Efficient *in vitro* regeneration of *Vigna radiata* (L.) Wilczek

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Abstract: Efficient *in vitro* plant regeneration protocol was developed using nodal and cotyledonary node explant of *Vigna radiata* (L.) Wilczek. Maximum response in terms of shoot regeneration was observed in MS medium supplemented with BAP (0.5 ppm) and NAA (0.25ppm). The regenerated shoots were cultures on rooting medium supplemented with different doses of IBA. The best response was observed in 3 ppm IBA. *In vitro* regenerated plantlets with well developed roots were successfully established in soil.
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Key Words: *Vigna radiata;* NAA; regenerated shoots; IBA; Plantlets

1. Introduction

Vigna radiata (L.) Wilczek (Fabaceae), commonly called as mungbean is an important grain legume crop (Betal and Raychaudhuri ,1999). It is widely cultivated pulse crop and used as major source of dietary protein all over the world. Several factors, biotic and abiotic, together with low genetic variability due to self pollination are supposed to be responsible for lowering the production of this important crop using conventional methods of breeding. Biotechnological manipulations could help breeders to overcome these problems at the cellular and molecular level. However, recalcitrant nature of leguminous tissues under *in vitro* conditions impedes crop improvement.

Attempts have been made to establish *in vitro* regeneration protocol for *V. radiata* using different explants (Goel et al. 1983; Gulati and Jaiwal, 1990; Mendoza et al. 1992; Chandra and Pal, 1995; Amutha et al. 2003; Rao et al. 2005). However, low multiple shoot induction and percentage response have been reported in many cases. There has always been a need for an efficient *in vitro* plant regeneration system for successful crop improvement programs through genetic engineering. Viewing the immense importance of this nutritive pulse crop the present study was done to develop reproducible *in vitro* plant regeneration system.

Material and methods

Surface sterilization

The seeds of *V. radiata* were washed in running tap water and surface sterilized with mercuric chloride (0.1%, w/v) containing laboline (0.1%, v/v) for 8 min and finally rinsed five times with of sterile water. The seeds were inoculated for germination on basal MS medium. Cultures were kept in a 16h photo period (80µmol m^{-2} sec^{-1}) at 25±2 ºC with 60% humidity.

Shoot multiplication

Nodal (1-2 cm) and cotyledonary nodes were used as explants from the germinated seedlings. The explants were cultures on Basal MS medium supplemented with various concentrations of BAP and NAA, either alone or in combinations. The cultures were observed and examined every day and final data were recorded. The shoot response of different explants was investigated for effective and reproducible multiplication. The pH of the media was adjusted to 5.8 with 0.1 N NaOH before autoclaving at 15psi and 121°C for 20 min. All culture media contained 3% sucrose (w/v) and solidified with 0.8% agar (Himedia, India). Explants were placed vertically in 150ml Erlenmeyer flasks containing 25 ml of culture medium and plugged tightly with non absorbent cotton.

Rooting and transfer of plantlets to soil

Regenerated Shoots regenerated from different explants were excised and transferred to ½ MS medium and full MS medium fortified with various concentrations IBA. After incubating in rooting medium the rooted microshoots were removed from the culture medium and washed in sterile distilled water to remove all traces of agar. Then the plantlets were transferred to plastic pots containing sterile vermiculite, solarite, and sterile soil (1:1:1). A glass beaker was inverted over plant to ensure high humidity during the first few days and kept in a culture room at 25±2◦C temperature and 16h day illumination with cool fluorescent light (40µmol $m^{-2}s^{-1}$) for 2-3 weeks. The plantlets were sprinkled with water every alternate day. Finally the plants were individually transferred to pots containing soil, sand and farmyard manure (1:1:1) and were reared in the greenhouse.

Results and Discussion

One of the most important applications of plant tissue culture is micropropagation. It is now

established that different concentrations of hormones in media results in differential response of explants (Vats and Kamal, 2013a; Vats and Kamal, 2013b; Vats and Kamal, 2013c; Vats et al., 2012; Vats, 2012). Seeds of *V. radiata* were inoculated on basal MS media. The germination took place after 2-3 days. Cotyledonary node and nodal explants was studied for their organogenetic potential. MS medium supplemented with various concentrations of BAP and NAA, alone and in combinations was used for shoot generation (Table-1and2). BAP alone with dose range 0.5-5ppm gave 3-4 shoots per explant from both the explants studied (Fig. 1). It was observed that with the increased concentrations of BAP the percentage response decreased significantly. Using BAP (0.5-5ppm) in combination with NAA (0.25) showed differential shoot response. Lower mean shoot response together with low percentage response and shoot length was observed on increasing the concentration of BAP in combination with NAA. In both the explants used the best response was observed in BAP (0.5ppm) and NAA (0.25ppm). The mean number of shoots was observed to be 7.4 with 92.5 percentage response and 3.8 cm in length from cotyledonary node (Fig. 2). In terms of nodal explant the mean 5.3 with 4.8 cm length and 90.6 percentage response (Fig. 3). Basal callusing with each treatment was also observed. Amutha et al. (2003) reported regeneration of shoots from cotyledon derived callus. The best response in terms of shoot induction was observed in media supplemented with 6.6 µM BAP and 2.5 µM TDZ but the shoot length and percentage response was considerably low than the present investigation. Hoque et al. (2007) performed shoot regeneration from cotyledons and the maximum numbers of shoot were 5.5. In yet another study (Rao et al. 2005) the number of shoots observed was less than the present investigation. Number of shoots, shoot length and percentage response are important parameters for efficient plant regeneration methodology. The present study reveals a better shoot regeneration capacity of explants tried using above parameters as compared to other studies. Healthy root induction from the regenerated shoots is an essential part for *in vitro* propagation of plants. The in the present study the regenerated shoots were cultured on half MS medium and IBA (3 and 4 ppm), separately. The response were significantly different ($p<0.05$) from each other (Table-3). The least response was observed in half MS medium with mean number of roots 15.2 and root length 3.2 cm and percentage response 63.2. The best response was observed in MS medium fortified with 3ppm IBA with mean number of roots 55.6, 5.5 cm root length and 94.6 percentage response (Fig. 4). On increasing the concentration of IBA the response deteriorated. The *in vitro* generated plantlets with well developed roots were removed from the cultured flask and transferred in plastic pots containing sterile vermiculite, solarite and sterile soil (1:1:1). A glass beaker was inverted over each plant to ensure high humidity. 85% of plantlets survived and resumed growth after planting in field condition.

Table 1: Effect of different concentrations of BAP and NAA on regeneration of shoots from cotyledonary node of *V. radiata*

BAP (ppm)	NAA (ppm)	Mean No. of shoots	Shoot length (cm)	% response
0.5 ppm	-	3.6 [d]	3.5 cm [a]	80.1 [b]
1.0 ppm	-	3.3 [e]	3.2 cm [a]	75.3 [d]
2.0 ppm	-	3.7 [d]	2.8 cm [b]	65.4 [d]
5.0 ppm	-	4.2 [c]	2.3 cm [b]	68.1 [e]
0.5 ppm	0.25 ppm	7.4 [a]	3.8 cm [c]	92.5 [a]
1.0 ppm	0.25 ppm	4.2 [c]	2.9 cm [bd]	82.6 [b]
2.0 ppm	0.25 ppm	2.8 [f]	1.7 cm [e]	78.2 [c]
5.0 ppm	0.25 ppm	4.8 [b]	1.9 cm [e]	70.3 [f]

[a–f] Mean values represented by the same letters within the same column are not significantly different at $p<0.05$.

Table 2: Effect of different concentrations of BAP and NAA on regeneration of shoots from nodal explant of *V. radiata*

BAP (ppm)	NAA (ppm)	Mean No. of shoots	Shoot length (cm)	% response
0.5	-	2.7 [e]	2.4 [d]	75.2 [d]
1.0	-	3.2 [d]	2.6 [d]	70.3 [e]
2.0	-	3.8 [c]	3.5 [c]	65.1 [f]
0.5	0.25	5.3 [a]	4.8 [a]	90.6 [a]
1.0	0.25	4.2 [b]	3.1 [c]	85.0 [b]
2.0	0.25	2.6 [e]	1.6 [e]	83.2 [b]
5.0	0.25	4.4 [b]	4.0 [b]	79.5 [c]

[a–f] Mean values represented by the same letters within the same column are not significantly different at $p<0.05$.

Table 3: Effect of half MS medium and different concentrations of IBA on regeneration of roots of *V. radiata*

Media	Mean no. of roots	Root length (cm)	% response
½ MS	15.2 [c]	3.2 [b]	63.2 [c]
3ppm IBA	55.6 [a]	5.5 [a]	94.6 [a]
4 ppm IBA	25.6 [b]	4.1 [c]	86.2 [b]

[a–c] Mean values ± standard deviation represented by the same letters within the same column are not significantly different at $p<0.05$.

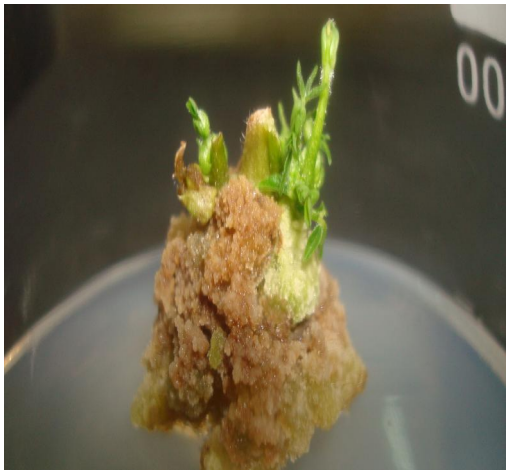

Fig 1: Multiple shoots from cotyledonary node on 5ppm BAP

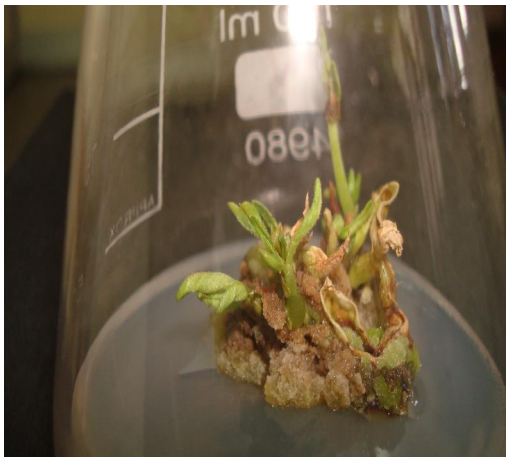

Fig 2: Multiple shoots from cotyledonary node explant on BAP (0.5 ppm) + NAA (0.25 ppm)

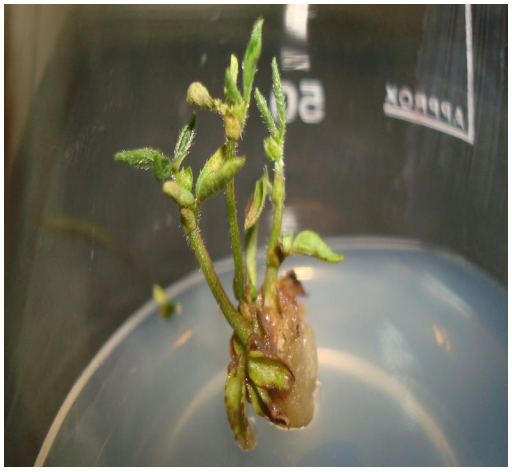

Fig 3: Multiple shoots and basal callusing nodal explant on BAP (0.5 ppm) + NAA (0.25 ppm)

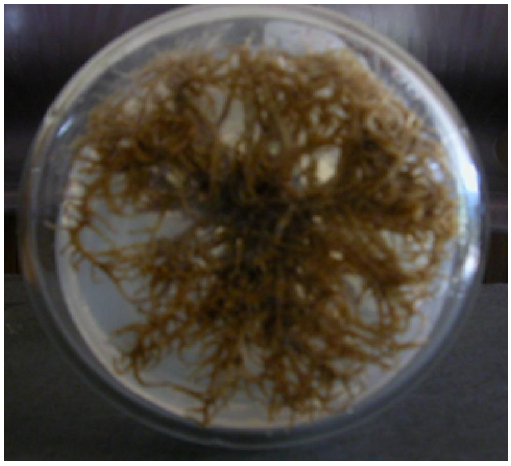

Fig 4: Rooting from regenerated shoot on IBA (3 ppm)

Conclusion

The present investigation gives the establishment of a reliable *in vitro* regeneration protocol for *V. radiata*. Various experiments related to genetic engineering to transfer gene of interest to *V. radiata* to confer disease resistance, stress resistance, improve protein content and quality and others can be taken up using this regeneration protocol for reproducible and better results.

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