TISSUE CULTURE STUDIES IN MOTHBEAN

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ABSTRACT

Hypocotyl, stem, leaf and cotyledon explants of mothbean were cultured on Murashige and Skoog's basal medium supplemented with different auxins (2,4-D or IAA) in factorial combination with cytokinins (BA or Kn) at 1,2 and 4 mg/l. Callusing of explants occurred in response to all the combination treatments. Differentiation of shoot buds was predominant in cultures transferred to basal medium devoid of phytohormones. Complete plants obtained were transferred to soil and reared to maturity.

INTRODUCTION

DESPITE persistent efforts, tissue culture techniques can be applied only to a limited number of grain legumes[1]. We initiated a detailed study on the cell and tissue culture of mothbean—a drought-resistant grain legume to look for somaclonal variants and also for use in other related studies. The present communication describes our results on morphogenetic response of hypocotyl, stem, leaf and cotyledons.

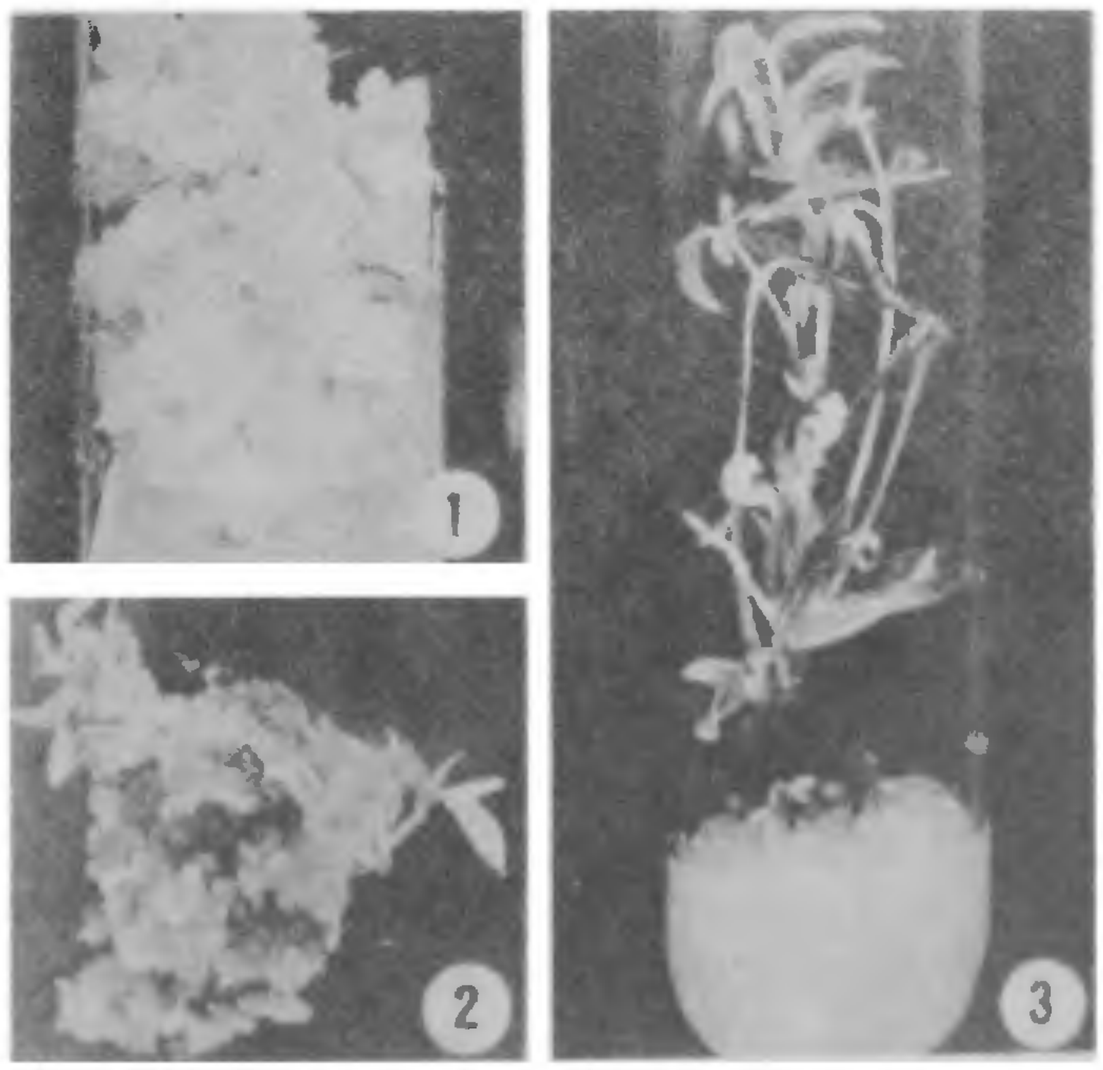
MATERIALS AND METHODS

Mothbean (*Vigna aconitifolia* Jacq marechal) cv No.88 was used as the source material. Seeds were surface-sterilized with 70% ethanol for 30 sec, followed by treatment with 0.1% mercuric chloride for 5 min. The seeds were rinsed five times with sterile distilled water and aseptically germinated on Murashige and Skoog's basal medium[2]. Various explants such as hypocotyl, cotyledon, leaves and stem were carefully excised from 5–7 day old seedlings taking care to avoid the cotyledonary node, shoot tip and axillary buds. The basal medium (BM) used for culture included inorganic nutrients of Murashige and Skoog[2], vitamins of Lin and Staba's medium[3] and 3% sucrose. The medium was supplemented with cytokinins like benzyladenine (BA) and kinetin (Kn) in factorial combination and with auxins such as indoleacetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations of 1,2 and 4 mg/l. For inducing differentiation, callus cultures were transferred to basal medium devoid of growth regulators. The pH of the medium was adjusted to 5.8 and gelled with 0.7% agar (SISCO labs, Bombay) before autoclaving. All cultures were incubated in continuous light (950 lux) at 25 ± 2°C and R.H. of 55–60%.

RESULTS

Effect of 2,4-D and BA

When hypocotyl and stem explants were cultured on a medium devoid of any growth regulators callus was initiated from the cut ends which gradually covered the entire explant. Addition of 2,4-D or 2,4-D in combination with BA resulted in more profuse callus (figure 1). Transfer of calli resulting from different treatments to BM devoid of growth regulators resulted in the differentiation of shoot buds (figure 2). In the controls



Figures 1–3. Cultures of *Vigna aconitifolia*. **1.** Callus obtained from hypocotyl on MS + 2,4-D (2 mg/l) + BA (1 mg/l). **2.** Regeneration of shoot buds from hypocotyl callus devoid of growth regulators. **3.** A regenerated plant which has flowered *in vitro*.

(only BM), 60 and 20 % of hypocotyl and stem explants differentiated the shoot buds respectively. However, cotyledon and leaf cultures did not show any differentiation in the absence of growth regulators. Addition of BA or BA in combination with 2,4-D induced shoot bud differentiation in cotyledon and leaf cultures and also favoured regeneration from stem and hypocotyl cultures. One hundred per cent of stem cultures differentiated shoot buds in response to treatment with 1 mg/l of BA.

Effect of Kn + 2,4-D

One hundred per cent of the hypocotyl explants and 25 % of stem cultures differentiated shoot buds in a treatment with 2 mg/l of Kn. Kinetin in combination with 2,4-D was found favourable for shoot bud induction in hypocotyl, cotyledon and leaf cultures.

Effect of IAA + BA

IAA either alone or in combination with BA proved very effective in inducing differentiation from cultured explants of hypocotyl, stem, root and leaf (table 1).

Effect of IAA + Kn

IAA in combination with Kn induced good callus in hypocotyl and stem explants and showed differentiation of shoot buds on subsequent transfer to basal medium (table 2). For leaf and cotyledon explants, some of the combinations of Kn and IAA were found useful for differentiation.

Table 1 *Effect of IAA and BA pretreatment on the frequency of regeneration from different explants of mothbean on transfer to basal medium*

Hormone supplement in (mg/l)	Regeneration percentage on basal medium			
	Hypocotyl	Stem	Cotyledon	Leaf
IAA(1)	50.0	50.0	Nil	Nil
IAA(2)	100.0	75.0	40.0	Nil
IAA(4)	80.0	80.0	8.3	8.3
BA(1) + IAA(1)	86.0	60.0	8.3	80.0
BA(1) + IAA(2)	50.0	100.0	83.3	50.0
BA(1) + IAA(4)	50.0	5.0	16.6	66.6
BA(2) + IAA(1)	66.6	50.0	20.0	66.6
BA(2) + IAA(2)	45.0	33.3	20.0	80.0
BA(2) + IAA(4)	40.0	50.0	33.3	50.0
BA(4) + IAA(1)	60.0	33.3	5.8	40.0
BA(4) + IAA(2)	80.0	50.0	75.0	75.0
BA(4) + IAA(4)	100.0	100.0	80.0	50.0

Table 2 *Effect of IAA and Kn pretreatment on the frequency of regeneration from different explants of mothbean on transfer to basal medium*

Hormone supplement in (mg/l)	Regeneration percentage			
	Hypocotyl	Stem	Cotyledon	Leaf
Kn(1) + IAA(1)	66.6	73.3	20.0	Nil
Kn(1) + IAA(2)	60.0	70.0	Nil	Nil
Kn(1) + IAA(4)	42.8	100.0	Nil	8.3
Kn(2) + IAA(1)	10.0	80.0	Nil	Nil
Kn(2) + IAA(2)	22.2	50.0	Nil	8.3
Kn(2) + IAA(4)	100.0	50.0	6.6	10.0
Kn(4) + IAA(1)	86.0	75.0	16.6	Nil
Kn(4) + IAA(2)	36.3	59.0	6.6	Nil
Kn(4) + IAA(4)	53.3	37.6	Nil	Nil

The plantlets developed a good root system on medium supplemented with NAA (1 mg/l) and also flowered *in vitro* (figure 3). About 70–90 % of the plants transferred to soil survived transplantation.

DISCUSSION

The above results demonstrate the regeneration potential in callus cultures of hypocotyl, stem, leaf and cotyledon of mothbean. In the case of hypocotyl and stem explants, callus induction and plant regeneration could be obtained on BM devoid of phytohormones. However, cultures grown on auxin and cytokinin media on subsequent transfer to basal media showed an enhanced frequency of shoot bud differentiation. In a previous paper on mothbean[4], hypocotyl and root derived callus cultures were reported to differentiate only in the presence of BA or Kn alone or in combination with IAA. In the present experiment, different auxins and cytokinins were used for callus induction. For obtaining shoot differentiation, the cultures had to be transferred to basal medium. In *Medicago sativa* also shoot differentiation is reported to occur only in a hormone-free medium[5,6].

In the present study a specific ratio of cytokinin to auxin was essential for differentiation of shoot buds in the callus derived from the cotyledon and leaf explants (although this was not so in the case of hypocotyl and stem explants) as is also reported in the case of callus cultures of *Indigofera enneaphylla*[7], *Stylosanthes guianensis*[8] and alfalfa[9]. Of the several cytokinin-auxin combinations tested, BA + IAA was found to enhance shoot bud differentiation in cotyledon and leaf explants. The usefulness of IAA + BA combination for regeneration has been reported in pea[10] and winged bean[11] cultures. Another interesting aspect of the

present study was that different explants required different levels of auxins and cytokinin combination to obtain regeneration. This is probably due to the differential endogenous levels of phytohormones. In *Medicago* species[12] petioles derived from seedlings had the highest capacity for somatic embryogenesis and plant formation.

The procedure of obtaining regeneration from callus cultures of different explants of mothbean may help in the studies directed to somaclonal variation.

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ANNOUNCEMENT

INTERNATIONAL CONFERENCE ON METALLIC AND SEMICONDUCTING GLASSES

An International Conference on "Metallic and Semiconducting Glasses" will be held at the University of Hyderabad from Tuesday, *December 16 to* Saturday, *December 20, 1986*, with the aim of providing a common forum for Indian and foreign scientists actively involved in various facets of these glasses to: (i) review the current status of these technologically important materials (ii) facilitate exchange of ideas and (iii) identify various potential areas for future research and development and technical and applications with particular reference to India and the South-east Asian region.

The scientific program will comprise plenary and poster sessions. The plenary sessions will consist of invited talks and a few contributed papers selected for oral presentation. Majority of contributed papers will be included in poster sessions to promote extensive discussion. The proceedings of the conference will be published.

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