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In vitro REGENERATION OF MUNGBEAN (*Vigna radiata* L.) FROM DIFFERENT EXPLANTS

M. K. Khatun, M. S. Haque, S. Islam and K. M. Nasiruddin
Department of Biotechnology, Bangladesh Agricultural University
Mymensingh-2202, Bangladesh

ABSTRACT

To regenerate plantlets both directly and indirectly, different explants (cotyledon, hypocotyls, root tip and shoot tip) of mungbean were cultured on medium supplemented with different concentrations and combinations of BAP (0, 1.0 and 5.0 mgL^{-1}) and NAA (0, 0.5 and 2.5 mgL^{-1}). Cotyledon explants performed best in callus induction (90.0%) at the combination of 1 mgL^{-1} BAP and 2.5 mgL^{-1} NAA for both the varieties (BINA mung 5 and BINA mung 7). The calli derived from cotyledon, hypocotyls and root tip were cultured in MS medium supplemented with different concentrations of Kn, BAP and/or NAA for shoot induction. Regeneration was achieved only from cotyledon calli at a frequency of 62.50% on 5 mgL^{-1} BAP and 0.05 mgL^{-1} NAA. Shoot tip cultured for direct regeneration in the same media containing 5 mgL^{-1} BAP and 0.05 mgL^{-1} NAA resulted in 90.0% shoot differentiation in BINA mung 7. The regenerated shoots rooted on MS medium with 0.2 mgL^{-1} NAA (90.0).

Key words : Callus, Plantlet, Regeneration, Mungbean, Seedling explants

INTRODUCTION

Mungbean (*Vigna radiata* L.) also known as green gram is one of the most important pulse crops, widely cultivated in a large number of countries. It has tremendous value in agriculture as a good source of plant protein for its high digestibility, good flavour, and high protein content and free from flatulent effects which are common to pulses (Ahmaed *et al*., 1978). It is cultivated most extensively in India, Myanmar, Bangladesh, Sri-Lanka, Pakistan, Thailand, Philippines, China, Japan, Korea, Iran, Indonesia, parts of East and Central Africa, West Indies, USA and Australia. The genus *Vigna* currently includes around 80 species distributed throughout the tropics (Pasquet, 2001).

In Bangladesh pulses are considered to be the poor man's meat, particularly because of its high protein content and low price as compared to animal proteins like meat, fish, egg, milk and milk products. Pulses have three or four times more protein content than rice and ten to fifteen times more than potatoes (Mian, 1976). The whole seed of the crop contains 51% carbohydrate, 26% protein, 3% minerals, 3% vitamins and 10% moisture (Kaul, 1982). The high lysine content makes it a good complementary food for rice based diets because lysine is the fist limiting amino acids (Chen *et al*., 1987).

Regeneration and transformation procedure of mungbean is not well developed compared to the success achieved in other grain legume crops. Successful plant regeneration from tissue culture of grain legumes is rather limited (Mroginski and Kartha, 1984). Considering the above facts the present investigation was undertaken to find out suitable explant and medium for regeneration, to develop a stable, reproducible and efficient protocol for the *in vitro* regeneration of mungbean.

MATERIALS AND METHODS

Seeds of the variety BINA mung 5 and BINA mung 7 were collected from Bangladesh Institute of Nuclear Agriculture. Healthy seeds were washed in tap water. The seeds were then sterilized with 70% ethanol for one minute and then rinsed with sterile distilled water. Afterwards, the seeds were soaked in 0.1% $HgCl_2$ plus two drops of a liquid detergent (Tween-20) solution and then agitated gently for 15-20 minutes, followed by 4 rinses in sterilized distilled water. The seeds were aseptically germinated on half MS (Murashige and Skoog, 1962) medium. Cotyledons, hypocotyls and root tips were excised aseptically from 5 to 7 days old seedlings and cultured on MS medium containing different concentrations and combinations of BAP (0, 1.0 and 5.0 mgL^{-1}) and NAA (0, 0.5 and 2.5 mgL^{-1}). Subcultures were carried out at 21 days interval and finally the calli were transferred to MS medium with different concentrations of cytokinins (BAP and Kn) and auxin (NAA) singly or in combinations. Shoot tips were also cultured on the same media. The regenerated shoots were rooted in MS media with different concentrations of NAA (0, 0.1, 0.2 and 0.5 mgL^{-1}) or IAA (0, 0.1, 0.2 and 0.5 mgL^{-1}).

The pH of the medium was adjusted to 5.8 prior to the addition of agar @ 8.0 g/L and the medium was autoclaved at 121^{o}C for 30 minutes. Twenty explants were cultured per treatment and each treatment was replicated 4 times. All cultures were maintained at 26±1^{0}C under white fluorescent tubes with a 16h photoperiod. The data collected were analyzed and the means were using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results revealed that a high variation in callus induction and plantlet regeneration ability was demonstrated by varieties, explants and hormone under present study. The media supplemented with 1.0 mgL^{-1} BAP and 2.5 mgL^{-1} NAA exhibited highest callus induction ability in all the varieties. The BINA mung 7 variety showed higher callus induction from all the treatments (Fig. 1). It is interesting to note that the media devoid of BAP failed to induce any callus from cotyledonary node explants (Fig. 1). It reveals that for the induction of callus from cotyledonary nodes, cytokinin might be indispensable. Among the explants used, cotyledon showed the highest percentage (90.00%) of callus induction in MS medium with 1.0 mgL^{-1} BAP + 2.5 mgL^{-1} NAA (Plate 1A) and minimum number of (14.50) days required for callus initiation. The highest callus weight (2.77 g) was observed from cotyledon in same media.

Remarkable variation in respect of regeneration was observed between the varieties in different concentrations of hormone. Among the induced calli, only cotyledon derived calli produced shoots. Hypocotyl and root tip derived calli failed in shoot induction. Shoot tip explants were also cultured for direct shoot regeneration. Anju and Pawan (1992) reported that completew plants were regenerated directly without in intervening callus phase from shoot tips of *Vigna radiata* on basal medium (MS salts + B_5 vitamins). Considering the combined effects of all factors, the highest percentage of shoot (90.00%) was produced in BINA mung 7 on media containing 5.0 mgL^{-1} BAP and 0.05 mgL^{-1} NAA from shoot tip explants which required minimum number of days (14.75) for shoot initiation (Plate 1B). It was revealed that supplementation of the medium with BAP or Kn alone or a combination of BAP and NAA induced shoots. However, Kn had higher efficiency than BAP while a combination of BAP and NAA was more efficient than the single application of either BAP or Kn. A suitable combination of an auxin and a cytokinin is always considered to be ideal for *in vitro* morphogenesis from cultured explants. Single application of auxin or cytokinin was reported to regenerate no shoots from garlic root tips (Haque *et al.*, 1997). Similar shooting frequency was also reported by Ignacimuthu and Franklin (1998). Cotyledon showed 62.50% shoot regeneration and it required 42.50 days for shoot initiation from callus (Plate 1C). Highest number of shoots per explant (7.00) was observed from shoot tip with shoot length of 4.85 cm. Cotyledon produced 5.50 shoots per explant and highest shoot length (5.07 cm) (Table 1).

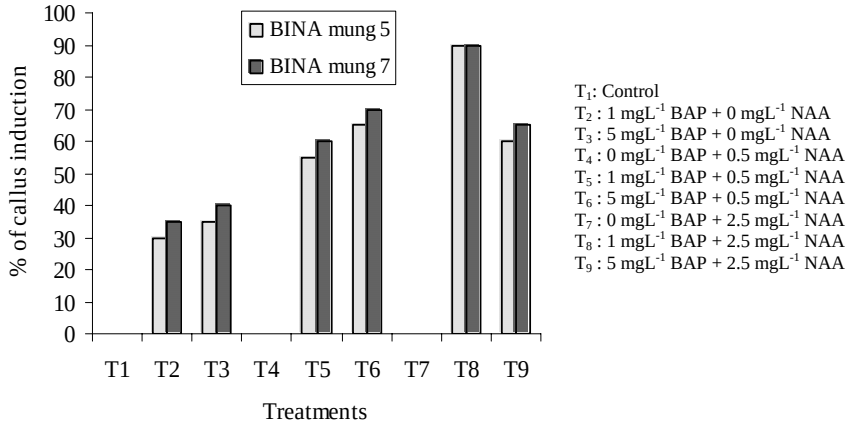

Fig. 1. Frequency of callus induction from cotyledon explants of mungbean as influenced by variety and growth regulators

Regenerated shoots were transferred to MS medium with different concentrations of NAA and IAA to induce root. Considering the combined effects of all factors, BINA mung 7 showed highest percentage (90.0 %) of root from both explants on MS medium supplemented with 0.2 mgL^{-1} NAA (Plate 1D). Brar *et al.* (1997) also reported that *in vitro* produced shoots could be rooted on MS media supplemented with NAA. The cotyledon explants required minimum number of days (9.50) but shoot tip required 11.25 days for root initiation in the same medium. Highest number (7.75) of roots per plantlet was

observed in BINA mung 7 from cotyledon on MS medium supplemented with 0.2 mgL^{-1} NAA while the shoot tip produced 6.50 roots per plantlet in the same variety on same media. Maximum root length (5.07 cm) was found from shoot tip in BINA mung 7 on MS medium supplemented with 0.2 mgL^{-1} NAA while cotyledon showed 4.87 cm root length in same variety and in same medium (Table 2). A lower percentage of rooting was observed in shoots regenerated from cotyledone explant on the growth regulator free medium, although the other explant failed to root on this medium. Garlic shoots were reported to induce roots on growth regulator free medium (Haque *et al*., 1997).

Table 1. Combined effect of Variety, Hormone and Explant on different characters of shoot regeneration

Variety	Hormone concentration (mgL^{-1})	Explants	Percent shoot induction	Days required for shoot induction	No. of shoot/ explant	Shoot length (cm)
BINA mung 5	$T_{1(Hormone\ free)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	15.00 i	24.50 d	2.50	2.35
	$T_{2\ (5\ BAP)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	17.50 i	23.00 e	2.75	3.00
	$T_{3\ (10\ BAP)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	35.00 g	20.50 fg	3.50	3.62
	$T_{4\ (5\ kn)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	40.00 fg	19.00 h	4.25	4.00
	$T_{5\ (10\ kn)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	55.00 e	17.50 i	5.00	3.55
	$T_{6\ (5\ BAP+\ 0.05\ NAA)}$	Cotyledon	57.50 de	44.25 b	4.25	4.67
		Shoot tip	87.50 ab	15.50 kl	6.50	4.75
	$T_{7\ (3\ BAP+\ 0.2\ NAA)}$	Cotyledon	37.50 fg	46.00 a	3.25	4.27
		Shoot tip	82.50 b	16.50 j	5.50	3.90
BINA mung 7	$T_{1\ Hormone\ free)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	17.50 i	22.75 e	2.75	2.40
	$T_{2\ (5\ BAP)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	25.00 h	21.00 f	3.00	3.12
	$T_{3\ (10\ BAP)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	42.50 f	20.00 g	3.75	3.82
	$T_{4\ (5\ kn)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	55.00 e	18.50 h	4.50	3.85
	$T_{5\ (10\ kn)}$	Cotyledon	0.00 j	0.00 m	0.00	0.00
		Shoot tip	65.00 c	16.50 j	5.25	3.85
	$T_{6(5\ BAP+\ 0.05\ NAA)}$	Cotyledon	62.50 cd	42.50 c	5.50	5.07
		Shoot tip	90.00 a	14.75 l	7.00	4.85
	$T_{7\ (3\ BAP+\ 0.2\ NAA)}$	Cotyledon	52.50 e	44.25 b	4.50	4.90
		Shoot tip	85.00 ab	16.00 jk	6.25	4.47

From the above result it can be concluded that BINA mung 7 responded better for *in vitro* regeneration than BINA mung 5. Among the explants, cotyledon was better than any other explants for plantlet regeneration. Among the hormonal combinations used, a combination of supplemented with 1.0 mgL^{-1} BAP and 2.5 mgL^{-1} NAA was best for callus induction while MS medium supplemented with 5.0 mgL^{-1} BAP and 0.05 mgL^{-1} NAA was best for shoot initiation and MS medium supplemented with 0.2 mgL^{-1} NAA was best for rooting of mungbean.

Table 2. Combined effect of variety, hormone and explant on different characters of root regeneration

Variety	Hormone concentrations	Explants	Per cent root induction	Days required for root induction	No. of root/ plantlet	Root length (cm)
BINA mung 5	T_{1} Hormone free)	Cotyledon	5.00	5.00	0.50	0.52
		Shoot tip	0.00	0.00	0.00	0.00
	T_{2} (0.1 mg/L NAA)	Cotyledon	30.00	15.75	2.75	2.37
		Shoot tip	25.00	19.00	1.75	2.02
	T_{3} (0.2 mg/LNAA)	Cotyledon	85.00	10.50	7.50	4.70
		Shoot tip	80.00	11.50	6.25	4.92
	T_{4} (0.5 mg/L NAA)	Cotyledon	70.00	13.00	2.25	3.00
		Shoot tip	55.00	14.50	2.50	3.75
	T_{5} (0.1 mg/L IAA)	Cotyledon	25.00	13.75	1.50	1.45
		Shoot tip	30.00	15.50	3.00	3.45
	T_{6} (0.2 mg/L IAA)	Cotyledon	30.00	15.50	4.00	2.10
		Shoot tip	50.00	14.00	3.75	2.62
	T_{7} (0.5 mg/L IAA)	Cotyledon	75.00	13.50	6.25	2.35
		Shoot tip	70.00	12.25	5.50	2.42
BINA mung 7	T_{1} Hormone free)	Cotyledon	10.00	9.25	1.00	1.07
		Shoot tip	0.00	0.00	0.00	0.00
	T_{2} (0.1 mg/L NAA)	Cotyledon	40.00	14.50	3.00	2.40
		Shoot tip	25.00	18.50	2.00	1.90
	T_{3} (0.2 mg/LNAA)	Cotyledon	90.00	9.50	7.75	4.87
		Shoot tip	90.00	11.25	6.50	5.07
	T_{4} (0.5 mg/L NAA)	Cotyledon	70.00	13.25	2.50	3.37
		Shoot tip	55.00	14.50	2.50	4.32
	T_{5} (0.1 mg/L IAA)	Cotyledon	30.00	17.50	2.00	1.95
		Shoot tip	30.00	15.25	2.50	3.47
	T_{6} (0.2 mg/L IAA)	Cotyledon	40.00	15.00	4.25	2.17
		Shoot tip	45.00	13.50	3.75	2.65
	T_{7} (0.5 mg/L IAA)	Cotyledon	80.00	11.00	6.50	2.55
		Shoot tip	75.00	11.50	6.25	2.42

In conclusion, for the creation of genetic variability in crop plants it is very important to regenerate plants via callus. With this obvious reasons it is suggested that in future tissue culture programme of mungbean some more cytokinin`s additives viz; 2-ip, zeatin, coconut water, ascorbic acid, glutamine may be used for successful plantlet regeneration from calli. Here a direct *in vitro* regeneration protocol was developed. However, further study is needed with different explants to standardize the protocol of regeneration from calli. The protocol developed here could be used for future improvement of mungbean through *in vitro* culture and genetic transformation.

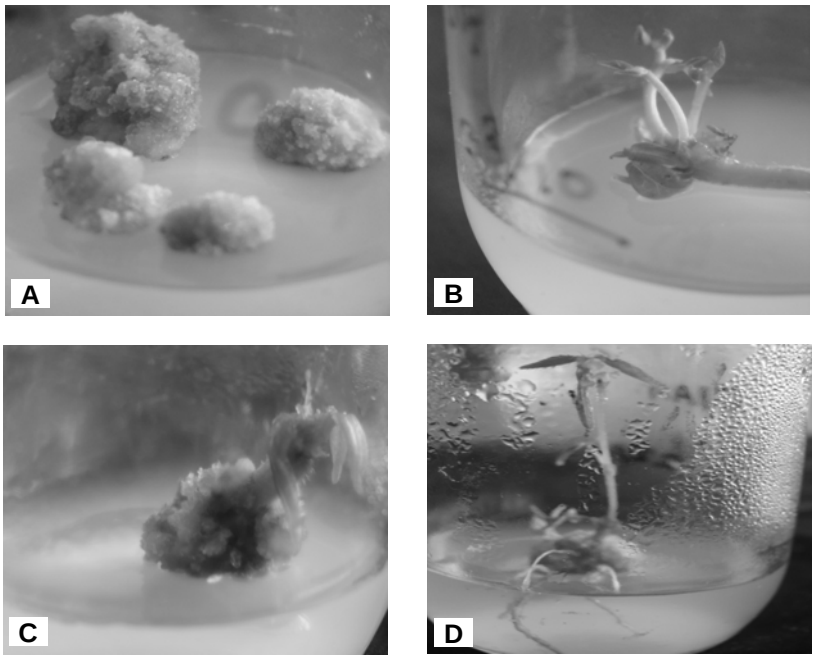

Plate 1. A) Callus induced from cotyledons explants of BINA mung 7 on MS + 1.0 mgL^{-1} BAP + 2.5 mgL^{-1} NAA. B) Initiation of multiple shoot from shoot tip of BINA mung 5. C) Initiation of shoot from cotyledon explants of BINA mung 7. D) Initiation of roots from regenerated shoots derived from shoot tip of BINA mung 7

REFERENCES

Ahmed, Z. U., Shaikh, M. A. Q., Khan A. I. and Kaul A. K. 1978. Evaluation of local, exotic and mutant germplasm of mungbean for varietal characters and grain yield in Bangladesh. *SABRAO J.*, 10(1): 40-48.

Anju, G. and Pawan, K. J. 1992. *In vitro* induction of multiple shoots and plant regeneration from shoot tips of mungbean (*Vigna radiata* (L.) Wilczek). *Plant Cell Tiss. Org. Cult.*, 29(3): 199-205.

Brar, M. S., Al-Khayri, J. M., Shamblin, C. E., McNew, R. W., Morelock, T. E. and Anderson, E. J. 1997. *In vitro* shoot tip multiplication o cowpea *Vigna unguiculata* (L.) Walp. *In Vitro* Cell Dev. *Biol. Plant*., 33(2): 114-118.

Chen, C. Y., Tsou, S. C. S. and Wang, H. H. 1987. Utilazation pattern of mungbean in the Chinese diet. I. Sw. shanmugasundaram (ed.), Mungbean: Proceedings of the second international symposium. Shatua, Taiwan : Asian Vegetable Research and Development Center, AVRDC Pub. No. 88-304. pp. 498-507.

Haque, M. S., Wada, T. and Hattori, K. 1997. High frequency shoot regeneration and plantlet formation from root tip of garlic. *Plant Cell Tiss. Org. Cult.,* 50: 83-89.

Ignacimuthu, S. and Franklin, G. 1998. Regeneration of plantlets from cotyledon and embryonal axis explants of *Vigna mungo* L. Hepper. *Plant Cell Tiss. Org. Cult*., 55(1): 75-78.

Kaul, A. K. 1982. Pulses in Bangladesh. BARC. Farmgate, Dhaka. p. 27.

Mian, A. L. 1976. *Grow more pulses to keep your pulse well an assay of Bangladesh pulses*. Department of Agronomy. Bangladesh Agricultural University. Mymensingh. pp. 1-8.

Mroginski, L. A. and Kartha, K. K. 1984. Tissue Culture of Legumes for Crop Improvement. In. Plant Breeding Reviews Ed. J. Janick. The AVI Publishing Co. Inc. USA. pp. 215-264.

Murashinge, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 470-497.

Pasquet, R. 2001. *Vigna* Savi. ***In***: Mackinder B., Pasquet R., Polhil Poly-R and Verdcourt B. (eds), Flora zambesiaca, volume part Phaseoleae. Royal Botanic Garedens, kew, pp. 121-156.