CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2014 Vol. 3 (4) October-December, pp.11-15/Himabindu et al.
Research Article

IN VITRO REGENERATION OF GREEN GRAM [*VIGNA RADIATA* (L.) WILCZEK] CULTIVAR VAMBAN-2 USING COTYLEDONARY NODES

Himabindu Y.[1,2], Madhava C Reddy[2] and *Chandrasekhar T.[1]
[1]*Department of Environmental Science, Yogi Vemana University, Kadapa-516003, Andhra Pradesh, India*
[2]*Department of Biotechnology & Bioinformatics, Yogi Vemana University, Kadapa-516003, Andhra Pradesh, India*
**Author for Correspondence*

ABSTRACT
Efficient protocols have been developed to induce shoot multiplication from cotyledonary node cultures of green gram [*Vigna radiata* (L.) Wilczek] cultivar Vamban-2. Cotyledonary nodal segments cultured on Gamborg's medium (B5) containing 0.5 mg/l 6-benzyladenine (BA) induced on an average of nine multiple shoots. The effect of agar on shoot multiplication was studied and we did not found much difference between the treatments. Regenerated shoots showed 65 % rooting on full strength B5 basal medium without any plant growth regulators. *In vitro* regenerated plantlets were successfully acclimatized to greenhouse conditions.

Keywords: *Green Gram, Vamban, Agar, Cotyledonary Node, B5 Medium*

INTRODUCTION

Green gram or mung bean [*Vigna radiata*(L.) Wilczek] is one of the most important pulse crops in India and cultivated in different parts of the world. Protein rich edible seeds, sprouts rich in vitamins and amino acids are used directly and apart from this the crop is widely used as forage (Kaviraj *et al.,* 2006). However, the productivity and quality of the grain is severely reduced due to different stress factors in many parts of the country. Despite its great economic importance little information regarding its degree of stress tolerance is available through conventional studies, although yield losses are considerable when subjected to different stress conditions. But using genetic engineering methods we can achieve any kind of stress tolerance by producing transgenic green gram plants using different resistant genes (Yadav *et al.,* 2012). But before that the establishment of *in vitro* regeneration protocol is prerequisite for genetic transformation methods.

Micropropagation is an alternative to the conventional method of vegetative propagation with the objective of enhancing the rate of multiplication. Most of the green gram *in vitro* micropropagation focuses on different explants including cotyledonary node multiplication, which is ideal for high clonal fidelity and efficiency (Khatun *et al.,* 2008). Protocols have been established for multiplication from different green gram cultivars using cotyledonary node explants (Gulati *et al.,* 1994; Janaki and Manoharan, 2012; Vats *et al.,* 2014). However existing regeneration protocols for green gram are not repeatable across different species, i.e. are to a significant extent species-specific.

There are a limited number of laboratories running mass production of green gram at commercial scale due to technical and commercial reasons.Keeping in view the importance of clonal propagation through cotyledonary node multiplication and lack of information on this protocol with Vamban-2 species, we are interested in establishing a highly reproducible protocol for large-scale micropropagation of this cultivar.

MATERIALS AND METHODS

Healthy and uniform seeds of mung bean cultivar Vamban-2 were rinsed with distilled water for 5-6 times and then with 70% ethanol for 1 min. The seeds were then surface sterilized with 0.1% HgCl2 for 8-10 min followed by four to five times repeated washings with sterile distilled water. The surface sterilized seeds were asceptically germinated on water agar medium. Cotyledonary node segments from 2-3 day old seedlings were cultured on shoot multiplication medium.

The basal medium used in the present study consisted of the salts and vitamins of B5 medium at full strength for cotyledonary node multiplication with different plant growth regulators (PGR) such as BA, kinetin (Kn), α–naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) and the concentration starting with 0.2 mg/l to 4.0 mg/l in alone and combinations. For rooting experiments we used B5 basal medium without plant growth regulators. Unless otherwise specified, all media were fortified with 3% sucrose and here we used two concentrations of agar i.e. 0.6% to 0.8%. The pH of the medium was adjusted to 5.7-5.8 prior to being autoclaved at 121°C for 15 min. After the completion of autoclaving, they were removed and autoclaved medium was dispensed into 25x150 mm glass tubes (15 ml) and the tubes were placed in a slight slanting position to get more surface area. Before inoculation, the laminar air flow chamber was smeared with ethanol and all the requirements for inoculation were transferred inside the chamber. Sterilization of the chamber was done by switching on the ultraviolet lamp for half an hour before inoculation. Inoculation was carried out near the spirit lamp. Hands were frequently cleaned with ethanol from time to time to minimize contamination. All the inoculated cultures were incubated in a culture room at 25 ± 2°C with a relative humidity of 50-60% and around 16 h photo period.

In order to achieve acclimatization, rooted plantlets were removed from the rooting medium and washed with sterile distilled water. Plantlets were transferred to polythene bags containing vermiculite, covered with polythene bags for high humidity and kept under the culture room conditions for 15 days. Plants with newly formed leaves were then transferred to the greenhouse. Visual observations of the cultures were done and a minimum of ten replicates were involved in each experiment and conducted thrice. The mean and excel programming techniques performed using personal computer for different parameters.

RESULTS AND DISCUSSION

At the beginning, 10-20 % cultures showed initial contamination with cotyledonary nodal segments even though explants initiated from *in vitro* culture. Initially we started with MS medium and we did not observed much encouragement results with cotyledonary nodes and then we proceed with B5 medium. Similar favorable results were observed with B5 medium as per Gulati *et al.,* (1994) but they obtained less number of multiple shoots with cotyledonary nodes and also they worked in different green gram cultivar. In contrast Janaki and Manoharan (2012) and Vats *et al.,* (2014) observed best results in MS medium and this is also may be the reason that they used different cultivar.

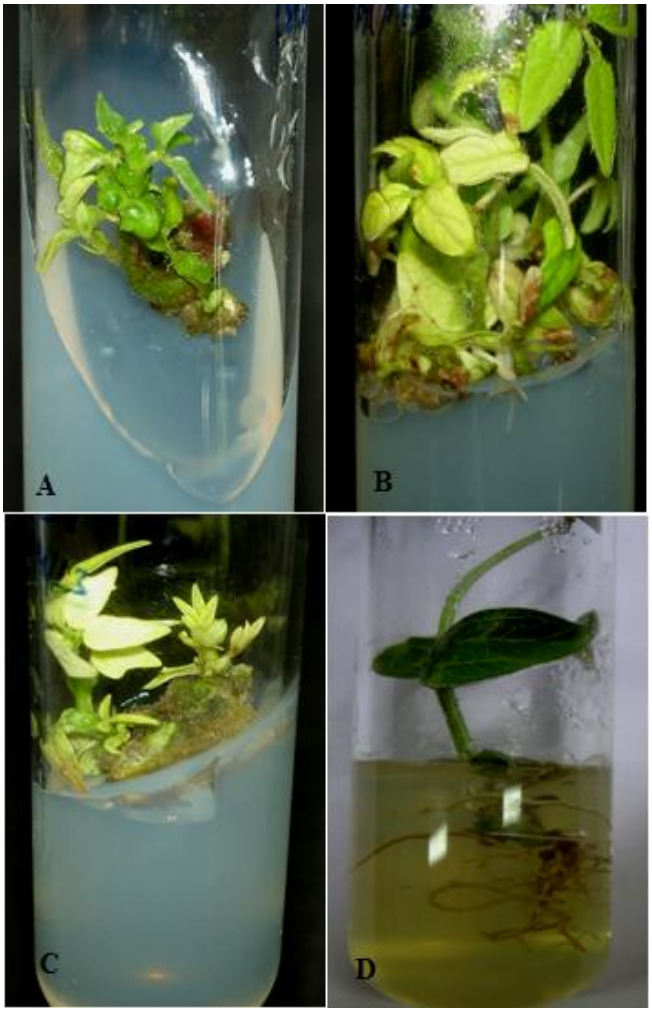
The present study indicates that multiple shoots can be induced from cotyledonary node explants on B5 medium containing BA or Kn alone and in combination with NAA or 2, 4-D. Between the two cytokinins BA and Kn used, BA at most of the low concentration levels tested, induced multiple shoot development within 2 weeks (Table.1). B5 along with 0.5 mg/l BA proved on an average nine multiple shoots per culture (Figure1A&B). BA along with NAA or 2,4-D exhibited more than two shoots sometimes but callus interfere with these cultures. The promotive effect of BA for inducing multiple shoots has been previously reported in mung bean (Gulati *et al.*, 1994; Vats *et al.*, 2014). However 2-iP also proved best in multiple shoot induction in green gram as per Gulati *et al.,* (1994). B5 medium along with 2.0 mg/l Kn also exhibited four shoots on an average (Figure 1C).

Both 0.6% and 0.8% agar concentrations did not showed much difference in multiple shoot induction except plants in 0.6% agar responded quickly and the problem with 0.6% agar is that the explants cannot be positioned stably because of bit semi solid condition. Both the conditions proved minute differences with the case of percentage of cultures showing response and shoot multiplication (data not shown). Multiple shoot production increased and was proliferated further by repeated subculturing of cotyledonary nodes at every 4-week intervals. Sixty five percentage of rooting was achieved in B5 medium without any plant growth regulators (Figure1D). This is in contrast with results of Khatun *et al.,* (2008) and Janaki and Manoharan (2012) because they got rooting in MS or half strength MS medium along with NAA or IBA. The different results observed may be due to the response of different species to *in vitro* conditions, specifically to growth regulators. The interaction between species and growth medium has been reported in many plant species.

Table 1: Production of multiple shoots on Gamborg'sB5 media along with different plant growth regulators using cotyledonary nodes

Plant growth regulators (mg/l)				Per cent of cultures showing response	Number of multiple shoots
BA	**Kn**	**NAA**	**2,4-D**		
0.5				70.0	9
1.0				65.0	4
2.0				55.0	2
4.0				60.0	2
0.5		0.2		60.0	4 (with callus)
1.0		0.2		60.0	2 (with callus)
2.0		0.2		50.0	2 (with callus)
4.0		0.2		50.0	2 (with callus)
0.5			0.2	65.0	3 (with callus)
1.0			0.2	60.0	3 (with callus)
2.0			0.2	65.0	2 (with callus)
4.0			0.2	50.0	2 (with callus)
	0.5			60.0	2
	1.0			50.0	2
	2.0			60.0	4
	4.0			55.0	3
	0.5	0.2		55.0	2 (with callus)
	1.0	0.2		50.0	2 (with callus)
	2.0	0.2		55.0	3 (with callus)
	4.0	0.2		50.0	2 (with callus)
	0.5		0.2	65.0	2 (with callus)
	1.0		0.2	60.0	2 (with callus)
	2.0		0.2	50.0	2 (with callus)
	4.0		0.2	55.0	2 (with callus)

Mean of 10 replicates conducted thrice. Observation after 4-6 weeks



A & B: Multiple shoot initiation from the cotyledonary nodal portion on B5 medium along with 0.5mg/l BA (A-two weeks and B-6 weeks of observation)
C: Multiple shoot induction from cotyledonary node on B5 medium along with 2.0 mg/l Kn
D: Rooting from regenerated shoots on B5 medium without plant growth regulators
Figure 1: Multiple shoot production from cotyledonary nodes of green gram on B5 medium using different plant growth regulators

To acclimatize these *in vitro* developed plantlets, they were first washed with sterile distilled water and plants were removed from tubes and transferred to plastic bags containing autoclaved vermiculite. Plants were covered with polyethylene bags to maintain humidity and watered with autoclaved tap water and after one month they were transferred to green house.

ACKNOLEDGEMENT
The authors are thankful to the Department of Science and Technology (DST) funding in the form of INSPIRE fellowship to YHB and partial support from the Council of Scientific and Industrial Research (CSIR), Government of India, for this research work

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