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BRIEF COMMUNICATION

Differential morphogenetic responses of cotyledonary explants of *Vigna mungo*

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Abstract

The morphogenetic responses of cotyledonary nodal explants of *Vigna mungo* (L.) Hepper cv. VBN1 cultured on the same Murashige and Skoog's medium, B5 vitamins, and 13.31 μM N^6-benzylaminopurine showed variations in the pattern of multiple shooting and morphology of leaves in dependence on initial explants (presence/absence of cotyledons). The regenerated shoots elongated in the initial medium and most of them rooted in the presence of 2.41 μM indole-3-butyric acid, and flowered *in vitro*. Rooted plants could be transferred to the field after hardening.

Additional key words: apical dominance, *in vitro* flowering, regeneration, somaclonal variation.

Variation in tissue culture is more a rule than exception. There are examples of variations in tissue culture that have been found in different parts of just one plant in a population (Meins *et al.* 1983). Studies also show that variation of enzyme pattern among sublines from the same tissue is greater than the variation in lines from different tissues of the same plant (Haddon and Northcote 1976). In many instances, heritable variations were found in culture (somaclonal variation). Somaclonal variation in cotyledonary plants of *Vigna radiata* has also already been reported (Mathews *et al.* 1986). In our studies with *Vigna mungo*, we tried to find variations in the pattern of shoot morphogenesis and leaf structure in physiologically different explants of a single cultivar (VBN1) grown under identical culture conditions. We also tried to decide if apical dominance is only in the primary shoot or also exists in axillary shoots.

Seeds of the *Vigna mungo* (L.) Hepper cultivar VBN1 were obtained from National Pulses Research Centre, Vamban, Pudukkottai, Tamilnadu, India. For *in vitro* germination, the seeds were washed several times in running tap water and treated with 70 % ethyl alcohol for 30 s, sterilised with a 0.1 % of mercuric chloride and sodium dodecyl sulphate for 180 s and repeatedly washed with sterilised double distilled water. Then the seeds were allowed to imbibe in sterilised double distilled water over night and inoculated on MS basal medium for germination. The cotyledonary explants with or without cotyledon were prepared from 6-d- old seedlings.

MS medium (Murashige and Skoog 1962) containing B5 vitamins (Gamborg *et al.* 1968) supplemented with 13.31 μM BAP was used for induction and subsequent development of shoots. Half strength MS medium containing 2.41 μM IBA was used for rooting. The pH was adjusted to 5.8 by using either 0.1 M NaOH or 0.1 M HCl. The medium was solidified with 0.7 % *Bacto* agar (*Hi-media*, Mumbai, India) before autoclaving. Each culture tube containing 20 cm^3 of medium was inoculated with one explant and plugged with non-absorbent cotton. The cultures were incubated in 16 h photoperiod of cool fluorescent light providing a quantum flux density of 30 μmol m^{-2} s^{-1} at 25 ± 2 °C. For every trial 20 replicates were maintained and each experiment was repeated twice. All the regenerated shoots were visually examined for variations.

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Abbreviations: B5 vitamins - vitamins after Gamborg *et al.* (1968), BAP - N^6-benzylaminopurine, IBA - indole-3-butyric acid, MS medium - medium after Murashige and Skoog (1962).
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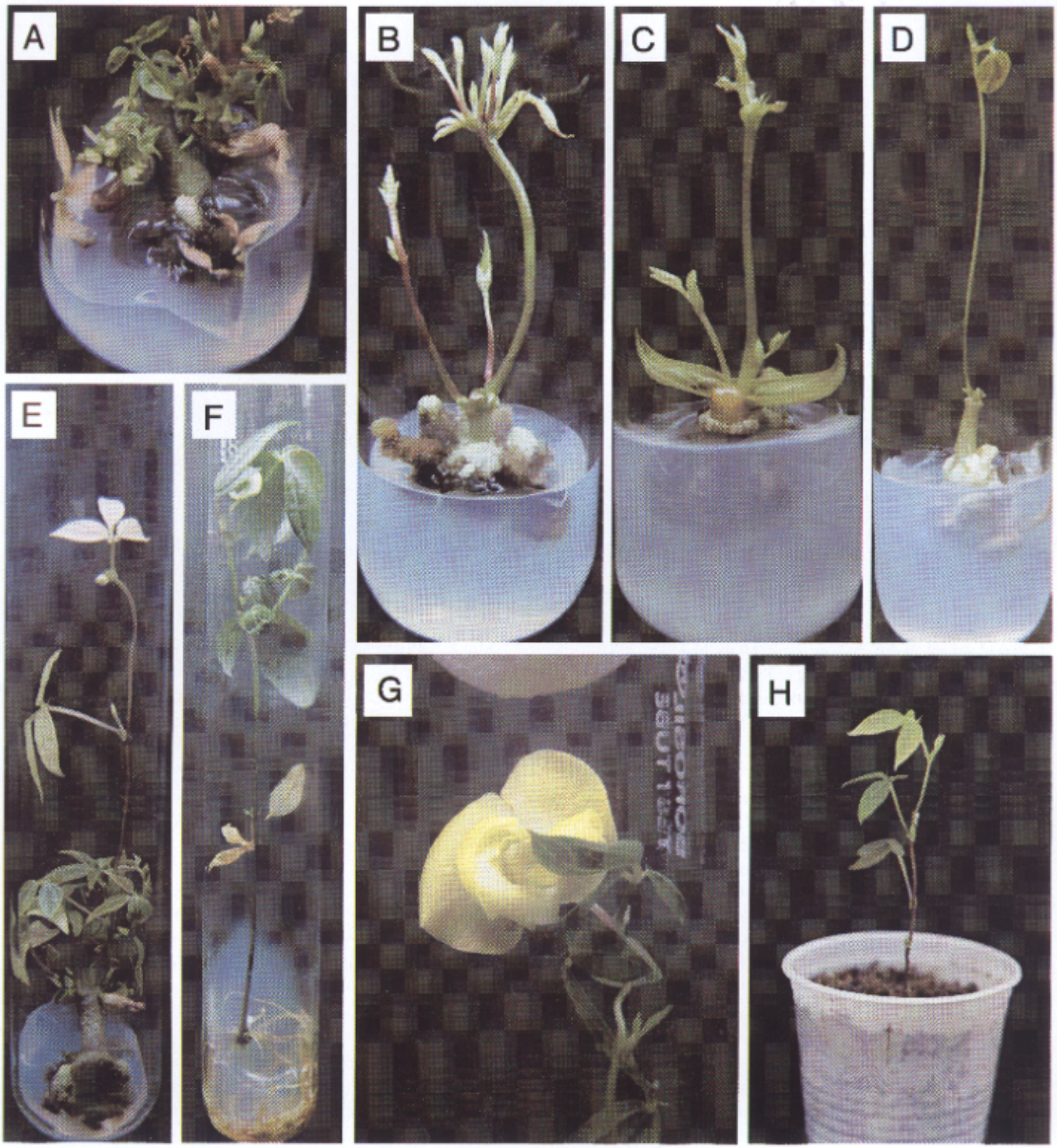

Fig. 1. Variations found during *in vitro* culture of *Vigna mungo* L. Hepper cv. VBN1 on MS medium containing B5 vitamins and supplemented with 13.31 μM BAP: *A* - variation in the frequency of shoot regeneration between two axis of the cotyledonary node, *B* - one of the axillary shoot regenerated from cotyledonary node producing many shoots at its apical region like an umbel, *C* - healthy shoot possessing trifoliate leaf produced from the primary leaf node when the cotyledon was included with the explant, *D* - weak shoot possessing a simple leaf regenerated from the primary leaf node when the cotyledon was not included with the explant, *E* - shoot elongated from cotyledonary node having trifoliate leaves when cotyledon was included with the explant, *F* - rooting and subsequent *in vitro* flower bud formation, *G* - well developed *in vitro* flower, and *H* - regenerated plantlet transferred to plastic cup containing farm manure and soil for hardening.

Rooted plantlets were transferred to plastic cups containing sterile vermiculite, watered with sterile half-strength Hoagland's solution and covered with a plastic sheet. The plants were acclimatised by gradually exposing them to the external environment over a period of 10 d. Hardened plants were transferred to soil in the greenhouse and then transplanted to the field.

When cotyledonary nodes with intact cotyledons were cultured on MS medium containing B5 vitamins and augmented with 13.31 μM BAP, shoots regenerated from the axis of cotyledon and embryonal axis. However, the number of shoots produced from one side (12.5 ± 2.4) was significantly higher than from the other side (4.3 ± 1.5) (Fig. 1*A*). Chandra and Pal (1996) also observed similar variation in mature seeds of *Vigna radiata*. The reason was seen in fact that one of the cotyledons was closely attached to the embryonic axis, while the other cotyledon was more distantly attached. Shoot regeneration was faster and higher efficiency of the cotyledon more closely attached to the embryonic axis was similar to our observation.

In one of the cultures, a single axillary shoot originated from cotyledonary node produced many shoots (7.0) at its apical region like an umbel (Fig. 1*B*). When the regular path of translocation to the apex of primary shoot is blocked by decapitating the primary shoot, translocation of cytokinin (BAP) may take place from the culture medium to the apical region of axillary shoot. This observation substantiates the cytokinin-controlled apical dominance and suggests that apical dominance is not only confined to primary shoots, but also exists in axillary shoots.

Single shoot was produced on either side of the primary leaf node when the shoot tip also included with the explant in the presence and absence of cotyledons. In the presence of cotyledons, the axillary shoot produced from the primary leaf node was healthy and possessed trifoliate leaves (Fig. 1*C*). Whereas, in the absence of cotyledons the shoot from primary leaf node was very weak and possessed a simple leaf (Fig. 1*D*). So it may be concluded that some morphogenetic signal translocated from cotyledon to regenerating shoots in addition to reserve food material, may confer the leaf characteristics in culture. There are many published reports available for the support of this theory of translocation of nutrients and morphogenetic signal from cotyledon to shoots (Mathews and Rao 1984, Gulati and Jaiwal 1990, Franklin *et al.* 1991).

Similar to the simple-leafed shoot of the present study, variations in the leaf morphology such as multifoliate and pentafoliate has also already been reported in *Vigna radiata* (Santos 1969, Mathews *et al.* 1986). Thakare *et al* (1980) reported variation in cotyledonary leaf of the same plant due to mutation (green cotyledon mutant). Franklin *et al.* (2000) also reported a sequential progression of trifoliate leaves from simple *via* bifoliate leaves in *Cajanus cajan*, when culturing mature embryonal axes explants. These reports substantiates the high levels of variability in legumes (in particular *Vigna* species) in culture.

Regeneration in tissue culture is a genetically controlled trait and as such may be genotype dependent (Bhojwani *et al.* 1984, Templenton-Somers and Collins 1986). However, the present results shows that the frequency and pattern of shoot regeneration varies with the status of cotyledonary explants (presence or absence of cotyledons) of a genotype under similar cultural conditions (physical and chemical). Thus, the reason for variation in the morphogenetic responses is rather physiological than genetical.

All the micro-shoots elongated in the shoot induction medium (Fig. 1*E*). Elongated shoots were rooted on half strength MS medium supplemented with 2.41μM IBA. Many of the shoots regenerated from the cotyledonary nodes resulted in *in vitro* flowering immediately after rooting as reported earlier in *Vigna mungo* (Ignacimuthu *et al.* 1997) and *Pisum sativum* (Franklin and Ignacimuthu 2000) (Figs. 1*F* and 1*G*). Whereas, the plantlets obtained from the culture variants (umbellate multiple shoots and simple leafed shoot) did not root and flower. Rooted plantlets were successfully hardened (Fig. 1*H*) and established in the field as reported earlier (Ignacimuthu and Franklin 1998).

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