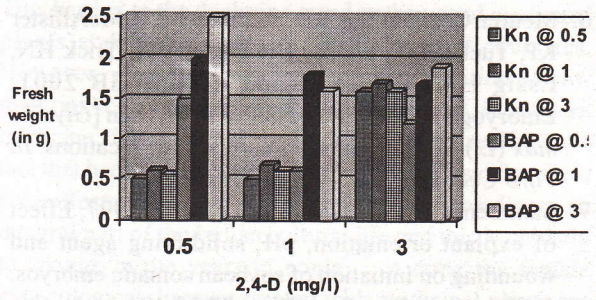

Fig.3. Effect of 2,4-D + Kn/BAP on fresh weight of hypocotyl callus of *G.max*

combinations of IBA (0.5-3.0 mg/l) + Kn (0.5 and 1.0 mg/l) in 60-100% of the cultures. Several roots with increased growth of callus (white, compact and nodular) were noted on high levels of IBA (3.0 mg/l) + lower levels Kn (0.5 and 1.0 mg/l). All levels of IBA (0.5-3.0 mg/l) in combination with high levels of Kn (3.0 mg/l) evoked callus growth without roots where maximum growth of callus (green and compact) was recorded on IBA (1.0 mg/l) + Kn (3.0 mg/l) (Fig. 6).

IBA + BAP: Fresh weight of the callus increased on all the combinations of IBA + BAP. Maximum growth was recorded on higher levels of IBA (3.0 mg/l) + BAP (3.0 mg/l). In some of the cultures, shoots and shoot buds in callus were also observed on medium with lower levels of IBA (0.5 and 1.0 mg/l) + BAP (1.0 and 3.0 mg/l) (Fig. 6).

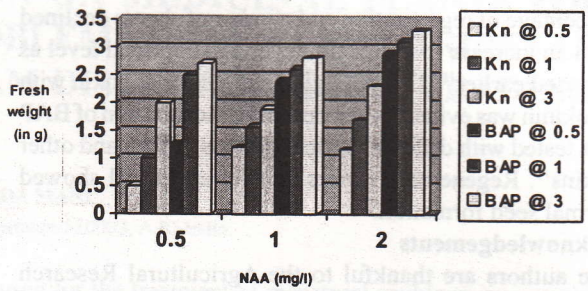

Fig.4. Effect of NAA + Kn/BAP on fresh weight of hypocotyl callus of *G. max*

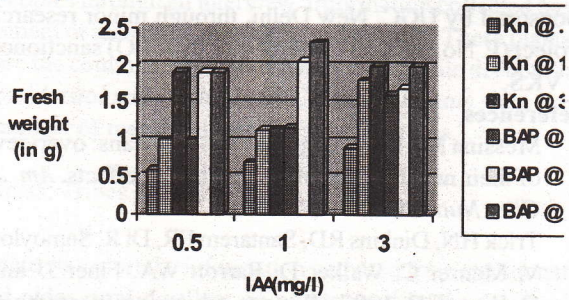

Fig.5. Effect of IAA + Kn/BAP on fresh weight of hypocotyl callus of *G. max*

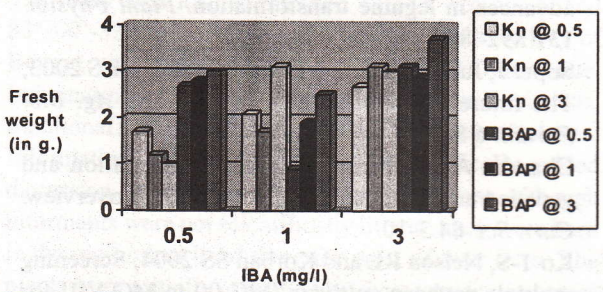

Fig.6. Effect of IBA + Kn/BAP on fresh weight of hypocotyl callas of *G. max*

Regeneration in callus cultures- The callus, 3-4 weeks of subculturing, in medium with some combinations of IBA + BAP, exhibited the formation of few shoot buds/shoots. Among the cultures, only 22% calli produced shoot buds on medium with IBA (0.5 and 1.0 mg/l) + BAP (1.0 and 3.0 mg/l). The maximum number of shoots with callus proliferation was observed on IBA (1.0 mg/l) + BAP (3.0 mg/l). Number of shoots increased upon repeated subculturing upto 3 subcultures on the same medium. It has been observed that various leguminous crops respond to auxin/cytokinin ratio showing morphogenesis[5]. In the present study also simultaneous inclusion of auxin and cytokinin was essential for shoot regeneration. BAP was the effective cytokinin for shoot induction as well as shoot proliferation. The stimulating effect of BAP on shoot bud

induction has been reported earlier in soybean[13, 15]. The percentage of regeneration and number of shoots declined with an increase in cytokinin beyond the optimal level as reported earlier[11]. The synergistic influence of auxin with cytokinin was evident when optimal concentration of BAP was tested with different concentrations of IBA and other auxins[13]. Regenerated shoots transferred to soil showed normal seed formation.

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