Theor Appl Genet (1986) 72:384–387



# Regeneration of plants from cultured root explants of mothbean (*Vigna aconitifolia* L. Jacq. Marechal)

**S. Eapen and R. Gill**

Plant Biotechnology Section, Bio-Organic Division, Bhabha Atomic Research Centre, Bombay-400 085, India

Received November 7, 1985; Accepted January 5, 1986
Communicated by G. S. Khush

**Summary.** Decapitated seedling root explants of seven cultivars of mothbean (*Vigna aconitifolia*) cultured on Murashige and Skoog's basal medium without any phytohormone gave rise to plantlets at the end of 4–5 weeks. Addition of cytokinins such as BA, Z, Kn and 2,i-P enhanced the frequency of plant regeneration and also the average number of shoot buds/culture. The buds originated directly from cortical cells or through callussing and subsequent differentiation from the surface. The plantlets obtained were successfully transferred to the field.

**Key words:** Mothbean – Plant regeneration – Root culture – *Vigna aconitifolia*

## Introduction

Shoot development and subsequent plantlet formation directly from excised roots is of interest from the practical point of view as it offers a new source of somaclonal variation yet unexplored. A large number of weeds are known to multiply in nature by shoot differentiation directly from roots thus enabling them to survive adverse conditions (Raju et al. 1966). Though grain legumes are known to differentiate from such aerial parts as the hypocotyl, epicotyl, cotyledon, cotyledonary node, leaf and stem explants (see Bajaj and Gosal 1981; Mroginski and Kartha 1984, for review), plant differentiation directly from cultured roots of grain legumes is a rare phenomenon. In this report, we present the conditions essential for direct plant differentiation from cultured seedling roots of Mothbean – a drought resistant grain legume widely cultivated in the tropics.

*Abbreviations:* BA = benzyladenine; 2,i-P = 6-$\gamma$-$\gamma$-dimethylallylaminopurine; Kn = kinetin; TIBA = 2,3,5-Triiodobenzoic acid; Z = zeatin

## Material and methods

Mothbean (*Vigna aconitifolia* L. Jacq Marechal) cvs – IPCMO – 131, IPCMO-186, IPCMO-560, IPCMO-909, IPCMO-760, MG-1 and No-88 were used as source material. The seeds were surface-sterilized with 70% alcohol (V/V) for 30 s followed by 0.1% $HgCl_2$ (W/V) for 5 min. After sterilization, the seeds were rinsed five times with sterile distilled water and aseptically implanted on Murashige and Skoog's (1962) medium, for germination. At the end of 6–7 days of growth, the tap root was excised, cut into 4 segments of 1 cm each, taking care to remove the root tip. The explants were cultured on medium containing Murashige and Skoog's mineral elements (1962), vitamins after Lin and Staba (1961) and 3% sucrose. Different cytokinins such as BA, Z, 2,i-P and Kn (1 mg/l) and antiauxins like TIBA (1 mg/l) were incorporated into the medium. In a separate experiment, the effect of different concentrations of BA (1, 2, 5 and 10mg/l) was investigated. All media were adjusted to pH 5.8 and were solidified with 0.8% agar (Sisco Labs, Bombay). The cultures were incubated at $25 \pm 2$ °C in continuous light of 950 lux. Final observations were taken at the end of 3 months during the second passage and average number of plantlets/explant and frequency of regeneration were recorded.

For histological studies, root explants at the end of different periods of culture were fixed in formalin-acetic-alcohol and processed in an alcohol-xylol series. Sections were cut at 14 μm and stained with toluidine blue.

## Results

Root explants cultured on basal medium enlarged considerably, accompanied by enormous growth of secondary and tertiary roots. Eventually the entire

**Table 1.** Effect of different cytokinins on the frequency of shoot bud regeneration and average number of shoot buds/explant from cultured root segments of different cultivars of mothbean grown of MS medium at the end of two months of culture*

| Cultivar | Cytokinin treatment | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nil | | Z (1 mg/l) | | 2iP (1 mg/l) | | Kn (1 mg/l) | | BA (1 mg/l) | | BA (2.0 mg/l) | | BA (5.0 mg/l) | | BA (10.0 mg/l) | |
| | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| No. 88 | 25.0 | 3.8±0.02 | 83.3 | 3.0±0.04 | 83.3 | 3.4±0.01 | 33.3 | 4.0±0.06 | 83.3 | 4.0±0.05 | 79.1 | 3.0±0.06 | 62.5 | 2.1±0.02 | 75.0 | 9.5±0.08 |
| IPCMO-909 | 12.6 | 1.0±0 | 100.0 | 4.0±0.06 | 100.0 | 3.0±0.03 | 100.0 | 3.0±0.04 | 20.8 | 3.2±0.03 | 41.6 | 4.0±0.05 | 50.0 | 4.0±0.07 | 50.0 | 3.0±0.04 |
| IPCMO-186 | 8.3 | 1.0±0 | 50.0 | 3.0±0.03 | 12.6 | 1.0±0 | 8.6 | 1.0±0 | 16.6 | 3.0±0.01 | 66.6 | 2.0±0.01 | 37.5 | 3.7±0.05 | 16.6 | 2.0±0.01 |
| IPCMO-131 | 25.0 | 2.0±0.01 | 25.0 | 2.0±0.01 | 4.1 | 1.0±0 | 83.3 | 5.2±0.07 | 83.3 | 2.6±0.004 | 25.0 | 2.0±0.02 | 16.6 | 3.5±0.04 | 33.3 | 3.0±0.02 |
| MG-1 | 16.6 | 1.0±0 | 33.3 | 2.5±0.004 | 29.1 | 2.5±0.01 | 12.5 | 1.0±0 | 16.6 | 4.4±0.06 | 12.5 | 1.3±0.01 | 4.1 | 1.0±0 | 4.1 | 1.0±0 |
| IPCMO-760 | 25.0 | 3.0±0.02 | 100.0 | 1.5±0.001 | 83.3 | 4.3±0.01 | 100.0 | 6.0±0.4 | 83.3 | 4.0±0.06 | 83.3 | 2.0±0.04 | 83.3 | 2.0±0.01 | 83.3 | 2.0±0.01 |
| IPCMO-560 | 45.8 | 3.0±0.03 | 83.3 | 3.0±0.03 | 66.6 | 3.5±0.04 | 50.0 | 5.0±0.08 | 62.5 | 4.0±0.04 | 33.3 | 2.0±0.01 | 33.3 | 2.0±0.02 | 66.6 | 3.0±0.02 |

* Average of 24 replicates
a = % of cultures showing response; b = average number of shoot buds/regenerating root explant ± SE

medium was covered with secondary and tertiary roots. The surface of the root explant split open at random sites and this was followed by the emergence of greenish patches with or without callussing. Within a period of 4–6 weeks, the greenish patches gradually developed into distinct shoot meristems flanked by leaves (Fig. 1). Shoot buds developed further and this was accompanied by roots from the base (Fig. 2). Such of the shoot buds which did not root on the same medium were rooted on basal medium with 1 mg/l of NAA. Complete plants thus obtained were initially established in paper cups and later grown in pots in the field (Fig. 4).

Supplementing the basal medium with different cytokinins such as Z, Kn, BA and 2,i-P resulted in either a considerable reduction or the complete inhibition of secondary and tertiary roots. The proliferation induced in the presence of cytokinins was more intense compared to that grown only on basal medium. Such calli developed greenish patches which developed into numerous shoot buds (Fig. 3).

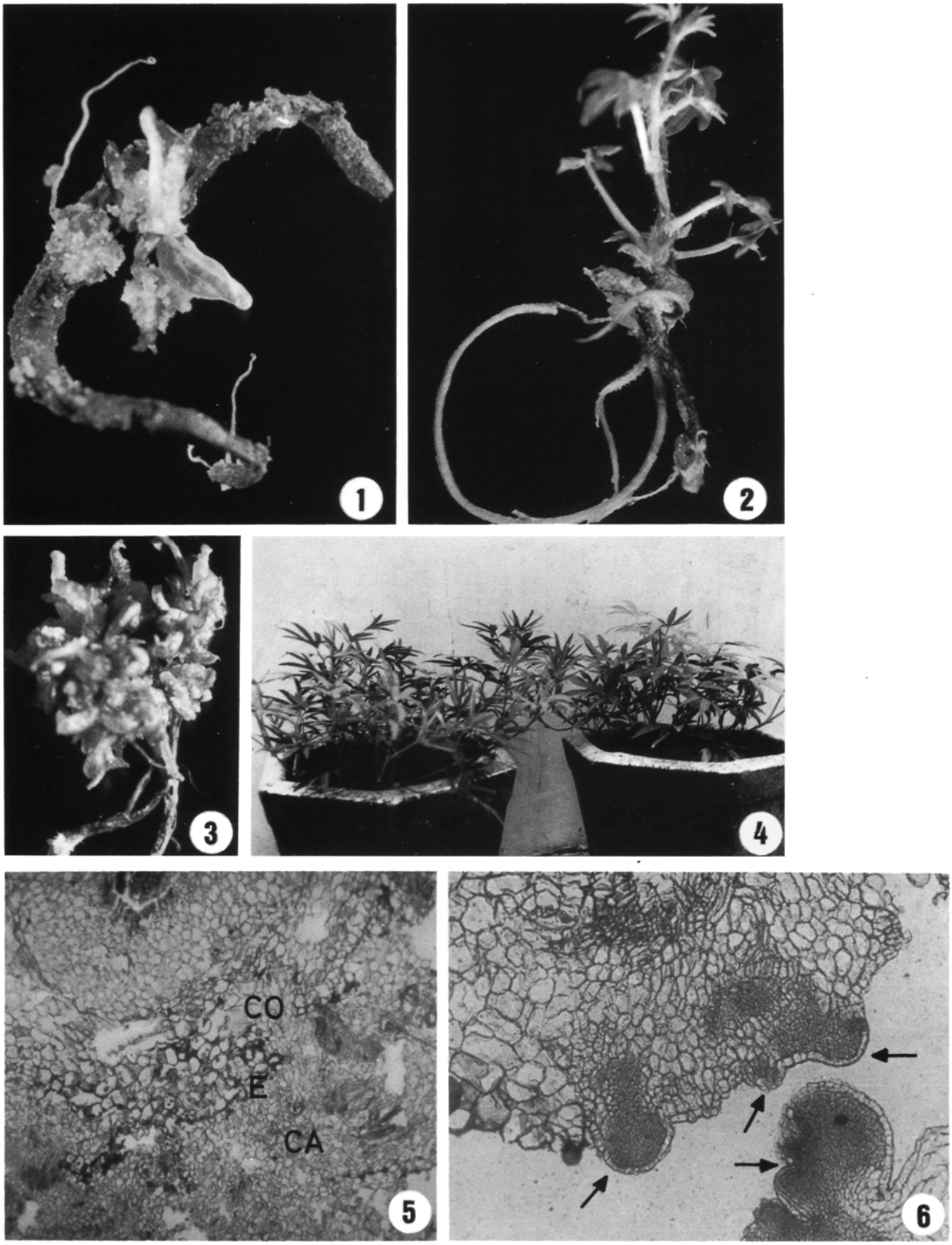
The frequency of regeneration was enhanced on cytokinin supplemented medium in the majority of the cultivars (Table 1). The frequency of response varied with the cultivar. Addition of cytokinins also enhanced the average number of shoot buds/culture in some of the treatments (Table 1).

Histological examination of the root at the time of culture showed an epidermis consisting of isodiametric cells surrounded by a cortex of 6–7 layers of loosely packed parenchymatous cells. A distinct endodermis and pericycle were seen surrounding the stele. Callusing was initiated at the cortical region giving rise to a small callus mass which split open the epidermis and emerged (Fig. 5). The shoot buds were formed from the surface of the calli (Fig. 6). In some cases the cortical cells directly produced shoot meristems unaccompanied by the callus phase. The shoot buds differentiated vascular connections with the central vascular cylinder. Structures resembling early dicotyledonous embryos were also observed, but further differentiation was not obtained.

## Discussion

The foregoing observations have revealed that it is possible to induce differentiation of plantlets directly from cultured seedling root explants of mothbean.

Bud formation in root explants has been reported in *Isatis* (Danckwardt-Lillestrom 1957), *Convolvulus* (Torrey 1958; Bonnett and Torrey 1965), *Linaria* (Charlton 1965), *Comptonia* (Goforth and Torrey 1977), *Limnophila* (Rao and Mohan Ram 1981) and *Dalbergia* (Mukhopadhyay and Mohan Ram 1981). A significant observation of the present study was that basal medium devoid of phytohormones could induce plantlet




**Figs. 1–6.** Stages in plant differentiation from cultured root segments of *Vigna aconitifolia*. **1** Differentiation of shoot buds from root segments cultured on basal medium at the end of 4 weeks of culture. **2** A plant with well-developed roots at the end of 6 weeks of culture on basal medium. **3** Differentiation of numerous shoot buds from calli initiated from root segments cultured on MS+Kn (1 mg/l). **4** Regenerated plants growing in pots, one month after transplantation. **5** A cross section of root showing development of calli from cortical cells. CA-Callus; CO-Cortex; E-Epidermis. **6** Differentiation of shoot buds from the surface of calli. *Arrows* indicate shoot buds

formation in excised seedling explants of mothbean. Such a situation has also been reported in *Linaria* (Charlton 1965). Presumably the decapitated seedling explants may have sufficient endogeneous cytokinins to induce differentiation. However, there are also reports that cytokinin alone or in combination with auxin enhances the frequency of plant differentiation (Danckwardt-Lillestrom 1957; Peterson 1969; Mukhopadhyay and Mohan Ram 1981; Bhargava and Chandra 1983). This was also observed by us.

Though some early stages of embryogenesis were observed in the present study, their further differentiation was somehow impaired and mature embryos were never obtained. A somewhat parallel situation has been reported in *Limnophila indica* where a pattern of division reminescent of early stages of embryogenesis was observed though no true embryoids developed (Rao and Mohan Ram 1981).

Multiplication by root-bud formation is a natural phenomenon to overcome adverse conditions in many of the weeds (Raju et al. 1966). *Vigna aconitifolia* being a drought resistant plant also has the ability to produce buds from cultured roots, though in nature they are seldom produced.

Our findings that plants can be obtained from cultured roots of *V. aconitifolia* provides an opportunity for studying an additional source of somaclonal variation, which may find use in plant breeding programmes.

*Acknowledgements.* Our gratitude is acknowledged to Dr. P. S. Rao for critical evaluations of the manuscript and Mr. S. E. Pawar, Nuclear Agriculture Division, for the supply of seeds.

## References

Bajaj YPS, Gosal SS (1981) Induction of genetic variability in grain legumes through tissue culture. In: Rao AN (ed) Proc Costed Symp Tissue Culture of Economically Important Plants. Singapore, pp 25–41

Bhargava S, Chandra N (1983) In vitro differentiation in callus cultures of mothbean, *Vigna aconitifolia.* Plant Cell Rep 2:47–50

Bonnett HJ, Torrey JG (1965) Chemical control of organ formation in root segments of *Convolvulus* cultured in vitro. Plant Physiol 40:1228–1236

Charlton WA (1965) Bud initiation in excised roots of *Linaria vulgaris.* Nature 207:781–782

Danckwardt-Lillestrom C (1957) Kinetin-induced shoot formation in isolated roots of *Isatis tinctoria.* Physiol Plant 10:794–797

Goforth PL, Torrey JG (1977) The development of isolated roots of *Comptonia peregrina* (Myriaceae) in culture. Am J Bot 64:476–482

Lin M, Staba J (1961) Peppermint and spearmint tissue cultures. 1. Callus formation and submerged culture. Lloydia 24:139–145

Mroginski LA, Kartha KK (1984) Tissue culture of legumes for crop improvement. In: Janick J (ed) Plant breeding reviews, vol 2. Avi, West Fort (USA), pp 215–264

Mukhopadhyay A, Mohan Ram HY (1981) Regeneration of plantlets from excised roots of *Dalbergia sissoo.* Indian J Exp Biol 19:1113–1115

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:471–497

Peterson RL (1969) Bud formation in root segments of *Ophioglossum petiolatum:* Effect of application site of cytokinins and auxin. Can J Bot 47:1285–1287

Raju MVS, Coupland RT, and Steeves TA (1966) On the occurrence of root buds on perennial plants in Saskatchewan. Can J Bot 44:33–37

Rao S, Mohan Ram HY (1981) Regeneration of plants from cultured root tips of *Limnophila indica.* Can J Bot 59: 969–973

Torrey JG (1958) Endogenous bud and root formation by isolated roots of *Convolvulus* grown in vitro. Plant Physiol 33:258–263