Short Communication



## In vitro organogenesis from shoot tip in blackgram

## P. K. Das, M. Roy and N. Mandal

Department of Genetics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741 252

(Received: April 2001; Accepted: November 2001)

To develop suitable protocol for *in vitro* organogenesis directly from the explant source in blackgram (*Vigna mungo* (L.) Hepper) an experiment was designed with a view to rapid multiplication of true to type elite strain. In recent past such approach has assumed significance in number of grain legumes like Mungbean [1], Cowpea [2] and Winged bean [3].

Shoot tips with leaf primordia (3-4 mm) were excised from asceptically raised 12 days old seedlings in Phytohormone free MS basal medium [4] of two blackgram genotypes T-9 and L-13, a mutant of T-9 [5]. They were cultured in the modified MS containing MS salts + B5 vitamins [6] + 3mg I<sup>-1</sup> BAP+1mg/I<sup>-1</sup> NAA. The light intensity was maintained at 2500-3000 Lux and the temperature was  $25 \pm 1^{\circ}$ C. Within 7 days of culturing little callus mass was observed at the explant base (Fig. 1a). After another 7-10 days culturing, 2-3 multiple shoots appeared (Fig. 1b). Percent shoot induction was as high as 80 in both the genotypes. Multiple shoots were separated and each individual shoot was transferred to the rooting medium containing MS salts + B5 vitamins + IBA 3 mg  $l^{-1}$ . By another 15-17 days of culturing plantlets showed good rooting with 4-5 trifoliate leaves (Fig. 1c). T-9 displayed a little higher success percent in rooting (Table 1). In this case the level of cytokinin constantly remained higher and cytokinin : auxin was 3 : 1. Kartha et al. [7] in tomato found a complete reverse situation where auxin: cytokinin was 10:1 in MS with B5 vitamins. It can be

Table 1. Direct organogenesis from shoot-tip explant in modified MS\*

Geno- type	No. of explant	Days to shoot formation	No. of multiple shoots	Success (%)	Days to rooting	Root formed (%)
L13	20	14-17	2-3	80	15-17	70
Т9	20	14-17	2-3	80	15-17	75
*Shoot in	duction ·	MS salt +	B5 vitam	nin + BAP	3mal <sup>-1</sup>	+ NAA 1

mgl<sup>-1</sup>

\*Rooting : MS salt + B5 vitamin + IBA 3mgl<sup>-1</sup>.



## Fig. 1 a, b and c

suggested that in grain legumes like blackgram growth hormone formulation could be very different from that of other non-legumes. The study also suggests that auxin NAA in MS salts + B5 vitamins would be a better choice for successful in vitro shoot organogenesis directly from the explant in blackgram if the ratio between auxin and cytokinin mentioned is maintained. In the present study IBA has been found to be desirable root inducing auxin for in-vitro produced shoots. The results are in agreement with Jaiwal and Gulati [8] in mungbean and Geetha et al., [9] in the blackgram. However, full strength MS has been found to be unfavourable for shoot organogenesis from stem explants of blackgram [10]. In the present study shoot-tips were used as explants and the type of explant is known to influence the frequency of shoot organogenesis in a number of legumes [11]. Moreover the use of B5 vitamins in this case might have registered positive influence in the shoot organogenesis along with the specific auxin and cytokinin maintained at the right proportion.

## References

- Singh B. D., Singh R. P., Singh R. B. and Sing R. M. 1980. Plantlet regeneration from macerated shoot tips of mung. *In:* Plant tissue culture, genetic manipulation and somatic hybridization of plant cells (P. S. Rao, M. R. Meble and M. S. Chadha, eds), pp. 335-340.
- Pellegrineschi A. 1997. Plant regeneration via organogenesis of cowpea (*Vigna unguiculata* L. Walp.) Pl. Cell Rep., 17: 89-95.
- Gupta S. D., Ahmed R., De D. N. 1997. Direct somatic embryogenesis and plantlet regeneration from seedling leaves of winged bean (*Psophocarpus tetragonolobus* 1-DC) Pl. Cell Rep., 16: 628-631.
- Murashige T and Skoog F. 1962. A revised medium for rapid growth and biossay with tobacco tissue cultures. Pl. Physiol., 15: 473-497.
- Biswas P. K. 1989. Induction of variability for productivity and nodulation in blackgram (*Vigna mungo* L. Hepper) Unpubl. Ph. D Thesis, BCKV, Mohanpur, W. Bengal.
- Gamborg O. L., Miller R. A. and Ojima K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Expt. Cell Res., 50: 151-158.

- Kartha K. K., Champaux S., Gamborg O. L. and Pahl K. 1997. *In vitro* propagation of tomato by shoot apical meristem culture. J. Am. Soc. Hort Sci., 102: 346-349.
- Jalwal P. K. and Gulati A. 1995. Current status and future strategies of *in vitro* culture techniques of genetic improvements of mung (*Vigna radiata* L. Wilczek) Euphytica, 86: 167-181.
- Geetha N, Venkatachalam P., Rao G. R. 1997. Plant regeneration and propagation of black gram (*Vigna mungo* L. Hepper) through tissue culture. Trop. Agric., 74: 73-76.
- Das D. K., Prakash N. S. and Bhalla Sarin N. 1998. An efficient regeneration system of blackgram (*Vigna mungo* L. Hepper) through organogenesis. Plant Sci., 134: 199-206.
- 11. Kaneda Y., Tabei Y., Nishimura S., Harada K., Akihama T. and Kitamura K. 1997. Combination of thidiazuron and basal media with low salt concentrations increases the frequency of shoot organogenesis in soybean. Pl. Cell Rep., 17: 8-12.