AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not. Bot. Hort. Agrobot. Cluj 38 (3) 2010, 140-145

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Regeneration of Blackgram (*Vigna mungo* L.) on Changes of Hormonal Condition

Shariful Alam MONY[1)], Md. Shahidul HAQUE[1)], Md. Mahabub ALAM[2)], Mirza HASANUZZAMAN[2)], Kamrun NAHAR[3)]

[1)] *Bangladesh Agricultural University, Department of Biotechnology, Mymensingh-2202, Bangladesh; sa_mony07@yahoo.com*

[2)] *Sher-e-Bangla Agricultural University, Department of Agronomy, Dhaka-1207, Bangladesh; mhzsauag@yahoo.com*

[3)] *Sher-e-Bangla Agricultural University, Department of Agricultural Botany, Dhaka-1207, Bangladesh; knahar84@yahoo.com*

Abstract

The study comprised of experiments for shoot regeneration and plantlet formation from cotyledonary node of *Vigna mungo* by culturing them on low concentration of BAP followed by transfer to hormone free MS medium. Cotyledonary node explants were cultured on different concentrations of BAP (0, 1, 2.5 and 50 mg l^{-1}). Shoot regeneration occurred from cotyledonary nodes irrespective of the presence or absence of BAP in the medium. However, culture of cotyledonary node explants for 10 days on medium containing 1.0 mg l^{-1} BAP followed by transfer to hormone free medium gave higher number of shoots (9.33/explant) compared to culture of the explants on hormone free medium for 15 days followed by transfer to medium containing 1.0 mg l^{-1} BAP (8.33/explants). The regenerated shoots were transferred to rooting medium supplemented with different concentrations of IBA and NAA. The high frequency (100.0%) of rooting was observed with MS medium supplemented with 0.5 mg l^{-1} IBA. The rooted plants were transferred to pots for hardening.

Keywords: blackgram regeneration, cotyledonary node hormone free medium

Abbreviations: BAP: 6-benzylaminopurine; IBA: Indolebutyric acid; DAI: Days after inoculation; DAT: Days after transfer; MS: Murashige and Skoog; NAA: α- naphthaleneacetic acid

Introduction

Blackgram (*Vigna mungo* L. Hepper, syn. *Phaseolus mungo* L.) is one of the most important pulse crops grown in Bangladesh. It belongs to the family *Fabaceae*. Blackgram is an annual food legume. It is also grown in Southern Asia like India, Pakistan, Bangladesh, Afghanistan, Myanmar. Like mungbean, it is a member of Asiatic *Vigna* crop group. It is mainly a day neutral warm season crop commonly grown in semi-arid to sub-humid low land tropics and sub-tropics. Blackgram originated in India where it has been in cultivation from ancient times and is the largest producer and consumer in the world.

Cultivated grain legumes, a valuable source of protein, are unfortunately recalcitrant and a challenge to regeneration (Hammat *et al*., 1986). Exceptionally, select cultivars of soybean can be made to regenerate through somatic embryos (Hammat and Davey, 1997; Parrot *et al*., 1998). Many reported claims of regeneration in grain legumes are either non-reproducible or show poor results.

Morphogenesis is concerned with the shapes of tissues, organs and entire organisms and the positions of the various specialized cell types. Morphogenetic responses may be induced in organisms by hormones, or by environmental chemicals ranging from substances produced by other organisms to toxic chemicals or radionuclides released as pollutants, and other plants. Morphogenesis arises because of changes in the cellular structure or how cells interact in tissues (Scott, 2000). Efficient *in vitro* morphogenesis from any explant requires an appropriate concentration of suitable growth regulator(s).

The study was, therefore, undertaken to determine the optimum concentrations of growth regulators for *in vitro* morphogenesis study in Blackgram and to determine the effect of BAP concentrations for multiplication.

Materials and methods

The seeds of *Vigna mungo* (*2n=22*) were collected from Bangladesh Institute of Nuclear Agriculture. Cotyledonary nodes were used as explants in the present study. Surface sterilization of mature seeds was carried out under laminar air flow cabinet. Seeds were washed by sterile distilled water for 3 to 5 minutes. Later, they were rinsed in 70% ethyl alcohol followed by washing with sterile distilled water for 3 times. Finally, surface disinfection was done with 0.1% $HgCl_2$ for five minutes with continuous agitation. The seeds were then washed 5 times with sterile distilled water to remove the sterilant. The mature seeds were placed on a solidified agar medium containing no growth regulators in sterile vials. Seven to ten days after germination, cotyledonary nodes of seedlings were dis-

sected and cultured on MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of BAP (0, 1, 2.5, 5 and 10 mg l^{-1}) for direct shoot regeneration. Shoots regenerated on medium without growth regulator were transferred to medium containing different concentration of BAP (1, 2.5 and 5 mg l^{-1}). Again, shoots regenerated on medium containing different concentration of BAP were transferred to medium without BAP. For rooting, 5-6 cm shoots excised from multiple shoots were implanted to rooting medium consisting of MS medium supplemented with IBA (0, 0.25, 0.5, 1, 2, 3, 4 or 5 mg l^{-1}) and NAA (0.2 or 0.5 mg l^{-1}).

MS medium supplemented with different concentrations and combinations of growth regulators as per treatments was used for shoot induction, shoot multiplication and rooting of shoots. Hormones were added separately to different media according to their requirements. pH of the media was adjusted to 5.8 and then agar 9.0 g l^{-1} was added to solidify the medium. All instruments, glass wares and culture media were sterilized by autoclaving at 1.16 kg cm^{-2} pressure at 121°C for 30 min. The temperature of the growth room was maintained at 25±2°C. A 16 h light period was maintained with light intensity of 2000 lux. At least 20 explants were cultured in each treatment and all treatments were repeated thrice. One month old adequately rooted shoots when reached a height of 7-8 cm were brought out from culture room and kept in room temperature for 7 days. Plantlets were then taken out from the vials and agar was washed out gently from the roots. These plantlets were transplanted in small pots containing a mixture of compost, soil and sand (1:2:1) and were covered with transparent sheets. They were nurtured under room temperature in presence of sufficient light. In that conditions the plantlets were watered every alternate day.

The data for the parameters recorded in the present study were statistically analyzed by the programmed MSTATC (Russel, 1994). The experiment was conducted following Completely Randomized Design (CRD). The one way analysis of variances for different parameters was performed and the means were compared by Duncan's multiple range test (DMRT) at 5% level of probability for interpretation of results (Gomez and Gomez, 1984) using MSTATC Computer programmed.

Results and discussion

Effect of BAP on shoot initiation from cotyledonary node explants

Cotyledonary node explants were collected from *in vitro* germinated seedlings and cultured on MS medium supplemented with different concentrations of BAP. After few days, the cultured explants were transferred to other hormonal concentrations. The results on the effect of different concentrations of BAP for shoot proliferation have been discussed under different heads.

Days required for shoot initiation

Days required for shoot initiation from cotyledonary node explants were significantly influenced by different BAP concentrations (Tab. 1). The least number of days required for shoot induction was 9.33 on MS medium without BAP followed by 1 mg l^{-1} BAP. The maximum number of days required for shoot induction was 50.67 on MS medium containing 10 mg l^{-1} BAP. There was a dose depended delay in the number of days required for shoot initiation.

Tab. 1. Effect of different levels of BAP on shoot initiation and callus induction from cotyledonary node explants

Concentrations of BAP (mg l^{-1})	Percentage of explants showing shoot initiation	Days required for shoot initiation	Percentage of explants showing callus with shoot initiation
0.0	84.73b	9.33e	19.83d
1.0	93.13a	11.67d	71.67c
2.5	61.13c	15.67c	83.33b
5.0	57.67d	30.67b	84.63a
10.0	43.33e	50.67a	94.67a
CV%	6.63	3.64	6.50
LSD(0.05)	8.334	1.753	8.663

Means having common letter(s) are statistically identical at 5% level of probability

Percentage of explants showing shoot initiation

The results showed that the highest percentage (93.13%) of shoot regenerated on MS medium with 1 mg l^{-1} BAP followed by MS medium without growth regulators (84.73%) (Tab. 1). The lowest percentage (43.33%) was observed in MS medium with 10.0 mg l^{-1} BAP. The percentage of shoot regeneration decreased gradually with the increase of BAP concentration in the medium. The best response towards shoot regeneration from primary leaf callus of Blackgram on MS medium supplemented with 3 mg l^{-1} BAP and 1.5 mg l^{-1} NAA (Geetha *et al*., 1998). We recorded the lowest concentration of BAP as the best treatment in our study. The difference might be due to the difference in species, genotype and explants used. Shoot formation from cotyledonary nodes of legumes cultured on growth regulator free medium is not uncommon. We also found a high percentage of shoot regeneration from cotyledonary nodes on hormone free medium.

Percentage of explants showing callus with shoot initiation

There was a certain percentage of callus formation in addition to shoot regeneration from the explants (Tab. 1). The results showed that the highest percentage (94.67) of callus with shoot regeneration observed in MS medium with 10 mg l^{-1} BAP which was followed by MS medium with 5 mg l^{-1} BAP. The lowest percentage (19.83%) was ob-

served in MS medium without growth regulators. Geetha *et al*. (1998) reported that callus development was best in shoot tip explants with concentration of 3 mg l^{-1} BAP and 1.5 mg l^{-1} NAA. Mathur and Prakash (1997) reported that MS medium supplemented with 0.5 mg l^{-1} kinetin and 2 mg l^{-1} 2, 4-D gave the maximum callus induction in *Vigna mungo*. In the present research, addition of growth regulators in the medium had favorable an influence on callus induction. The percentage of callus induction increased with an increase in the BAP concentration. This also exhibited a concomitant decrease in shoot initiation. Similar results were found with shoot apical meristem cultures of grain legumes, using combinations of an auxin and a cytokinin for callus induction and differentiation (Kartha *et al*., 1981; Rubluo and Kartha, 1985; Gulati and Jaiwal, 1992; Venkatachalam *et al*., 1994).

Number of shoots explant^{-1}

The number of shoots explant^{-1} varied with the BAP concentrations of the shoot initiation and multiplication medium redundant at different DAI (Tab. 2). The number of shoots explant^{-1} was carefully observed at different DAT from shoot initiation medium to shoot multiplication medium. At 5 DAT, the number of shoots explant^{-1} was 2.33 as observed in culture of cotyledonary nodes for 15 days on MS medium without hormone followed by transfer to MS medium containing 1 mg l^{-1} BAP and statistically similar results were observed in culture of explants for 5 days on MS medium containing 1 mg l^{-1} BAP followed by transfer to hormone free MS medium and culture of explants for 10 days on MS medium containing 1 mg l^{-1} BAP followed by transfer to MS medium compared to culture of explants for 15 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium (2.00). Culture of explants for 5, 10 and 15 days on MS medium containing 2.5, 5 and 10 mg l^{-1} BAP followed by transfer to hormone free MS medium produced only 1.0 shoots explant^{-1} (Fig. 1). Significant variations in number of shoots explant^{-1} was observed with the passage of time on the shoot multiplication medium.

At 10 DAT, the higher number of shoots explant^{-1} (4.33) was observed in culture of cotyledonary node explants for 10 days on MS medium containing 1 mg l^{-1} BAP followed by transfer to MS medium without BAP compared to culture of explants for 15 days on MS medium followed by transfer to MS medium containing 1 mg l^{-1} BAP (3.67) (Tab. 2). The lowest number (1.00) was observed in culture of explants for 15 days on MS medium containing 10 mg l^{-1} BAP followed by transfer to hormone free MS medium.

At 15 DAT, the higher number of shoots explant^{-1} (6.33) was observed in culture of cotyledonary node explants for 10 days on MS medium containing 1.0 mg l^{-1} BAP followed by transfer to MS medium without BAP compared to culture of explants for 15 days on MS medium followed by transfer to MS medium containing 1 mg l^{-1} BAP (5.33) (Tab. 2). The lowest number (1.00) was observed in culture of explants for 15 days on MS medium containing 10.0 mg l^{-1} BAP followed by transfer to hormone free MS medium.

Tab. 2. Effect of BAP concentrations of shoot initiation and Shoot multiplication medium on shoot proliferation

BAP Concentration (mg l^{-1})		Days on initiation medium	No. of shoots explant^{-1} at different days after transfer (DAT)				Length of shoots (cm) at different days after transfer (DAT)				No. of leaves shoot^{-1} at 20 DAT
Initiation medium	Multiplication medium		5 DAT	10 DAT	15 DAT	20 DAT	5 DAT	10 DAT	15 DAT	20 DAT	
0.0	1.0	15	2.33a	3.67b	5.33b	8.33b	3.33a	4.20c	5.17e	6.67f	2.67c
0.0	2.5	15	1.00c	2.33cd	2.67fg	3.33f	1.00ef	3.83cd	4.77e-g	5.37g	2.00c
0.0	5.0	15	1.00c	1.33ef	2.00gh	3.00fg	0.93f	3.17e	4.33gh	4.43hi	2.33c
1.0	0.0	5	2.33a	3.33b	4.67bc	6.67c	1.63c	6.40b	8.27b	10.90b	5.33a
1.0	0.0	10	2.33a	4.33a	6.33a	9.33a	1.90b	7.80a	10.97a	12.90a	5.67a
1.0	0.0	15	2.00ab	3.33b	4.33cd	5.33d	1.70c	6.17b	7.67c	10.00c	5.67a
1.0	2.5	5	1.00c	2.00cd	3.33ef	4.33e	1.30d	3.93cd	5.87d	8.90d	4.00b
1.0	2.5	10	1.00c	2.67c	3.67de	5.33d	1.60c	4.07cd	6.20d	10.00c	2.67c
1.0	2.5	15	1.67b	2.33cd	3.33ef	3.67ef	1.17de	3.83cd	5.90d	9.97c	2.00c
1.0	5.0	5	1.00c	1.67de	2.67fg	3.00fg	0.93f	3.17e	4.57f-h	8.50d	2.00c
1.0	5.0	10	1.00c	1.00f	1.67hi	3.67ef	1.03ef	3.67d	4.87ef	8.90d	2.33c
1.0	5.0	15	1.00c	1.00f	1.33hi	2.33gh	1.07ef	2.50f	4.17hi	7.83e	2.00c
1.0	10.0	5	1.00c	1.67f	1.33hi	2.00h	0.53g	1.67g	2.53k	4.00i	2.00c
1.0	10.0	10	1.00c	1.67f	1.33hi	2.00h	0.90f	2.50f	3.83i	5.03gh	2.00c
1.0	10.0	15	1.00c	1.00g	1.00i	2.00h	0.47g	1.77g	2.97j	4.37hi	2.00c
	CV%		29.66	18.59	16.59	11.04	8.75	5.68	4.57	4.95	12.83
	LSD(0.05)		0.4847	0.6280	0.8329	0.7791	0.1907	0.3702	0.4198	0.6499	0.6391

Means having common letter(s) are statistically identical at 5% level of probability

Fig. 1. (a) Cotyledonary node explants of Blackgram after in vitro germination of seeds. Note: Origin of shoot regeneration (see arrow). (b) Shoots and roots initiation from explants on MS medium after 8 days of incubation. (c) Root initiation starts from lower portion of cotyledonary roots on MS medium after 8 days of incubation. (d) Callus induction on MS medium with 5.0 mg l-1. (e-h) Shoots from callus. (e) MS medium supplemented with 1.0 mg l-1 BAP. (f) MS medium supplemented with 2.5 mg l-1 BAP. (g) MS medium supplemented with 5.0 mg l-1 BAP. (h) MS medium supplemented with 10.0 mg l-1 BAP. (i-j) Increasing the shoot number, length and leaf number by culture of explants for 15 days on MS medium followed by transferred to MS medium supplemented with 1 mg l-1 BAP. (i) At 5 days after transfer. (j) At 20 days after transfer. (j-k) Increasing the shoot number, length and leaf number by culture of explants for 10 days on MS medium supplemented with 1.0 mg l-1 BAP followed by transferred to MS medium. (j) At 5 days after transfer. (k) At 20 days after transfer. (m) Root formation on MS medium supplemented with 0.5 mg l-1 IBA. (n) Length of root after 23 days of culture. (o) Transfer of rooted plantlet into plastic pot. (p) An established plantlet of *Vigna mungo*

At 20 DAT, the higher number of shoots explant^{-1} (9.33) was observed in culture of cotyledonary node explants for 10 days on MS medium containing 1 mg l^{-1} BAP followed by transfer to MS medium without BAP compared to culture of explants for 15 days on MS medium followed by transfer to MS medium containing 1.0 mg l^{-1} BAP (8.33) (Tab. 2). The lowest number (2.00) was observed in culture of explants for 15 days on MS medium containing 10.0 mg l^{-1} BAP followed by transfer to hormone free MS medium (Fig. 1i, j, k, l). Similar results were also obtained in various grain legumes (Kartha *et al*., 1981; Rubluo and Kartha, 1985; Rao and Chopra, 1989; Gulati and Jaiwal, 1992; Venkatachalam *et al*., 1994). Culture of explants for a brief period on shoot initiation medium containing BAP followed by transfer to growth regulator free MS medium might be ideal for efficient shoot multiplication. Regenerated shoots become abnormal when cultured for long time on medium containing cytokinin.

Length of shoots

The length of shoots varied with the concentrations of BAP on shoot induction and multiplication medium at different DAI (Tab. 2). The length of shoots was carefully measured at different DAT to the shoot multiplication medium. At 5 DAT, the longest shoots (3.33 cm) was observed in culture of cotyledonary node explants for 15 days on MS medium followed by transfer to MS medium containing 1 mg l^{-1} BAP compared to culture of explants on MS medium containing 1 mg l^{-1} BAP for 10 days followed by transfer to hormone free MS medium (1.90 cm). The shortest shoots (0.47 cm) was observed in culture of explants for 15 days on MS medium containing 10 mg l^{-1} BAP followed by transfer to hormone free MS medium.

At 10 DAT, the longest shoots (7.80 cm) was observed in culture of cotyledonary node explants for 10 days on MS medium containing 1 mg l^{-1} BAP followed by transfer to hormone free MS medium compared to culture of explants for 5 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium (6.40 cm) (Tab. 2). The shortest shoots (1.77 cm) was observed in culture of explants for 15 days on MS medium containing 10 mg l^{-1} BAP followed by transfer to hormone free MS medium. The highest concentration of BAP in shoot initiation medium might have a suppressive effect on shoot length.

At 15 DAT, the longest shoots (10.97 cm) was observed in culture of cotyledonary node explants for 10 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium compared to culture of explants for 5 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium (8.27 cm) (Tab. 2). The shortest shoots (2.97 cm) was observed in culture of explants for 15 days on MS medium containing 10 mg l^{-1} BAP followed by transfer to hormone free MS medium.

At 20 DAT, the longest shoots (12.90 cm) was observed in culture of cotyledonary node explants for 10 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium compared to culture of explants for 5 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium (10.90 cm) (Tab. 2). The shortest shoots (4.37 cm) was observed in culture of explants for 15 days on MS medium containing 10 mg l^{-1} BAP followed by transfer to hormone free MS medium.

Number of leaves shoot^{-1}

At 20 DAT, the highest number of leaves shoot^{-1} (5.67) was observed in culture of cotyledonary node explants for 10 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium and culture of explants for 15 days on MS medium containing 1 mg l^{-1} followed by transfer to hormone free MS medium compared to culture of explants for 5 days on MS medium containing 2.5 mg l^{-1} followed by transfer to hormone free MS medium (4.00) (Tab. 2). The lowest number of leaves shoot^{-1} (2.00) was observed in culture of explants for 15 days on hormone free medium followed by MS medium containing 2.5 mg l^{-1} BAP, 15 days on MS medium containing 2.5 mg l^{-1} BAP followed by hormone free MS medium, 5 days on MS medium containing 5 mg l^{-1} BAP followed by hormone free MS medium, 15 days on MS medium containing 5 mg l^{-1} BAP followed by hormone free MS medium, and 5, 10 and 15 days on MS medium containing 10 mg l^{-1} BAP followed by hormone free MS medium. From the parameters discussed above, it may be concluded that culture of cotyledonary node explants for 10 days on medium containing 1 mg l^{-1} BAP followed by transfer to hormone free medium performed best in term of number of shoots explant^{-1}, length of shoots and number of leaves shoot^{-1}.

Effect of different concentrations of IBA and NAA on rooting of regenerated shoots

The rooting of shoots was significantly affected by the auxin concentration (Tab. 3). MS medium supplemented with 0.25 mg l^{-1} IBA and 0.2 mg l^{-1} NAA performed better and required least number of days (8.33) for rooting. Khawar and Özcan (2002) reported that MS medium containing 0.25 mg l^{-1} IBA performed best and required four weeks for rooting. Geetha *et al*. (1998) reported that roots emerged within 15 days. A higher percentage of rooting (100%) was found with 0.5 mg l^{-1} IBA in the present study (Fig. 1m, n). Raman *et al*. (2004) reported that efficient rooting (100%) of the shoots on medium containing half MS salts, full MS vitamins and IBA (2.5 μM). Khawar and Özcan (2002) reported that medium containing 0.25 mg l^{-1} IBA obtained only 25% roots and Geetha *et al*. (1998) reported that medium containing 3 mg l^{-1} IBA showed 78.3% of rooting. The maximum number of roots (14.33) per shoot was recorded in medium containing 0.5 mg l^{-1} NAA. Geetha *et al*. (1998) reported that medium containing 3.0 mg l^{-1} IBA produced 14.5 roots/plants. It was clear from the above discussion that 0.5 mg l^{-1} IBA was better

Tab. 3. Main effect of different concentrations of hormones on root induction

	Hormone concentrations (mg l^{-1})	Days required for root initiation	Percentage of shoots showing roots	Number of roots $shoot^{-1}$
MS medium		13.33b	76.67b	6.67d
IBA	0.25	8.33f	96.67a	9.67c
	0.50	9.33e	100.0a	11.33b
	1.00	10.33d	90.00a	9.67c
	2.00	10.67d	73.33b	7.67d
	3.00	11.67c	73.33b	7.67d
	4.00	12.33c	70.00b	6.67d
	5.00	14.33a	66.67b	6.67d
NAA	0.20	9.33e	73.33b	14.33a
	0.50	10.67d	53.33c	9.33c
CV%		4.96	7.59	6.75
$LSD_{(0.05)}$		0.9396	10.07	1.039

Means having common letter(s) are statistically identical at 5% level of probability

for root formation than any other treatments. Das *et al*. (2002) and Geetha *et al*. (1998) reported that IBA was effective for rooting of blackgram, while Roy *et al*. (2007) reported that NAA was effective for rooting. In our study we found higher percentage of rooting using IBA while number of roots/plant was higher using NAA.

Healthy plantlets of 7-10 cm in height were planted in a mixture of garden soil, sand and cowdung at the ratio of 2:1:1. Immediately after transplantation, the plants along with pots were kept in diffused sunlight in the controlled environment of the growth room. Survival rate of the transplanted plantlets was 80-85% (Fig. 1 o, p).

References

Das, D. K., B. Prasanna, N. S. Prakash and N. Bhalla-Sarin (2002). Improvement method of regeneration of blackgram (*Vigna mungo* L.) through liquid culture. *In Vitro* Cell. Dev. Biol. Plant. 38(5):456-459.

Geetha, N., P. Venkatachalam and G. R. Rao (1998). *In vitro* plant regeneration from shoot tip culture of blackgram (*Vigna mungo* L. Hepper). J. Tropic. Agric. 36(1/2):6-11.

Gomez, K. A. and A. A. Gomez (1984). Statistical Procedude for Agricultural Research. 2nd Edition Intl. Rice Res. Inst. A willey InterScience Publications.

Gulati, A. and P. K. Jaiwal (1992). *In vitro* induction of multiple shoots and plant regeneration from shoot tips of mungbean (*Vigna radiata* L. Wilczek). Plant Cell Tissue Org. Cult 23:1-7.

Hammat, N. and M. R. Davey (1997). Somatic embryogenesis and plant regeneration from cultured zygotic embryo of soybean (*Glycine max*). J. Plant Physiol. 128:219-226.

Hammat, N., T. K. Ghosh and M. R. Davey (1986). Regeneration in legumes, pp. 66-95. In: I. K. Vasil (Eds.). Cell Culture and Somatic Cell Genetics of Plants, Vol. 3. Academic Press, New York.

Kartha, K. K., N. L. Leung and L. A. Mroginaki (1981). Plant regeneration from meristems of grain legumes; soybean, cowpea, peanut, chickpea and bean. Can. J. Bot. 59:1671-1979.

Khawar, K. M. and S. Özcan (2002). Effect of Indole-3-Butyric Acid on in vitro Root Development in Lentil (*Lens culinaris* Medik.). Turkey J. Bot. 26:109-111.

Mathur, V. L. and O. M. Prakash (1997). *In vitro* studies in *Vigna mungo* L. Hepper. Legume Res. 20(3/4):203-206.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Parrot, W. A., G. Dryden, S. Vogt, D. F. Hildebrandt, G. B. Collins and E. G. Willams (1998). Optimisation of somatic embryogenesis and embryo germination in soybean. *In Vitro* Cell Dev. Biol. Plant 24:87-820.

Raman, S., M. Sonia, M. Seema, B. Anila and P. K. Jaiwal (2004). An improved protocol of plant regeneration via somatic embryogenesis in cell suspension cultures of blackgram (*Vigna mungo* L. Hepper). Physiol. Mol. Biol. Plants 10(1):121-125.

Rao, B. G. and V. L. Chopra (1989). Regeneration from apical meristem, stem nodes and cotyledon of chickpea. Indian J. pulses Res. 2:20-24.

Roy, S. K., M. S. Haque and M. K. Siddiqua (2007). Plant regeneration through multiple shoot formation in blackgram. Progress. Agric. 18(2):11-16.

Rubluo, A. and K. K. Kartha (1985). *In vitro* culture of shoot apical meristem of various Phaseolus species and cultivars. J. Plant Physiol. 119:425-433.

Russel, O. F. (1994). MSTAT-C v.2.1 (a computer based data analysis software). Crop and Soil Science Department, Michigan State University, USA.

Scott, F. G. (2000). Morphogenesis and cell adhesion, in Developmental Biology, 6th edn. Sinauer Associates, Inc.

Venkatachalam, P., A. S. Pillai and N. Jayabalan (1994). Plant regeneration from cultured apical meristems of groundnut (*Arachis hypogea* L.). Proc. Nat. Acad. Sci. 64:99-103.