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IN VITRO Regeneration of Bina Mungbean Varieties

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Abstract

The experiment was conducted to develop an efficient protocol for in vitro regeneration of mungbean (Vignaradiata) on the aspect of regeneration potentiality of two mungbean varieties (BINA mung 5 and BINA mung 7) as influenced by different combinations of growth regulators supplemented with MS medium. Cotyledon explant of both varieties was used for the present study. Data were collected for various characters of callus initiation, shoot regeneration and root proliferation. Initiation of callus (%) and required days for its initiation and weight of callus were influenced significantly due to the effect of varieties where BINA mung 5 produced more callus induction (40.36%) at minimum requiring time (18.27 days) including heavier sizes of callus (1.54 g) than BINA mung 7 when BINA mung 5 further recorded the longest root (2.92 cm) compare to BINA mung 7. Effect of treatments of the present study were significantly influenced the whole characters regarding callus culture, shoot regeneration and root proliferation. The highest percentage of callus (88.44%) within minimum time (12.53 days) including larger sizes callus (3.521 g) were produced in 1.0 mg L–1 BAP + 2.5 mg L–1 NAA among the treatments while the highest percentage of regenerated shoot (83.44%) at minimum requiring time (17.59 days) and more shoots (7.69 callus–1) were obtained in 1.0 mg L–1 BAP + 2.0 mg L–1 NAA. Root induction (82.50%), number of roots plantlet–1 (8.469) with minimum requiring time for initiation (14.13 days) and root length (5.250 cm) were the highest in 0.2 mg L–1 IAA + 1.0 mg L–1 kinetin + 0.2 mg L–1 BAP. Incase of interaction, percentage of callus initiation (89.38 %) was the highest in BINA mung 5 treated by 1.0 mg L–1 BAP + 2.5 mg L–1 NAA at requiring minimum time (12.38 days) while same treatment produced the larger callus (3.581 g) among the interactions. The highest percentage (84.38%) and number (7.813 callus–1) of shoot with minimum requiring time (17.50 days) were found from BINA mung 5 treated by 1.0 mg L–1 BAP + 2.0 mg L–1 NAA. Similarly, the longest shoot (5.58 cm) was produced from the BINA mung 5 treated by 1.0 mg L–1 BAP + 2.0 mg L–1 NAA. However, root induction (%), roots plantlet–1, days required for root initiation and root length were statistically similar among the whole interaction treatments due to non significant variation. This result mentioned that the variety BINA mung 5 was better than BINA mung 7 for callus induction, shoot regeneration and root initiation while 1.0 mg L–1 BAP + 2.5 mg L–1 NAA, 1.0 mg L–1 BAP + 2.0 mg L–1 NAA and 0.2 mg L–1 IAA + 1.0 mg L–1 kinetin + 0.2 mg L–1 BAP supplemented with MS medium were the best combinations for better callusing, higher ability of shoot regeneration and root proliferation.

Key words: Callus, Mungbean, PGRs **(**BAP, NAA, IAA, Kinetin), Regeneration

Introduction

Mungbean (*Vignaradiata* (L.) Wilczek) is an important food grain legume crop grown all over the world. It has tremendous value in agriculture as a good source of plant protein for its high degestibility, good flavour, and high protein content and free from flatulent effects which are common to pulses (Ahmed *et al*., 1978). The whole seed of crop contains 51% carbohydrate, 26% protein, 3% minerals, 3% vitamins and 10% moisture (Kaul, 1982). In Bangladesh protein nutrition is alarmingly poor, particularly for children and the pregnant and lactating mothers while the daily consumption of pulses in Bangladesh is only 12 g head^{-1} compare to FAO recommended capita^{-1} consumption of 45 g (BARI, 2000). 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 (James 2004). So, 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; Chandra and Pal, 1995; Gulati and Jaiwal, 1994). However, the above *in vitro* regeneration protocols did not produce desired results using mungbean varieties from Bangladesh. Considering the above mentioned background, attempts were made in the present investigation to establish reproducible *in vitro* plant regeneration system in different mungbean varieties of Bangladesh. The present investigation was undertaken to develop a stable, reproducible and efficient protocol for *in vitro* regeneration of mungbean.

Materials and Methodology

In the present investigation, two varieties of *Vignaradiata*L. were used for growth and development of callus, *in vitro* regeneration of shoot and proliferation of root at different parameters. To achieve the goal, the experiment was conducted in the Lab of the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh during the period from July to November 2010. The seed materials of *Vignaradiata*L. were collected from Bangladesh Institute of Nuclear Agriculture (BINA). Half strength MS (Murashige and Skoog, 1962) medium was used for seed germination. For culture, cotyledon explants of two mungbean*viz.* BINA mung 5 and BINA mung 7 were used for the present study. Twelve treatments supplemented with MS medium *viz.* growth regulators free (T_1), 0 mg L^{-1} BAP + 1.0 mg $^{-1}$ NAA (T_2), 0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA (T_3), 1.0 mg L^{-1} BAP + 0 mg L^{-1} NAA (T_4), 1.0 mg L^{-1} BAP + 1.0 m0g/l NAA (T_5), 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA (T_6), 3.0 mg L^{-1} BAP + 0 mg L^{-1} NAA (T_7), 3.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (T_8), 3.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA (T_9), 5.0 mg L^{-1} BAP + 0 mg L^{-1} NAA (T_{10}), 5.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (T_{11}) and 5.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA (T_{12}) were used for callus induction and growth regulators free (T_1), 0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (T_2), 0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA (T_3), 1.0 mg L^{-1} BAP + 0 mg L^{-1} NAA (T_4), 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (T_5), 1.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA (T_6), 3.0 mg L^{-1} BAP + 0 mg L^{-1} NAA (T_7), 3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (T_8), 3.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA (T_9), 5.0 mg L^{-1} BAP + 0 mg L^{-1} NAA (T_{10}), 5.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (T_{11}) and 5.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA (T_{12}) were used for shoots regeneration. For root initiation MS medium supplemented with different hormonal combinations *viz.* Growth regulators free (T_1), 0.1 mg L^{-1} IAA + 0.5 mg L^{-1} kinetin + 0.1 BAP (T_2), 0.2 mg L^{-1} IAA + 0.5 mg L^{-1} kinetin + 0.1 BAP (T_3), 0.5 mg L^{-1} IAA + 0.5 mg L^{-1} kinetin + 0.1 BAP (T_4), 0.1 mg L^{-1} IAA + 1.0 mg L^{-1} kinetin + 0.2 BAP (T_5), 0.2 mg L^{-1} IAA + 1.0 mg L^{-1} kinetin + 0.2 BAP (T_6) and 0.5 mg L^{-1} IAA + 1.0 mg L^{-1} kinetin + 0.2 BAP (T_7) were also used for the present study. Data were recorded on percentage of callus induction, days required for callus induction and weight of callus, percentage of shoot initiation, days to shoot initiation, number of shoots per explant, shoot length (cm), percentage of root initiation, number of roots per plantlets, days required for root initiation and root length (cm). The data for the above characters were statistically analyzed by Completely Randomized Design (CRD) and the mean were adjusted by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Callus initiation

Effect of varieties on callus initiation (%), days to callus initiation and weight of callus showed significant variation. Between the varieties, BINA mung 5 produced more callus (40.36%) with an average minimum requiring time (18.27 days) than BINA mung 7 (37.71%) with and average requiring maximum time (19.90 days). The weight of callus had also the highest (1.541 g) in BINA mung 5 than BINA mung 7 (1.517 g) (Fig. 1). This result revealed that BINA mung 5 showed better performance than BINA mung 7 due to higher ability of callus formation while Sambaiah and Reddy *et al*. (2001) also developed efficient plant regeneration where callus induction ability was higher in WGG-2 than in MGG-295.

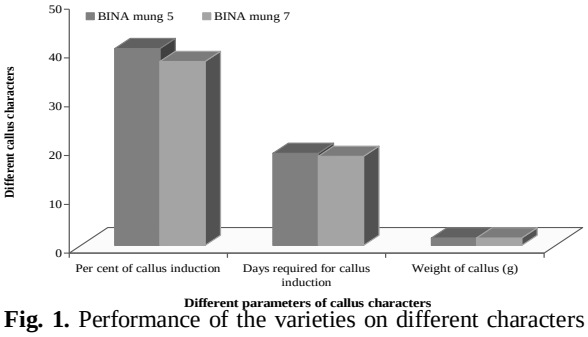


Fig. 1. Performance of the varieties on different characters of callus of *in vitro* regeneration of mungbean

Effect of treatments of twelve different combinations of BAP and NAA were found statistically significant effect on the percentage of callus induction, days required for callus induction and weight of callus. Among the treatments, the highest percentage (88.44%) and weight (3.521 g) of callus were found in 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA at required minimum time for callus initiation (12.53 days) followed by 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (80.00% at required 13.16 days for callus initiation). On the other hand, the lowest percentage (12.19%) and weight (0.452 g) of callus were observed in 5.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA at required maximum time for callus initiation (24.59). However, control treatment or without BAP and NAA unable to produced any induction of callus during the study. It was observed that percentage of callus and callus growth performance progressively decreased when different combinations of BAP and NAA were increased gradually. Similar trend were also found by Amutha *et al*. (2006 and 2003). Besides, higher formation of callus within minimum time and larger callus were produced due to 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA compare to other treatments of the study while higher doses of BAP and NAA were less efficient for getting the early initiation and higher formation of callus. The findings of the present study

were also similar to the findings of Amutha *et al*. (2003) who reported that organogenic calluses were induced from both cotyledon and hypocotyl explant of mungbean excised from 3 day old seedling on MS medium containing NAA (1.07 µM) and BA (2.22 µM) and 2, 4-D (0.90 µM) combinations respectively which are agreed to the present findings.

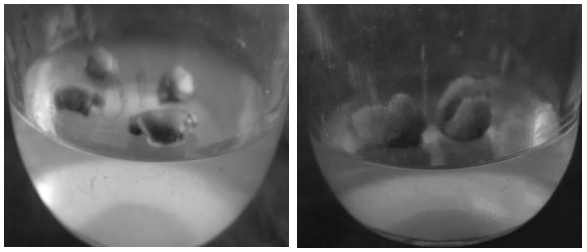
Table 1. Performance of different combinations of BAP and NAA on different callus characters of *in vitro* regeneration of mungbean

Treatment (mg L^{-1})		Callus induction (%)	Days required for callus initiation	Weight of callus (g)
BAP	NAA			
0	0	0.0000 j	0.000	0.0000 l
	1.0	22.50 g	23.66	0.8744 h
	2.5	52.19 c	19.91	2.060 d
1	0	30.94 f	22.22	1.229 g
	1.0	80.00 b	13.16	3.280 b
	2.5	88.44 a	12.53	3.521 a
3	0	42.19 e	21.28	1.724 e
	1.0	54.06 c	19.09	2.172 c
	2.5	46.88 d	19.69	1.702 f
5	0	22.19 g	23.50	0.643 j
	1.0	16.88 h	23.41	0.684 i
	2.5	12.19 i	24.59	0.454 k
CV (%)		**21.84**	**7.26**	**1.36**

Table 2. Combined effect of variety and different treatment combination of BAP and NAA on *in vitro* regeneration of mungbean

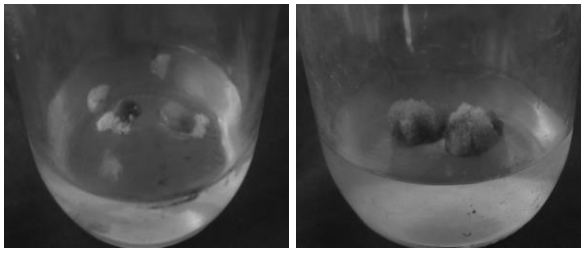
Varieties	Treatment (mg L^{-1})		Callus induction (%)	Days required for callus initiation	Weight of callus (g)
	BAP	NAA			
BINA mung 5	0	0	0.000 m	0.000	0.000 u
		1.0	20.00 jk	24.25	0.763 n
		2.5	48.75 de	21.56	1.894 i
	1.0	0	27.50 ghi	22.38	1.111 l
		1.0	83.13 ab	12.94	3.307 c
		2.5	89.38 a	12.38	3.581 a
	3.0	0	41.25 f	21.75	1.722 j
		1.0	58.13 c	19.19	2.340 e
		2.5	60.00 c	19.81	2.055 g
	5.0	0	26.88 hij	23.88	0.602 r
		1.0	17.50 kl	23.94	0.710 o
		2.5	11.88 l	24.75	0.403 t
BINA mung 7	0	0	0.000 m	0.000	0.000 u
		1.0	25.00 ij	23.06	0.986 m
		2.5	55.63cd	18.25	2.226 f
	1.0	0	34.38 g	22.06	1.347 k
		1.0	76.88 b	13.38	3.253 d
		2.5	87.50 a	12.69	3.461 b
	3.0	0	43.13 ef	20.81	1.726 j
		1.0	50.00 de	19.00	2.003 h
		2.5	33.75 gh	19.56	1.349 k
	5.0	0	17.50 kl	23.13	0.684 p
		1.0	16.25 kl	22.88	0.658 q
		2.5	12.50 l	24.44	0.506 s
CV (%)			**21.84**	**7.26**	**1.36**

A significant variation regarding callus induction, days required for callus induction and weight of callus were also obtained due to the interaction effect of varieties and various treatments of BAP and NAA. Among the interactions, the highest percentage of callus induction (89.38 %) and the highest weight of callus (3.58 g) were found in 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA at BINA mung 5 (required minimum time of 12.38 days for callus initiation) while BINA mung 7 produced statistically same percentage of callus induction (87.50%) at required 12.69 days for callus initiation at same treatment. Similarly, 5.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA both varieties of BINA produced statistically identical result i.e. the lowest percentage of callus induction (11.88 and 12.50%, respectively) at requiring maximum time for callus initiation (24.75 and 24.44 days, respectively) while 5.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA treated BINA mung 5 showed the lowest weight of callus (0.403 g) followed by the same treated plant of BINA mung 7 (0.506 g) (Table 2 and Plate 1 & 2). Above result indicated that the highly significant results were produced in BINA mung 5 than BINA mung 7 due to all the concentrations of BAP and NAA. Das *et al*. (2002) also found similar result with the present study where they found MS medium containing B_5 vitamins + 3 mg L^{-1} benzyladenine + 1 benzyladenine + 1 mg L^{-1} NAA treated cultivar T–9 found to be the highest result for callus formation than the cultivar T–13.



12 days after culture 35 days after culture

Plate 1. Initiated callus from MS medium supplemented with 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA from BINA mung 5



12 days after culture 35 days after culture

Plate 2. Initiated callus from MS medium supplemented with 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA from BINA mung 7 at 12 days after culture

'In vitro' regeneration of shoot

Effect of varieties did not vary significant among the callus characters *viz.* percentage of soot regeneration, days required for shoot initiation and length of shoot (cm). However, BINA mung 5 produced little bit higher results among the whole characters of shoot than BINA mung 7 (Fig. 2). This result revealed that shoot regeneration ability had higher in BINA mung 5 than that of BINA mung 7 due to higher cell elongation while similar trend were also obtained by Sambaiah and Reddy *et al*. (2001). They reported that the cultivar WGG-2 had more efficient than in MGG-295 for *in vitro* regeneration of shoot.

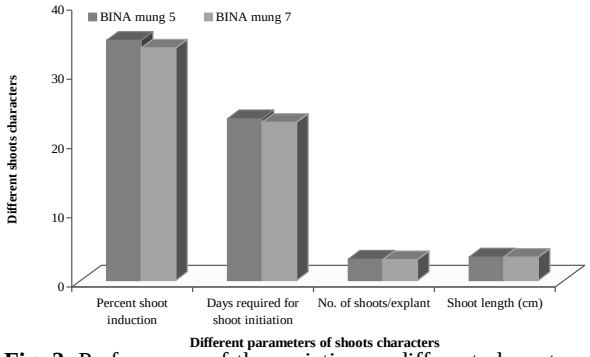


Fig. 2. Performance of the varieties on different characters of shoot of *in vitro* regeneration of mungbean

Effect of different treatment combinations of BAP and NAA was significantly influenced the percentage of shoot regeneration, days required for shoot initiation, no. of shoots callus^{-1} and shoot length (cm). The highest percentage of shoot regeneration (83.44%) at requiring minimum time (17.59 days) were observed in 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA while the lowest percentage of shoot regeneration (6.563%) at required maximum time (29.53 days). The maximum number of shoots callus^{-1} (7.688) and the lowest number of shoots callus^{-1} (0.5938) were observed in 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA and 5.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA, respectively (Table 3). From the above result, it was found that the 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA had more significant than that of other treatments of the study while without BAP and NAA were unable to produced any shoot. Similarly, Amutha *et al.* (2003) found that regeneration of adventitious shoot from cotyledon derived callus was achieved when they were cultured on MS medium supplemented with NAA, BA and 10% coconut water. Mahalaxmi *et al.* (2003) also found that BAP 5 µM and NAA 0.05 µMwere supplemented to the medium30-35% of induced buds developed into micro shoots while Savita *et al*. (2001) found that0.5 mg L^{-1} BAP, 0.5 mg L^{-1} N_6 adenine and 0.1 mg L^{-1} NAA induced differentiation at an average of 10 shoots in shoot tip explants, an optimum of 3 shoots were formed in explants in explants of embryonal axis in a treatment containing 0.5 mg L^{-1} BAP and 0.1 mg L^{-1} NAA. All the above findings were more or less similar with the present study.

Table 3. Performance of the different treatments combinations on shoots characters of *in vitro* regeneration of mungbean

Treatments (mg L^{-1})		Shoot regeneration (%)	Days required for shoot initiation	No. of shoots callus^{-1}	Shoot length (cm)
BAP	NAA				
0	0	0.0000 j	0.000	0.000 i	0.000 j
	2.0	17.50 g	28.66	1.250 g	2.874 g
	4.0	47.19 c	24.19	4.219 cd	4.060 d
1	0	25.94 f	27.09	2.063 f	3.229 f
	2.0	83.44 a	17.59	7.688 a	5.517 a
	4.0	77.19 b	18.19	7.250 b	5.280 b
3	0	37.50 e	26.28	3.250 e	3.724 e
	2.0	49.38 c	24.19	4.406 c	4.265 c
	4.0	41.88 d	24.72	3.875 d	3.702 e
5	0	11.25 h	28.50	0.9063 gh	2.640 h
	2.0	11.88 h	28.38	0.9688 gh	2.684 h
	4.0	6.563 i	29.53	0.5938 h	2.454 i
CV (%)		**17.71**	**7.85**	**23.96**	**4.59**

Shoot induction (%), days to shoot initiation, number of shoots callus^{-1} and shoot length varied significantly due to the combined effect of varieties and treatments. The highest percentage (84.38%) and number (7.813 callus^{-1}) of shoot and longest shoot (5.58 cm) were found from BINA mung 5 treated by 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA with an average requiring minimum time (17.50 days) while same treated plant of BINA mung 7 also produced statistically similar regeneration of shoot (82.50%) at statistically same requiring time for shoot initiation (17.69 days). However, 1.0 ml L^{-1} BAP + 2.0 ml L^{-1} NAA treated plant of BINA mung 7 and 1.0 ml L^{-1} BAP + 4.0 ml L^{-1} NAA treated plant of BINA mung 5 also produced statistically close maximum shoots callus^{-1} where but both the interaction treatments were also statistically similar regarding shoots production callus^{-1}. On the other hand, the lowest shoot regeneration (4.375%), shoots callus^{-1} (0.438) and the shortest shoot (2.40 cm) with an average maximum requiring time for shoot initiation (29.63 days) were observed from the variety BINA mung 5 treated by 5.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA while without BAP and NAA or control treatment unable to produced any shoots due to both BINA mung 5 and BINA mung 7 varieties (Table 4 and Plate 3, 4, 5 & 6). Above result revealed that BINA mung 5 had highly efficient than BINA mung 7 among the combination treatments of BAP and NAA while 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA were the best combinations due to both varieties. Similarly, Das *et al*. (2002) developed protocol for the *in vitro* organogenesis directly from the explants of black gram (*V. mungo*) cultivars T-9 and L-13 and cultured in MS medium containing B_5 vitamins + 3 mg L^{-1} benzyladenine + 1 benzyladenine + 1 mg L^{-1} NAA for multiple shoot induction where T–9 was the best for shoot regeneration due to treatments.

Table 4. Combined effect of variety and different combinations of BAP and NAA on shoots characters of *in vitro* regeneration of mungbean

Varieties	Treatments (mg L^{-1})		Shoot induction (%)	Days required for shoot initiation	No. of shoots $callus^{-1}$	Shoot length (cm)
	BAP	NAA				
BINA mung 5	0	0	0.000 k	0.000	0.000 n	0.00 p
		2.0	15.00 h	29.25	1.000 klm	2.76 l
		4.0	43.75 d	25.00	3.875 ef	3.89 g
	1.0	0	23.13 g	27.38	1.750 j	3.11 j
		2.0	84.38 a	17.50	7.813 a	5.58 a
		4.0	77.50 b	18.00	7.500 ab	5.31 c
	3.0	0	36.25 e	26.75	3.125 gh	3.72 h
		2.0	53.75 c	24.25	4.813 c	4.34 d
		4.0	55.00 c	24.81	5.000 c	4.06 f
	5.0	0	10.63 hi	28.88	0.688 lm	2.60 mn
		2.0	12.50 hi	28.94	0.938 klm	2.71 lm
		4.0	4.375 jk	29.63	0.438 mn	2.40 o
BINA mung 7	0	0	0.000 k	0.000	0.000 n	0.00 p
		2.0	20.00 g	28.06	1.500 jk	2.99 k
		4.0	50.63 c	23.38	4.563 cd	4.23 de
	1.0	0	28.75 f	26.81	2.375 i	3.35 i
		2.0	82.50 a	17.69	7.563 ab	5.45 b
		4.0	76.88 b	18.38	7.000 b	5.25 c
	3.0	0	38.75 e	25.81	3.375 fg	3.73 h
		2.0	45.00 d	24.13	4.000 de	4.19 e
		4.0	28.75 f	24.63	2.750 hi	3.35 i
	5.0	0	11.88 hi	28.13	1.125 kl	2.68 lm
		2.0	11.25 hi	27.81	1.000 klm	2.66 lm
		4.0	8.750 ij	29.44	0.750 lm	2.51 no
CV (%)			**17.71**	**7.85**	**23.96**	**4.59**

Plate 3. Initiated of multiple shoot on MS medium supplemented with 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA from BINA mung 5

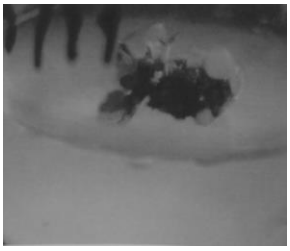


Plate 4. Initiated of multiple shoot on MS medium supplemented with 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA from BINA mung 7

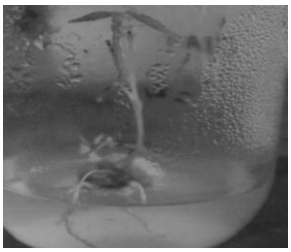


Plate 5. Shoot height was measured after 35 DAC from MS medium supplemented with 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA of BINA mung 5

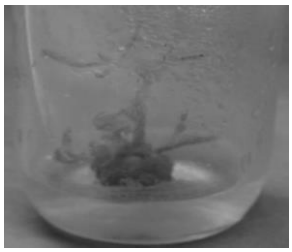


Plate 6. Shoot height was measured after 35 DAC from MS medium supplemented with 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA of BINA mung 7

Root initiation

Effect of different varieties showed non significant variation among the root characters except root length. As a result, percentage of root induction, number of roots $plantlet^{-1}$ and requiring days for root initiation were statistically similar between the varieties while the BINA mung 5 produced significantly the longest root (2.92 cm) than BINA mung 7 (Fig. 3). From the above results it was found that the variety BINA mung 5 had more significant than BINA mung 7 for getting the better proliferation of root during the study.

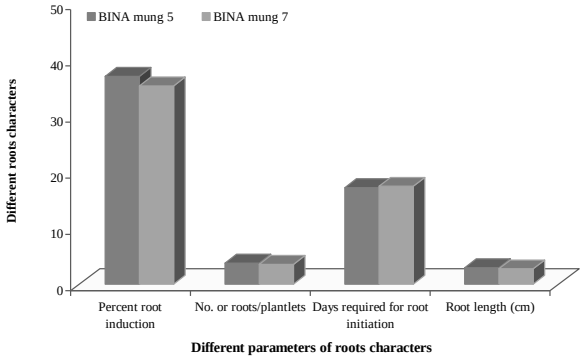


Fig. 3. Performance of the varieties on different characters of root of *in vitro* regeneration of mungbean

Effect of treatments was significantly influenced the whole characters of roots where the highest percentage of root induction (82.50%), maximum number of roots $plantlet^{-1}$ (8.469) with an average requiring minimum time for root initiation (14.13 days) and the longest root (5.250 cm) were observed in 0.2 mg L^{-1} IAA + 1.0 mg L^{-1} kinetin + 0.2 mg L^{-1} BAP. On the other hand, the lowest percentage of root induction (16.25%) with requiring time for root initiation of 22.16 days were obtained in 0.1 mg L^{-1} IAA + 1.0 mg L^{-1} kinetin + 0.2 mg L^{-1} BAP while same treatment also showed the minimum number of roots $plantlet^{-1}$ (1.656) and 0.1 mg L^{-1} IAA + 0.5 mg L^{-1} kinetin + 0.1 mg L^{-1} BAP observed the shortest root (1.688 cm). Percentage of root induction, number of roots $plantlet^{-1}$, days required for root initiation and root length (cm) did not vary significant due to the combined effect of varieties and different combination treatments of IAA, kinetin and NAA which indicated that all the combination treatments were produced statistically similar result among the above characters (Table 6 and Plate 7 & 8). Savita *et al*. (2001) found that the 0.5 mg L^{-1} IAA resulted in the formation of complete plantlets with root in 20 days while Teli and Maheshwari (2001) reported that roots were induced from *in vitro* produced shoot buds within a week in root induction medium (BM supplemented with NAA) in *V. radiata*.

Table 5. Performance of the different treatments combinations on roots characters of *in vitro* regeneration of mungbean

Treatment (mg L^{-1})			Root induction (%)	No. of roots $plantlet^{-1}$	Days required for root initiation	Root length (cm)
IAA	KN	BAP				
0	0	0	0.0000 f	0.0000 f	0.000	0.000 e
0.1	0.5	0.1	22.50 d	2.250 d	26.844	1.688 d
0.2	0.5	0.1	58.44 b	5.844 b	17.750	3.438 c
0.5	0.5	0.1	20.00 de	2.000 de	16.406	4.469 b
0.1	1.0	0.2	16.25 e	1.656 e	22.156	3.031 c
0.2	1.0	0.2	82.50 a	8.469 a	14.125	5.250 a
0.5	1.0	0.2	53.13 c	5.219 c	23.688	1.813 d
CV (%)			**23.23**	**18.75**	**10.39**	**26.17**

Table 6. Combined effect of variety and different combinations of BAP and NAA of *in vitro* root regeneration of mungbean

Varieties	Treatment (mg L^{-1})			Root induction (%)	No. of roots $plantlet^{-1}$	Days required for root initiation	Root length (cm)
	IAA	KN	BAP				
BINA mung 5	0	0	0	0.0000 f	0.0000 g	0.000	0.000 f
	0.1	0.5	0.1	23.75 d	2.375 e	26.750	1.81 e
	0.2	0.5	0.1	59.38 b	5.938 b	17.625	3.56 d
	0.5	0.5	0.1	21.25 de	2.125 ef	16.250	4.63 bc
	0.1	1.0	0.2	17.50 de	1.813 ef	22.000	3.13 d
	0.2	1.0	0.2	81.88 a	8.625 a	13.938	5.38 a
	0.5	1.0	0.2	55.00 bc	5.313 cd	23.563	1.94 e
BINA mung 7	0	0	0	0.0000 f	0.0000 g	0.000	0.000 f
	0.1	0.5	0.1	21.25 de	2.125 ef	26.938	1.56 e
	0.2	0.5	0.1	57.50 bc	5.750 bc	17.875	3.31 d
	0.5	0.5	0.1	18.75 de	1.875 ef	16.563	4.31 c
	0.1	1.0	0.2	15.00 e	1.500 f	22.313	2.94 d
	0.2	1.0	0.2	83.13 a	8.313 a	14.313	5.13 ab
	0.5	1.0	0.2	51.25 c	5.125 d	23.813	1.69 e
CV (%)				**23.23**	**18.75**	**10.39**	**26.17**

Plate 7. Initiated roots from regenerated shoots derived from BINA mung 5 at 35 days after initiation

Plate 8. Initiated roots from regenerated shoots derived from BINA mung 7 at 35 days after initiation

Conclusions

From the above results of the present study it could be concluded that the MS medium containing 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA, 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA and 0.2 mg L^{-1} IAA + 1.0 mg L^{-1} kinetin + 0.2 mg L^{-1} BAP were the best combinations for better callusing, higher ability of shoot regeneration and root proliferation due to both varieties while BINA mung 5 had highly significant than BINA mung 7.

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