Journal of Tropical Agriculture 36 (19981 : 6-11

IN VITRO PLANT REGENERATION FROM SHOOT TIP CULTURE OF BLACKGRAM | *VIGNA MUNGO* (L.) HEPPER]

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Abstract : *In vitro* culture of blackgram | *Vignamungo* (L.) Hepper] cv. LBG was initiated from 7 day old aseptic seedling shoot tips. Isolated shoot tips were cultured on MS medium containing various concentrations and combinations of cytokinins and auxins. High frequency of callusing and shoot differentiation was observed on MS medium supplemented with BAP (3.0 mg l^{-1}) and NAA (1.5 mg 1^{-1}). A single shoot tip produced more than 12 shoots. The regenerated shoots were rooted on MS medium supplemented with different concentrations of IBA. Seventy five per cent of the shoots produced r<x>ts, and 90-95% of them survived in the field condition.

Key words : Blackgram, growth regulators, multiple sh<x>t formation, plant regeneration, shoot tip cultures, *Vigna mungo* Hepper.

INTRODUCTION

Grain legumes (edible legumes or seed legumes) constitute an important human and animal dietary constituent. Seeds of leguminous crops in general, grain legumes in particular carry viruses within a growing crops usually results in extensive losses in seed quality and yield. Meristem or shoot tip culture has been successfully used in the elimination of viral pathogen, including seed-borne viral infections in forage and grain legumes (Kartha *et al.*, 1981; Kartha, 1981; Gulati and Jaiwal, 1992; Venkatachalam *et al.*, 1994). There has been only few attempts to regenerate blackgram plants via tissue culture (Hoque *el al.*, 1984). Recovery of single plants from blackgram meristems and shoot tips on basal medium supplemented with one auxin and one cytokinin was reported by Bajaj and Dhanju (1979), Goel *el al.* (1983).

Gene transfer into plants can be achieved by a number of ways. *Agrobacterium* based vectors have been successfully employed to transfer genes into a number of dicotyledonous plants. However, grain legumes, in general, remain recalcitrant to DNA transfer technology. To date, meristem based methods have been used successfully in *Agrobacterium* mediated genetic transformation of petunia (Lulsdorf *et al.*, 1991), sunflower (Gould *el al.*, 1991) and greenbean (Fraklin *et al.*, 1993).

In view of the economic importance of blackgram and potential to improve commercial cultivar by gene transfer technology, a search was initiated for alternative regeneration methods. This report describes protocols that were developed for the *in vitro* plant regeneration and multiple shoot production from shoot tips of *Vigna mungo.*

MATERIALSANDMETHODS

Blackgram [*Vigna mungo* (L.) Hepper (cv. LBG)] seeds were obtained from Tamil Nadu Agricultural University, Coimbatore, India. In order to minimise variation between seeds that were in uniform size and without cracks in the seed coats were used. To raise aseptic seedlings, seeds were washed thoroughly by adding a few drops of Tween-80. There were surface sterilized in 0.1% (w/v) mercuric chloride for 5 min and then washed three times in sterile distilled water. The rinsed seeds were kept on MS basal medium in 250 ml conical flasks and germinated in a 1 h photoperiod 25 ±2°C with 60% humidity.

Table 1. Effect of different concentrations of BAP + IAA and BAP + NAA on callus induction, shoot bud differentiation and multiple shoot development from blackgram [*Vigna mungo* (L.) Hepper] shoot tips*

MS + hormonal concentration (mg l ')		Calli formed (%) (Mean *i* SD)	Calli with shoots (%) (Mean ., SD)	Mean number of shoots / callus (Mean ± SD)
BAP	IAA			
1.0	0.5	28.5 + 6.04	26.3 t 4.40	1.6 + 0.48
2.0	1.0	49.4 ± 5.79	35.4 t 3.34	3.8 . 1.63
3.0	1.5	65.3 + 4.24	58.2 + 3.83	5.1 ± 1.22
4.0	2.0	58.6 ± 7.01	64.3 + 2.85	6.0 *i* 1.63
5.0	2.5	51.4 *i* 1.46	55.7 ± 4.32	8.8 *i* 0.81
BAP	NAA			
1.0	0.5	34.8 i 2.85	48.4 t 5.06	2.8 ± 0.48
2.0	1.0	55.6 t 2.85	69.8 *i* 4.81	5.1 ± 0.81
3.0	1.5	79.7 . 7.59	88.3 ± 2.85	7.8 t 1.14
4.0	2.0	68.5 . 4.16	71.5 + 2.84	8.9 t 1.63
5.0	2.5	61.4 ± 2.77	68.4 ± 2.53	11.5 ± 2.63

* 15-20 shoot tips were cultured for each treatment

Table 2. Effect of IBA on root induction in subcultured shoots of blackgram [*Vigna mungo* (L.) Hepper]

MS media + IBA (mg l^{-1})	Root induction, : % (Mean ± SD):	No. of roots per shoot (Mean + SD)
0.5	49.6 ± 2.04	8.3 ± 0.61
1.0	55.3 ± 2.85	10.4 t 0.89
2.0	69.8 + 2.20	12.7 ± 0.81
3.0	78.3 . 1.22	14.5 i 0.97
4.0	69.5 i 1.71	13.8 ± 1.30
5.0	63.8 ± 1.22	11.5 ± 1.14

Subsequent to germination, shoot tips measuring 0-5 - 0.8 cm (7 day old) served as explants. All leaves except a pair of leaf primordia were removed from the explants under a dissection microscope in a laminar flow cabinet. Additional tissue was removed to expose the base of the shoot tip (apical meristem). Shoot tips were transferred immediately to test tubes containing 15-50 ml of nutrient agar medium and plugged with non-absorbent cotton.

The nutrient medium consisted MS (Murashige and Skoog, 1962) salts, B_5 vitamins (Gamborg *et al.*, 1968), 3% (w/v) of sucrose and 0.8% (w/v) Difco Bacto Agar. 6-benzyl aminopurine (BAP), α-naphthalene acetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) were added in various combinations and concentrations. The pH of the medium was adjusted to 5.8 with 0.1*N* NaOH or HC1 prior to adding the agar. The medium was autoclaved at 1.46 kg cm^{-2} for 15 min. All cultures were maintained at a constant temperature of 25 ± 2° C, 16/8 hour light/dark

conditions. The light intensity of 2000 lux was provided by Phillips cool white fluorescent lamps. At least 15-20 shoot tips were cultured in each experiment and all experiments were repeated thrice. Plantlets with well developed roots were removed from the culture tubes and after washing their roots in running tap water, were transferred to pots containing red soil and sand in the ratio of 1:1. Each plant was covered by a polythene bag to ensure high humidity during the first week after transfer. Subsequently, the plants were transferred to field conditions.

RESULTS AND DISCUSSION

Isolated shoot tips cultured on MS medium containing different concentrations of BAP (1-5 mg l^{-1}) in combination with IAA or NAA (0.5 - 2.5 mg l^{-1}) showed varying frequency of callus induction at the cut end of explants within 2 weeks of culture. The effect of MS medium containing BAP in combination with NAA on callus induction is summarised in Table 1. The initial callus tissue formed was soft and greenish. The compact callus developed within soft and callus masses one week later. The compact callus tissues were increased considerably following 2 weeks incubation of the cultures on callusing medium. Callus induction was found to occur in all combinations and the callusing percentage varied depending upon the auxin and cytokinin concentrations and combinations. The highest frequency of callus initiation was 79.7% in BAP (3 mg l^{-1}) and NAA (1.5 mg l^{-1}) combination whereas it was 65.3% in BAP (3 mg l^{-1}) and IAA (1.5 mg l^{-1}) combination. Among the various concentrations of auxin used for callus induction, 1.5 mg l^{-1} was found to be the optimum concentration for maximum frequency of callus initiation while increasing the auxin concentration above this level reduced the callusing frequency (Table 1). NAA was, in general, more effective for callus induction than IAA and induced as much as 79.7% callus formation at a concentration of 1.5 mg l^{-1} NAA in combination with 3 mg l^{-1} BAP. In all the previous studies dealing with shoot apical meristem cultures of grain legumes, an auxin and a cytokinin were used in combination for callus induction and differentiation (Kartha *et al.*, 1981; Rubluo and Kartha, 1985; Gulati and Jaiwal, 1992; Venkatachalam *et al*, 1994).

The two to three week old callis were transferred to shooting medium for shoot bud differentiation. Green spots appeared on the compact callus tissues 6 to 7 days later. Shoots were formed on the green spot-forming calli 2 weeks later. Shoot bud regeneration was observed within 3 weeks. In order to increase the frequency of shoot bud regeneration, different concentrations and combinations of BAP (1.5 mg l^{-1}) together with IAA or NAA (0.5-2.5 mg l^{-1}) were used. The results are depicted in Table 1. Of the two combinations, NAA and BAP combination was more efficient for shoot bud regeneration. Among the combination of BAP and IAA tried, the combination of BAP (4 mg l^{-1}) and IAA (2 mg l^{-1}) was found to be superior for shoot differentiation. However, the best result for shoot bud differentiation was obtained by supplementing NAA at 1.5 mg l^{-1} and BAP 3 mg l^{-1} (88.3%), whereas it was 64.3% in BAP and IAA combination. The combination of BAP (5 mg l^{-1}) and NAA (2.5 mg l^{-1}) induced more number of shoots (11.5 shoots in callus) than the BAP and IAA combination (Table 1). These findings are similar to those reported for other grain legume meristems (Kartha *el al.*, 1981; Venkatachalam *et al.*, 1994). Multiple shoots were also induced in some cultures (BAP at 5 mg l^{-1}). Both the auxins, in combination with BAP, induced multiple shoots. Similar results were also obtained in various grain legumes (Rubluo and Kartha, 1985; Gulati and Jaiwal, 1992; Kartha *et al.*, 1981; Rao and Chopra, 1989; Venkatachalam *et al.*, 1994).

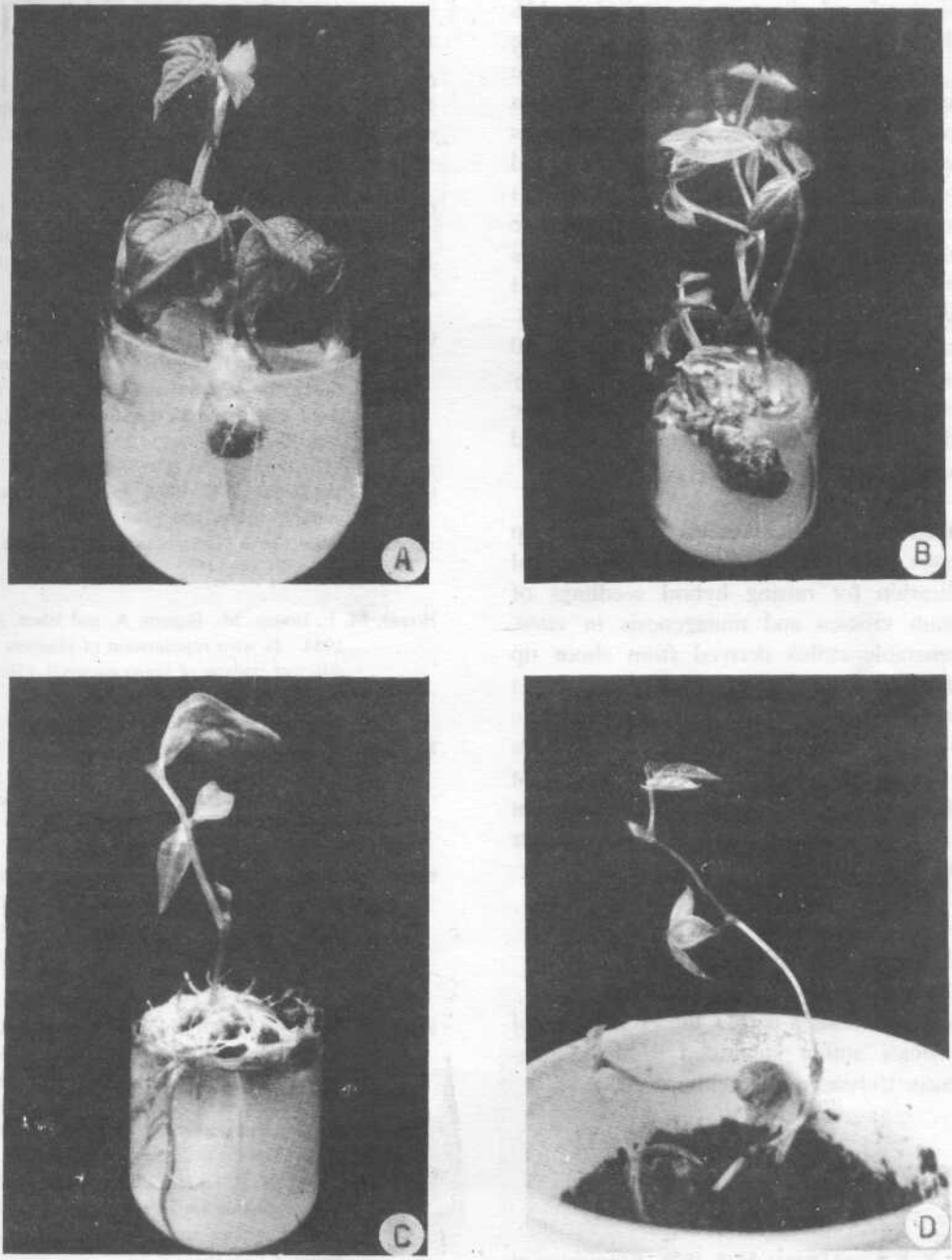

Fig 1. Plant regeneration from shoot tip culture of blackgram: (A). Plant regeneration from the shoot tip callus on MS medium containing NAA (1.5 mg l^{-1}) and BAP (3.0 mg l^{-1}); (B). Multiple shoots developing from the shoot tip callus; (C). Roots developed on MS medium containing 3.0 mg l^{-1} IBA; (D). *In vitro* raised potted plant of blackgram

Well developed shoots were rooted in MS medium containing 1BA (0.5-5 mg l^{-1}) (Table 2). Roots emerged from the cut end of the shoots within 15 days. The maximum frequency of root induction (78.3%) was observed in presence of 3 mg l^{-1} 1BA and more number of roots (14.5 roots per shoot) were induced with this **combination.** These results are in consonance with the previous reports by Moss *et al.* (1988), Gulati and Jaiwal (1992); Venkatachalam *el aI.* (1994). Plant regeneration was achieved within 50-60 days. The rooted plantlets were subsequently transferred to pots and later established in the field, where 90-95% of them survived and resumed growth (Fig 1).

Formation of multiple shoots from shoot tip cultures of *Vigna mungo* could be of practical application for raising hybrid seedlings of difficult crosses and mutagenesis *in vitro.* Regenerable callus derived from shoot tip explants has been shown to be a suitable target tissue for the transformation experiments. The present shoot tip based regeneration methods in blackgram can be applied to plant transformation, either by particle bombardment or *Agrobacterium* mediated gene transfer technology.

ACKNOWLEDGEMENT

This paper forms a part of the Ph.D thesis of the senior author submitted to the Bharathidasan University, Tiruchirappalli, 1995.

REFERENCES

Bajaj, Y. P. S. and Dhanju, M. S. 1979. Regeneration of plants from apical meristem tips of some legumes. *Curr. Sci.* 48 : 906-907

Franklin, C. I., Triew, T. N., Cassidy, B. G., Dixon, R. A. and Nelson, R. S. 1993. Genetic transformation of greenbean callus via. *Agrobacterium* mediated DNA transfer. *Pl. Cell Rep.* 12 : 74-79

Gamborg, O. L., Miller, R. A. and Ojima, K. 1968. Nutrient requirements for suspension cultures of soybean root cells. *Exp. Cell Res.* 50 : 151-158

Goel, S., Mudgal, A. K. and Gupta, S. C. 1983. Development of plants from *in vitro* cultured shoot tips of *Vigna mungo* and *V. radiata. Trop. Pl. Sci. Res.* 1 : 31-33

Gould, J., Banister, S., Hasegawa, O., Fathima, M. and Smith, R. H. 1991. Regeneration of *Gossypium hirsutum* and *G. barbadense* from shoot apex tissue for transformation. *Pl. Cell Rep.* 10 : 12-16

Gulati, A. and Jaiwal, P. K. 1992. *In vitro* induction of multiple shoots and plant regeneration from shoot tips of mungbean *(Vigna radiata* (L.) Wilczek).

Hoque, M. I., Hoque, M., Begum, A. and Islam, A. S. 1984. *In vitro* regeneration of plantlets from different explants of *Vigna mungo* (L.) Hepper. *Bangladesh J. Bot.* 13 : 45-51

Kartha, K. K., Leung, N. L. and Mroginaki, L. A. 1981. Plant regeneration from meristems of grain legumes; soybean, cowpea, peanut, chickpea and bean. *Can J. Rot.* 59 : 1671-1679

Kartha, K. K. 1981. Gene pool conservation through tissue culture. *Proc. Int. Sym. on Tissue Culture of Economically Important Plants.* (Ed. Rao, A. N.), National University, Singapore, p. 213-218

Lulsdorf, M. M., Rempel, H., Jackson, J. A., Baliski, D. S. and Hobbs, S. L. A. 1991. Optimizing the production of transformed pea (*Pisum sativum* L.) callus using disarmed, *Agrobacterium tumerfaciens* strains. *Pl. Cell Rep.* 9 : 479483

Moss, J. P., Dutt, N. R. G. and Lingamaneni, A. 1988. Root induction on *in vitro* grown shoots of *Arachis* species and a hybrid. *Int. Arachis Newsl.* 4 : 25-26

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15 : 473-497

Rao, B. G. and Chopra, V. L. 1989. Regeneration from

apical meristem, stem nodes and cotyledons of chickpea. *Indian J. Pulses Res. 2* : 20-24

Rubluo, A. and Kartha, K. K. 1985. *In vitro* culture of shoot apical nieristem of various *Phaseolus* species and cultivars. *J. Pl. Physiol.* 119 : 425-433

Vetlkatachalarn, P., Pillai, A. S. and Jayahalan, N. 1994. Plant regeneration from cultured apical meristems of groundnut *(Arachis hypogea* L.). *Proc. Nat. Acad. Sci.* 64 : 99-103