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# *In vitro* Plant Regeneration in Mungbean (*Vigna radiata* (L.) Wilczek)

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## Abstract

An efficient regeneration protocol without the intervention of callus using cotyledon explants of three mungbean varieties namely, BARI mug-3, BARI mug-5 and BINA mug-5 was developed. Best response toward multiple shoot regeneration in BARI mug-5 and BINA mug-5 was observed on MS supplemented with 0.5 and 0.5 mg/l Kn. However, for BARI mug-3, MS supplemented with 0.5 mg/l BAP and 0.1 mg/l Kn was found to be most effective for multiple shoot regeneration. Half-strength of MS supplemented with 0.5 mg/l IBA was found to induce healthy roots from the excised shoots in all the three mungbean varieties. *In vitro* regenerated plantlets with well developed roots were successfully established in soil.

## Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is an important food grain legume crop all over the world. This crop is regarded as a quality pulse in Bangladesh for its excellent protein quality (20 - 28.4%), high digestibility and freedom from flatulent effects associated with other pulses e.g., chickpea and lentil. The demand for this crop has been steadily increasing in the Indian subcontinent. However, this crop is characterized by low yield potential. Several biotic and abiotic factors as well as low genetic variability are supposed to be responsible for lowering the production of this important crop. In some growing seasons losses exceed more than 50% due to incidence of many pests and diseases (Poehlman 1991 and Bose 1991).

There is a need to increase productivity which enhances the nutritional value and other essential agronomic qualities of this crop. In any yield improvement program a broad based gene pool should be created by different methods. In the past several attempts have been made to develop disease resistant as well as high yielding varieties of mungbean through interspecific hybridization. However, due to interspecific cross-incompatibility and hybrid sterility it has not been

possible to develop such improved mungbean varieties. Thus low genetic variability of mungbean caused by high degree of self-pollination has imposed limitation for its improvement using conventional methods of breeding.

In recent years genetic engineering has been effectively used to develop desirable breeding lines of many important crop plants (Fisk and Dandekar 1993, James 2004, Wambugu 1999). A reproducible and reliable transformation system enables us to insert genes which are of interest in mungbean lines, are not available in the existing genotypes. Thus, it may be possible that, genetic transformation combined with traditional breeding may prove helpful in improving both the quality and yield of mungbean.

Efficient *in vitro* plant regeneration system is required for successful crop improvement programs through genetic engineering. In case of grain legumes, the crop improvement is mostly hampered due to the recalcitrant nature of leguminous tissues under *in vitro* condition. Several attempts have been made to establish *in vitro* regeneration protocol for mungbean. There are some reports on the *in vitro* plant regeneration in mungbean using different explants (Amutha et al. 2003, Mendoza et al. 1992, Singh et al. 1980, Goel et al. 1983, Avenido and Desiree 1990, Mathews 1987, Gulati and Jaiwal 1990, 1994, Chandra and Pal 1995). However, the *in vitro* regeneration protocols developed by the these workers did not produce desired results using mungbean varieties from Bangladesh. Considering the above mentioned background in the present investigation attempts were made to establish reproducible *in vitro* plant regeneration system in different mungbean varieties of Bangladesh.

## Materials and Methods

Three varieties of mungbean (*Vigna radiata* (L.) Wilczek) namely, BARI mug-3, BARI mug-5 and BINA mug-5 were used in the present investigation. BARI mug-3, BARI mug-5 were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur and BINA mug-5 was collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

Explants namely, cotyledonary nodes, cotyledons and decapitated mature embryos were used for shoot regeneration. Cotyledonary nodes were collected from three-four-day-old aseptically grown seedlings. Mature embryos were separated from overnight soaked sterilized seeds. Sterilized distilled water and media containing 3% sucrose with 0.8% agar were used for *in vitro* seed germination. After removing the seed coats the seeds were split open and the two cotyledons were separated. The shoot- and root meristems from these embryos were decapitated. In another set of experiments cotyledon explants were excised from the sterilized seeds. Two cotyledons were separated. Embryos

were found to remain with one part of the cotyledon. The embryos from the cotyledons were removed before inoculation.

For shoot initiation and development, MS supplemented with various combinations and concentrations of BAP Kn, IAA, $GA_3$ and additives such as tyrosine and coconut milk (CM) were used.

MS or half-strength MS supplemented with different concentrations and combinations of IBA and IAA were used for root development. About 2.0 - 4.0 cm long shoots were separated and cultured on freshly prepared medium containing different combinations and concentrations of IBA and IAA.

All cultures were maintained under fluorescent illumination with 16/8 h light/dark cycle (except co-culture experiments) at 25 ± 2˚C. The intensity of light was maintained at 1500 lux.

## Results and Discussion

Not much work has been done on the application of tissue culture in the improvement of mungbean. Plant regeneration among *Vigna* species is limited compared to those of other grain legumes. Shoot regeneration *via* callus formation was tried by some previous workers using nutrient media containing various concentrations and combinations of hormones. However, their attempts to regenerate shoots from the induced callus were not successful (Bhadra et al. 1989, Avenido and Desiree 1990, Bose et al. 1992, Sarker and Siddiqua 2004). Mathews and Rao (1984) and Mathews (1987) also failed to regenerate shoots from established callus cultures of mungbean.

There are a few reports of successful *in vitro* regeneration of mungbean through indirect organogenesis from cotyledon- and hypocotyl explants (Amutha et al. 2003), immature leaflet derived calli (Mendoza et al. 1992). But in most of the cases, these protocols were irreproducible, less efficient and very few of them are applicable in genetic transformation.

The callus phase and its duration are negatively correlated with the regeneration ability of explants. Moreover, somaclonal variation can influence the phenotype of regenerated plants in case of indirect organogenesis (Fontana et al. 1993). Therefore, emphasis was given on developing a direct regeneration protocol for mungbean using cotyledons, decapitated embryos and cotyledonary nodes.

In all the three varieties of mungbean, cotyledon showed better response than other explants toward regeneration. The number of shoots per explant was higher in all the three varieties than the remaining explants. Next to cotyledon, cotyledonary nodes showed better response toward *in vitro* shoot regeneration.

In the present study, different concentrations of BAP, Kn, IAA, $GA_3$, TDZ were used singly or in combinations in MS to observe their effect on initiation and development of shoots. MS supplemented with different concentrations of BAP (0.1 - 2.0 mg/l) was tried for shoot regeneration from cotyledon explants. It was observed that BAP alone in MS medium did not show optimum response toward shoot regeneration. Increased BAP concentration produced a small number of shoots, indicating the decreased regeneration efficiency.

When cotyledons were cultured on MS supplemented with BAP (0.1 - 1.0 mg/l) and IAA (0.5 - 1.0 mg/l) a considerable number of explants responded to shoot regeneration. However, the number of shoots per explants was low. A maximum of two - three shoots per explant was observed. The response of decapitated embryos toward shoot regeneration was studied on the same medium. In this case mostly single shoots developed and the regenerated shoots were thin and pale.

The influence of BAP (0.5 mg/l) and Kn (0.25 mg/l) or Kn alone was studied in the MS medium to determine their effect on shoot regeneration. In this case mostly single shoots were observed. In another set of experiments, only 0.5 mg/l BAP was used in combination with Kn (0.25 - 0.5 mg/l), $GA_3$ (0.1 mg/l) and tyrosine (0.0 - 5.5 mg/l) in MS medium for shoot regeneration and their development from decapitated embryo explants. In this medium mostly single shoots were observed in all the three varieties of mungbean. Sarker et al. (2003) used similar type of hormonal combinations in shoot regeneration medium and reported successful regeneration in lentil.

The combined effect of BAP and Kn in MS medium was tested to examine their effect in regenerating multiple shoots directly from cotyledon. Results of this experiment have been presented in Table 1. In case of BARI mug-3 maximum multiple shoot regeneration was obtained when cotyledon explants were cultured on MS containing 0.5 mg/l BAP and 0.1 mg/l Kn. However, MS supplemented with 0.5 mg/l BAP and 0.5 mg/l Kn was found the best in case of BARI mug-5 and BINA mug-5. Maximum mean number of shoots/explant was 4.8 in case of BARI mug-3 (Fig. 1). On the other hand, in case of BARI mug-5 and BINA mug-5 the maximum mean number of shoots/explant was 4.5 and 5.5, respectively (Fig. 2). Healthy green shoots with expanded leaves were obtained from cotyledonary explants in all the varieties. During this study, the length of the regenerated shoots was found to increase following subculturing on the same medium composition (Fig. 3).

Multiple shoots were found to develop from cotyledonary nodal explants on MS supplemented with BAP and CM. The maximum mean number of shoots/explant was 5 in observed BARI mug-3 whereas 4.5 and 3.5 were in BARI mug-5 and BINA mug-5, respectively on MS containing 2.0 mg/l BAP and 10%

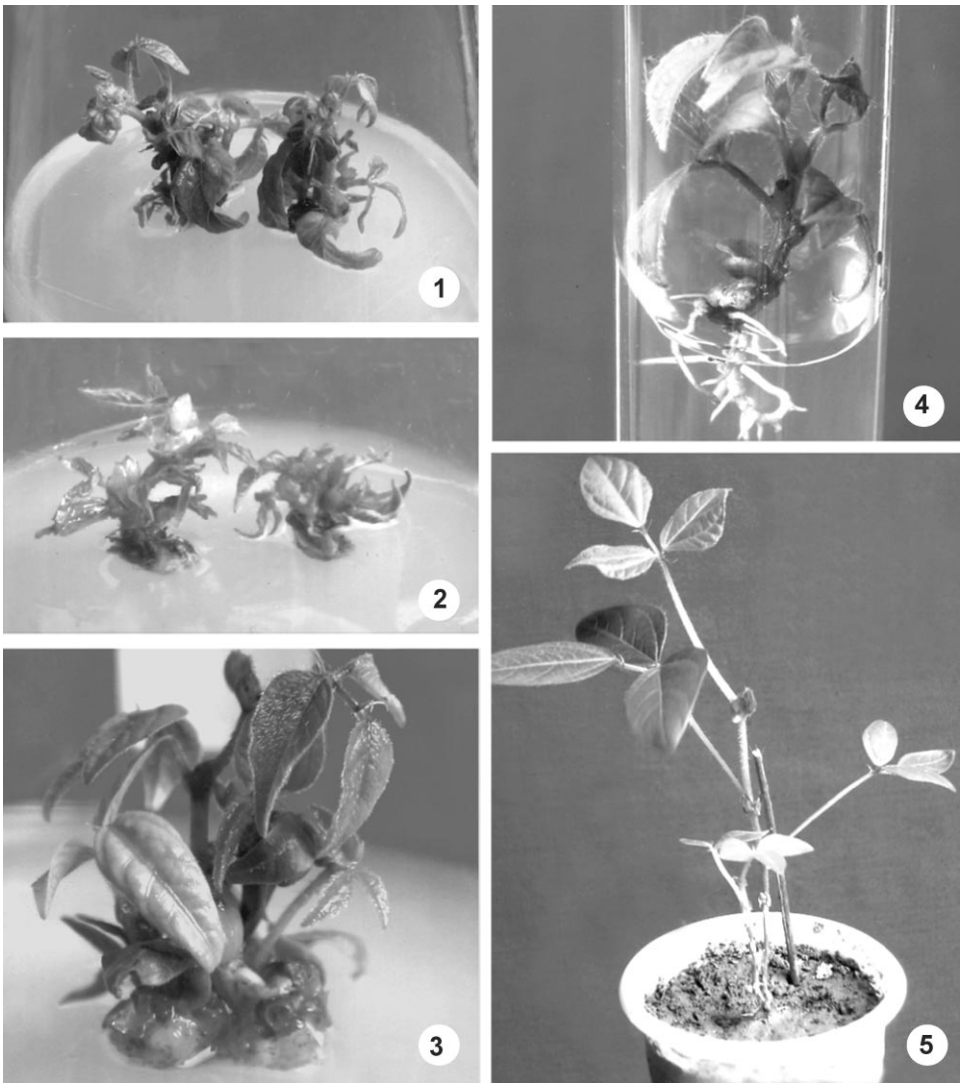
CM. Similar results were also reported by Sarker and Siddiqua (2004) in BARI mug-2, BARI mug-3, MK-72 and NM-92. However, the elongation of regenerated shoots was not so satisfactory on the medium containing 2.0 mg/l BAP and 10% CM.

Induction of healthy roots from the regenerated shoots is an essential part for stable development of plantlets and as such regenerated shoots were cultured on full as well as half the strength of MS supplemented with IAA and IBA for root induction. It was observed that half-strength of MS with 0.5 mg/l IBA was effective for root induction, more so for its subsequent development in BARI

**Table 1. Effect of different concentrations of BAP and Kn on regeneration of shoots from cotyledon of three varieties of mungbean.**

| Conc. of BAP (mg/l) | Conc. of Kn (mg/l) | No. of explants inoculated | % of responsive explants | Days to shoot initiation | Mean No. of shoots/ explant | Average length of shoots after eight weeks (cm) |
|---|---|---|---|---|---|---|
| **BARI mug-3** | | | | | | |
| 0.1 | 0.1 | 60 | 71 | 8 - 10 | 1.8 | 4.2 |
| 0.3 | 0.1 | 60 | 65 | 8 - 14 | 2.1 | 5.0 |
| 0.5 | 0.1 | 60 | 88 | 9 - 12 | 4.8 | 5.5 |
| 0.5 | 0.3 | 60 | 77 | 8 - 11 | 3.1 | 4.8 |
| 0.5 | 0.5 | 60 | 75 | 9 - 10 | 2.5 | 4.2 |
| 1.0 | 1.0 | 60 | 68 | 12 - 14 | 2.2 | 3.4 |
| **BARI mug-5** | | | | | | |
| 0.1 | 0.1 | 60 | 64 | 8 - 12 | 2.2 | 3.9 |
| 0.3 | 0.1 | 60 | 56 | 8 - 10 | 1.9 | 4.0 |
| 0.5 | 0.1 | 60 | 72 | 8 - 10 | 3.0 | 4.6 |
| 0.5 | 0.3 | 60 | 70 | 9 - 12 | 2.1 | 5.0 |
| 0.5 | 0.5 | 60 | 85 | 11 - 12 | 4.5 | 5.2 |
| 1.0 | 1.0 | 60 | 55 | 8 - 12 | 2.0 | 4.8 |
| **BINA mug-5** | | | | | | |
| 0.1 | 0.1 | 60 | 80 | 10 - 12 | 2.0 | 4.2 |
| 0.3 | 0.1 | 60 | 84 | 11 - 14 | 1.8 | 5.4 |
| 0.5 | 0.1 | 60 | 79 | 9 - 12 | 3.5 | 6.0 |
| 0.5 | 0.3 | 60 | 83 | 8 - 10 | 4.8 | 5.5 |
| 0.5 | 0.5 | 60 | 92 | 11 - 14 | 5.5 | 6.2 |
| 1.0 | 1.0 | 60 | 87 | 9 - 14 | 2.8 | 5.1 |

mug-3, BARI mug-5 and BINA mug-5 (Fig. 4). The present findings were similar with those of Bose (1991). The plantlets with well developed roots were transferred to small plastic pots. After proper hardening they were established in field condition (Fig. 5).




Figs. 1-5: 1. Multiple shoot regeneration from cotyledon of var. BARI mug-3 on MS with 0.5 mg/l BAP and 0.1 mg/l Kn. 2. Multiple shoot regeneration from cotyledon of BINA mug-5 on MS with 0.5 mg/l BAP and 0.5 mg/l Kn. 3. *In vitro* regenerated shoot proliferation of BARI mug-5 on MS with 0.5 mg/l BAP and 0.5 mg/l Kn. 4. Root induction in BINA mug-5 on half strength of MS containing 0.5 mg/l IBA. 5. Establishment of BARI mug-3 seedling in soil.

The results of the present investigation demonstrated the establishment of a reliable *in vitro* regeneration protocol for three selected Bangladeshi mungbean varieties. Using this protocol, further study can be conducted to transfer useful

candidate genes conferring disease, insect and pest resistance in these mungbean varieties of Bangladesh.

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