Reprint **ISSN 1991-3036 (Web Version)**

International Journal of Sustainable Crop Production (IJSCP)

(*Int. J. Sustain. Crop Prod.*)

Volume: 7 **Issue: 3** **November 2012**

Int. J. Sustain. Crop Prod. 7(3): 1-7 (November 2012)

IN VITRO PLANT REGENERATION OF LENTIL (*Lens culinaris* Medik.) USING LEAF AND NODAL EXPLANTS

S. SULTANA, M. AKTARUZZAMAN, U. HABIBA AND T. AFROZ

GGF
Nature is Power

An International Scientific Research Publisher
Green Global Foundation©
Publication and Bibliography Division
100 Leeward Glenway
Apartment # 1601
M3c2z1, Toronto, Canada
E-mails: publication@ggfagro.com, editor@ggfagro.com
http://ggfagro.com/ejournals/current issues

IJSCP** issn 1991-3036, HQ:19-10 cantral place, saskatoon, saskatchewan, s7n 2s2, Canada

ISSN-1991-3036 (Online)
Int. J. Sustain. Crop Prod. 7(3): 1-7 (November 2012)

IN VITRO PLANT REGENERATION OF LENTIL (*Lens culinaris* Medik.) USING LEAF AND NODAL EXPLANTS

S. SULTANA[1], M. AKTARUZZAMAN[2], U. HABIBA[3] AND T. AFROZ[4]

[1]Department of Genetics and Plant Breeding, Hajee Mohammand Danesh Science and Technology University, Dinajpur, Bangladesh; [2]Senior Agriculture officer, RDRS Bangladesh; [3]Department of Biotechnology, Bangladesh Agriculture University, Mymansingh, Bangladesh; [4]Department of Plant Pathology, Hajee Mohammand Danesh Science and Technology University, Dinajpur, Bangladesh.

Corresponding author & address: Md. Aktaruzzaman, E-mail: jewellhstu.plp@gmail.com
Accepted for publication on 25 October 2012

ABSTRACT

Sultana S, Aktaruzzaman M, Habiba U, Afroz T (2012) *In vitro* plant regeneration of lentil (*Lens culinaris* Medik.) using leaf and nodal explants. *Int. J. Sustain. Crop Prod.* 7(3), 1-7.

The investigation was carried out in the Tissue Culture Laboratory of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during November, 2009 to April, 2010 with a view to study *in vitro* regeneration of Lentil (*Lens culinaris* Medik.). For this purpose, three varieties of Lentil *viz.* BARI Masur-3, BARI Masur-4 and BARI Masur-5 & different concentrations and combinations of hormones (2, 4-D, NAA, BAP, Kn and IAA) were used to assess regeneration ability by using two explants (leaf disc and nodal segments). Leaf disc and nodal segments of the three genotypes of Lentil were cultured on MS medium with different concentrations and combinations of growth regulators. Among the three varieties, BARI Masur-4 showed early callusing (17.67 days) with maximum rate of callus induction (83.33%) incase of leaf disc culture and also showed highest percentage of callus induction (80.55%) with minimum days (17.33 days) with nodal segment as explant. Early and maximum rate of callusing appeared in (MS + 1.5 mg/L 2, 4-D + 0.5 mg/L BAP) and (MS + 2 mg/L 2, 4-D + 0.5 mg/L NAA) from leaf disc and nodal segment respectively. BARI Masur-4 had the highest percentage of shoot regeneration from leaf disc (69.44%) and nodal segment (66.66%) explants. Early and maximum rate of regeneration was found in (MS + 2.0 mg/L Kn + 0.2 mg/L NAA) and (MS medium + 0.5 mg/L BAP + 0.25 mg/L Kn). The highest number of roots per shoot was counted in BARI Masur-4 (47.23%), in (MS medium containing 10mg/L IAA) and (MS medium containing 15mg/L IBA) considering leaf disc and nodal segment as explant. Considering the overall performance, BARI Masur-4 appeared to be the best genotype for callus formation, shoot regeneration and root formation.

Key words: *in vitro, regeneration, tissue culture, lentil, Lens culinaris*

INTRODUCTION

Lentil (*Lens culinaris* Medik.) popularly known as Masur is an important grain legume of Bangladesh. This commonly grown as pulse crop belongs to the sub-family Faboideae (Papilionaceae) under the family Fabaceae (Leguminosae). According to Ladizinsky (1979) lentil has been originated in Southern Turkey. Cubero (1984) in a detailed review concluded that the region between western Turkey and Kurdis could be its place of origin. It is the oldest and is one of the valuable pulse crops of the world. It is grown in India, Bangladesh, Pakistan, Egypt, Greece, Italy, and countries in the Mediterranean basin, Switzerland, U.S.A.

Lentil (*Lens culinaris* Medik.) is the main supporting food supplement to rice in Bangladesh. But average lentil yield is poor and it cannot compete with cereal crops. Bangladesh is an agro-based country in the South-East Asia and lentil is one of the major pulses grown in Bangladesh. According to FAO (2009) the area harvested of pulses is 312 thousand ha and production is 258 thousand tons in Bangladesh. The cultivated area of lentil is 179354 acre and yield per acre is 399 kg and production is 71535 M. tons (BBS 2008).

Lentil is considered as the poor man's meat as it is the cheapest source of protein for under privilege people who cannot afford to buy animal protein (Gowda and Kaul, 1992). Pulses have three or four times more protein content than rice and ten to fifteen times more than potatoes (Mian 1976). The protein content of Lentil seeds is found to vary from 21.75% to 32.48% (Dimitrove 1973) while the protein contents of rice and wheat are 7.5% and 11.9% respectively. Not only the seeds but also bushy stem and leaf portion of the plant contain considerable amount of protein, which may be used as animal feed. Lentil may become the major source of income for farmers. As lentil has a significant contribution in our economy that is why, more and more attention should be given for improving its quality and higher production.

In addition to conventional breeding procedures, mutation breeding has been attempted in order to evolve high yielding varieties but none of the above methods were successful. The failure was attributed to lack of resistance sources in the available lentil germplasm.

Tissue culture techniques may also be utilized conveniently to overcome incompatibility barrier through fusion of protoplasts from vegetative cells of interspecific, intergenceric and interfamiliar group (Rao and Chadha, 1986; Rao 1985). The regeneration of plants from tissue culture is an important and essential component of biotechnological research and sometimes it is required for the genetic manipulation of plant. The techniques of plants tissue culture have been developed as a new and powerful tool for crop improvement (Carlson 1975; Razdan and Cocking, 1981). So, there is no doubt that in vitro regeneration in lentil has the great potentiality for improving lentil.

Copyright© 2012 Green Global Foundation (GGF)

MATERIALS AND METHODS

Location, time and duration of the experiment

The present study was conducted in the Tissue Culture Laboratory of the Department of Genetics and Plant Breeding, HSTU, Dinajpur, Bangladesh during November 2009 to April 2010.

Experimental materials

The experimental material used in the present investigation is the seed of three Lentil (*Lens culinaris* Medik.) varieties *viz.* BARI Masur-3, BARI Masur-4 and BARI Masur-5 were used to study different parameters associated with plant regeneration.

Sources of the experimental materials

The seed materials of three Lentil (*Lens culinaris* Medik.) varieties were collected from the Bangladesh Agricultural Development Corporation (BADC), Dinajpur, Bangladesh.

Methods

Various culture media were used in the present investigation depending on specific purposes. A list of them is given below:

A. For Seed Germination

Half strength MS medium (Murashige and Skoog, 1962) medium was used for seed germination.

B. For callus induction

I. T_1 = MS medium containing 1 mg/L 2, 4-D
II. T_2 = MS medium containing 1.5 mg/L 2, 4-D + 0.5 mg/L BAP
III. T_3 = MS medium containing 2 mg/L 2, 4-D + 0.5 mg/L NAA

C. For Shoot regeneration

I. T_1 = MS medium containing 1.5 mg/L Kn
II. T_2 = MS medium containing 2.0 mg/L Kn + 0.2 mg/L NAA
III. T_3 = MS medium containing 0.5 mg/L BAP+ 0.25 mg/L Kn

D. For root formation

I. T_1 = Hormone free 0.5 strength MS medium (Evans *et al.* 1981)
II. T_2 = MS medium containing 15 mg/L IBA
III. T_3 = MS medium containing 10 mg/L IAA

Sterilization of experimental materials (Seed)

Matured seeds of three varieties of lentil were washed in running tap water for 3-5 minutes for two or three times to remove the surface organism. The floating seeds were discarded. Later the seeds were dipped in 70% ethyl alcohol for 3-5 minutes with gentle shaking followed by washing with sterile distilled water. Surface disinfections was done by the use of sodium hypochlorite solutions (1% active chlorine) containing 1-2 drops of tween-20 for ten minutes with gentle shaking and then rinsed five times in sterile distilled water. These sterilized seeds were then ready for keeping in the MS (Murashige and Skoog, 1962) media.

Recording of Data

Number of explants with callus (Percent callus induction)

The number of explants producing in each vial was recorded. The percentage of callus induction was calculated on the basis of the number of explants placed and total number of callus induced.

$$Percent\ callus\ induction = \frac{No.\exp lants\ induced\ Calli}{Total\ no.of\ \exp lant\ \ incubated} \times 100$$

Number of callus with shoot (percent shoot regeneration)

Number of callus with shoot was recorded and the percentage of shoot regeneration was calculated by following formula.

$$Percent\ \ shoot\ regeneration = \frac{No.of\ \ calli\ with\ \ shoot}{Total\ no.of\ \ incubated\ \ calli} \times 100$$

Number of shoots with roots

Average number of shoots with roots calculated by using the following formula:

$$\overline{X} = \frac{\sum Xi}{n}$$

Where, $\overline{X}$ = mean of shoots with roots
Σ= Summation
Xi= number of shoots with roots
n = number of observations

Statistical analysis of data

The data for the parameters under present study were statistically analyzed wherever applicable. The Duncan's Multiple Range Test (DMRT) compared the analysis of variance for different parameters.

RESULTS AND DISCUSSION

Analysis of variance for total no. of explants showing callus, percentage of callus induction and days required for callus induction showed significant mean sum of square difference for both variety and treatment (Table 1).

Table 1. Analysis of variance for different parameters related to callus induction

Sources of variation	Degrees of freedom (df)	Leaf			Nodal segment		
		Total no. of explants showing callus	% Callus induction	Days required for callus initiation	Total no. of explants showing callus	% Callus induction	Days required for callus initiation
Variety	2	1.000 *	69.472 *	1.593 *	0.333 *	23.148 *	0.481 *
Treatment	2	4.778**	331.877**	29.481*	4.111**	285.691**	26.037*
Variety x treatment	4	0.111 **	7.724 **	0.370 *	0.444 **	30.877 **	0.593 *
Error	18	0.370	25.730	0.778	0.222	15.444	0.704

** Indicates significant at 1% level of probability and * indicates significant at 5% level of probability

Combined effects of different treatments and variety on callus induction of Lentil (*Lens culinaris* Medik)

Combined effect of different treatment and variety interactions on different parameter such as, number of explants with callus, percent callus induction and days required for callus initiations are presented in the Table 2.

Callus induction from leaf discs:

The variety BARI Masur-4 showed best performance with T_2 (MS + 1.5 mg/L 2, 4-D + 0.5 mg/L BAP) showing maximum number of explants showing callus (10.00) and percent callus formation (83.33%) followed by T_3 (MS + 2 mg/L 2, 4-D + 0.5 mg/L NAA) and T_1 (MS + 1 mg/L 2, 4-D).

Days required for callus initiation (17.67 days) was early in T_2 (MS + 1.5 mg/L 2, 4-D + 0.5 mg/L BAP) and callus initiation were late (21.00 days) in T_1 (MS + 1 mg/L 2, 4-D).

Callus induction from nodal segment:

The maximum number of explants showing callus (9.667) and percent callus formation (80.55%) was found in BARI Masur-4 genotype with T_3 (MS + 2 mg/L 2, 4-D + 0.5 mg/L NAA) followed by T_2 (MS + 1.5 mg/L 2, 4-D + 0.5mg/L BAP) and T_1 (MS + 1 mg/L 2, 4-D).

The treatment T_3 (MS + 2 mg/L2, 4-D + 0.5 mg/L NAA) also took minimum days (17.33 days) and shoot initiation (21.00 days) was late in T_1 (MS +2 mg/L 2, 4-D + 0.5 mg/L NAA). The present findings showed conformity with Mathur and Prakash (1997).

Table 2. Combined effects of different treatments and variety on callus induction of Lentil (*Lens culinaris* Medik.)

Interaction Treatment x Variety		Leaf			Nodal segment		
Treatment	Variety	Number of explants showing Callus	% Callus formation	Days required for callus initiation	Number of explants showing Callus	% Callus formation	Days required for callus initiation
T_1 MS+1 mg/L 2, 4-D	BARI Masur-3	8.000 d	66.66 d	21.00 a	8.000 b	66.66 b	21.33 a
	BARI Masur-4	9.667 ab	80.55 ab	17.33 b	8.533 b	71.44 b	17.33 b
	BARI Masur-5	8.667 b-d	72.22 b-d	19.67 a	8.333 b	69.44 b	19.33 a
T_2 MS+1.5 mg/L 2, 4-D+0.5 mg/L BAP	BARI Masur-3	9.000 a-d	75.00 a-d	20.00 a	8.000 b	66.66 b	20.00 a
	BARI Masur-4	10.00 a	83.33 a	17.67 b	8.667 a	78.55 ab	17.00 b
	BARI Masur-5	9.333 a-c	77.78 a-c	20.67 a	8.333 b	69.44 b	20.33 a
T_3 MS+2.0 mg/L 2, 4-D+0.5 mg/L NAA	BARI Masur-3	8.333 cd	69.44 c-d	20.67 a	8.333 b	69.44 b	20.33 a
	BARI Masur-4	9.00 a-d	81.33 ab	16.67 b	9.667 a	80.55 a	17.33 b
	BARI Masur-5	9.000 a-d	75.00 a-d	20.00 a	8.000 b	66.66 b	20.67 a
LSD at 0.05%		1.043	8.701	1.513	0.8082	6.741	1.439
CV (%)		6.68	6.68	4.57	5.51	5.51	4.37

Treatment and Variety interaction on shoot induction parameters of Lentil (*Lens culinaris* Medik.)

Results related to treatment and variety interaction for the characters of shoot regeneration such as percent shoot regeneration and days required for shoot initiation in different concentrations of growth regulators showed significant variations. The results are presented in Table 3.

Using Leaf as Explant

Best interaction was found in BARI Masur-4 on percent shoot regeneration (69.44) in T_2 (MS+2.0 mg/L Kn + 0.2 mg/L NAA) followed by BARI Masur-5 and BARI Masur-3 showed lowest performance (50.00%) on percent shoot regeneration in T_1 (MS medium + 1.5 mg/L Kn).

Time required for shoot initiation was minimum (25.67 days) on the interactions of T_2 (MS + 2.0 mg/L Kn + 0.2 mg/L NAA) with BARI Masur-4 and maximum (28.67days) on the interactions T_1 (MS medium + 1.5 mg/L Kn) with BARI Masur-3 (Table 3). All the genotypes showed satisfactory results against T_2 (MS + 2.0mg/L Kn + 0.2 mg/L NAA) treatment.

From the above results, it may be concluded that BARI Masur-4 with T_2 (MS + 2.0mg/L Kn + 0.2 mg/L NAA) showed the best performance on shoot regeneration.

Using Nodal Segment as Explant

BARI Masur-4 also give superior result on percent shoot regeneration (66.66%) with T_3 (MS medium +0.5 mg/L BAP + 0.25 mg/L Kn) (Table 3) followed by BARI Masur-5 and BARI Masur-3 showed lowest performance (50.00%) on percent shoot regeneration in T_1 (MS medium + 1.5 mg/L Kn).

The time requirement for Shoot initiation was minimum (25.30 days) on the interactions of T_3 (MS medium + 0.5 mg/L BAP + 0.25 mg/L Kn) with BARI Masur-4 and maximum (29.33 days) on the interactions T_1 (MS medium + 1.5 mg/L Kn) with BARI Masur-3 (Table 3). All the genotypes showed satisfactory results against T_3 (MS medium +0.5mg/L BAP + 0.25 mg/L Kn) treatment.

In the above investigation, it may be concluded that T_3 (MS medium + 0.5 mg/L BAP + 0.25 mg/L Kn) showed the best performance on shoot regeneration. Contrary to these findings, Raghuvanshi and Singh (1989), Khanam (1994) and Khanam *et al*. (1995) obtained best response in multiple shoot regeneration on MS medium containing 0.5 mg/l BAP + 0.5 mg/l Kn + 0.2 mg/l NAA + 100 mg/l CH. Moreover, Polanco *et al*. (1988) reported the formation of multiple shoots on MS medium with BAP (2.0 mg/l) and NAA (0.2 mg/l) in lentil.

Table 3. Treatment and Variety interaction on shoot induction parameters of Lentil (*Lens culinaris* Medik.)

Interaction Treatment x Variety		Leaf			Nodal segment		
Treatment	Variety	Total number of calli with shoot	% Shoot regeneration	Days required for shoot initiation	Total number of calli with shoot	% Shoot regeneration	Days required for shoot initiation
T_1 MS+1.5 mg/L Kn	BARI Masur-3	6.000 e	50.00 e	28.67 a	6.000 b	50.00 b	29.33 a
	BARI Masur-4	7.667 a-c	63.88 a- c	25.67 d	6.667 b	50.00 b	25.33 d
	BARI Masur-5	6.667 c-e	55.55 c-e	28.67 a	6.333 b	52.78 b	28.00 bc
T_2 MS+2.0 mg/L Kn+0.2 mg/L NAA	BARI Masur-3	7.000 b-e	58.33 b-e	27.00 c	6.000 b	50.00 b	28.67 ab
	BARI Masur-4	8.333 a	69.44 a	25.67 d	7.607 a	60.88 a	25.33 d
	BARI Masur-5	7.333 a-d	61.11 a-d	27.00 c	6.333 b	52.78 b	27.00 c
T_3 MS+0.5 mg/L BAP+0.25 mg/L Kn	BARI Masur-3	6.333 de	52.78 de	27.33 bc	6.333 b	52.78 b	29.00 ab
	BARI Masur-4	8.000 ab	63.88 a	25.30 d	7.667 a	66.66 ab	25.67 d
	BARI Masur-5	7.000 b-e	58.33 b-e	28.00 abc	6.000 b	63.66 a	28.33 c
LSD at 0.05%		1.094	9.122	0.9899	0.8082	6.736	0.9899
CV (%)		8.93	8.93	2.14	7.19	7.19	2.12

Treatment and Variety interaction on root formation of Lentil (*Lens culinaris* Medik.)

Treatment and variety interaction for the characters of root regeneration such as percent root formation and days required to root formation in different concentrations treatments showed significant variations. The results are presented in Table 4.

Using Leaf as Explant

The interaction of T_3 (MS medium containing 10mg/L IAA) with BARI Masur-4 was found more effective one in percent root regeneration (47.23%) followed by T_2 (MS medium containing 15mg/L IBA) and T_1 (hormone free 0.5 strength MS medium) with BARI Masur-3 (39.67%).

The time required for root initiation was minimum (15.30 days) on the interaction of T_3 (MS medium containing 10 mg/L IAA) with BARI Masur-4 and maximum (17.67 days) on the interaction T_1 (Hormone free 0.5 strength MS medium) with BARI Masur-3 (Table 4).

From the above results, it may be concluded that T_3 (MS medium containing 10 mg/L IAA) with BARI Masur-4 showed the best performance on root regeneration. Similar results also reported by Anju and Pawan (1992).

Using Nodal Segment as Explant

Among the three varieties, BARI Masur-4 showed best performance (47.23%) on percent root regeneration in T_2 (MS medium containing 15 mg/L IBA). But in contrast, BARI Masur-3 showed the lowest performance (37.27%) on percent root regeneration with T_1 (Hormone free 0.5 strength MS medium).

Days required for root initiation was minimum (15.33 days) on the interaction of T_2 (MS medium containing 15 mg/L IBA) with BARI Masur-4 and maximum (17.33 days) on the interaction T_1 (Hormone free 0.5 strength MS medium) with BARI Masur-3 (Table 4).

From the above discussion, it may be concluded that T_2 (MS medium containing 15 mg/L IBA)) with BARI Masur-4 showed the best performance on root regeneration. The investigation is similar with those of Das *et al.* (2002), Geetha *et al.* (1997).

Table 4. Treatment and Variety interaction on root formation of Lentil (*Lens culinaris* Medik.)

Interaction Treatment x Variety		Leaf			Nodal segment		
Treatment	Variety	No. of shoots with root	% Root formation	Days required root formation	No. of shoots with root	% Root formation	Days required root formation
T_1 (Hormone free 0.5 strength MS medium)	BARI Masur-3	4.000 c	39.67 c	17.67 a	3.000 c-d	37.27 c-d	17.33 a
	BARI Masur-4	5.667 a	44.22 a	15.67 c-d	5.637 a	45.22 a	15.67 b
	BARI Masur-5	4.990 b	43.01 b	16.67 a-c	4.303 ab	42.90 ab	15.67 b
T_2 (MS+15 mg/L IBA)	BARI Masur-3	5.000 c	40.67 c	17.60 a	3.020 c-b	39.67 c	17.67 a
	BARI Masur-4	5.667 a	45.22 a	15.33 d	5.687 a	47.20 a	15.33 b
	BARI Masur-5	5.323 b	42.45 b	16.33 b-d	5.310 ab	43.45 ab	16.00 b
T_3 (MS+10 mg/L IAA)	BARI Masur-3	5.030 c	41.67 c	17.00 a-b	3.000 c-d	40.67 ac	17.33 a
	BARI Masur-4	5.697 a	47.23 a	15.30 d	5.667 a	46.22 a	15.34 b
	BARI Masur-5	5.363 ab	44.45 b	16.00 b-d	4.333 ab	43.45 b	15.36 b
LSD at 0.05%		0.2082	6.236	1.190	0.5082	6.536	1.094
CV (%)		8.84	8.83	4.23	8.84	8.83	3.94

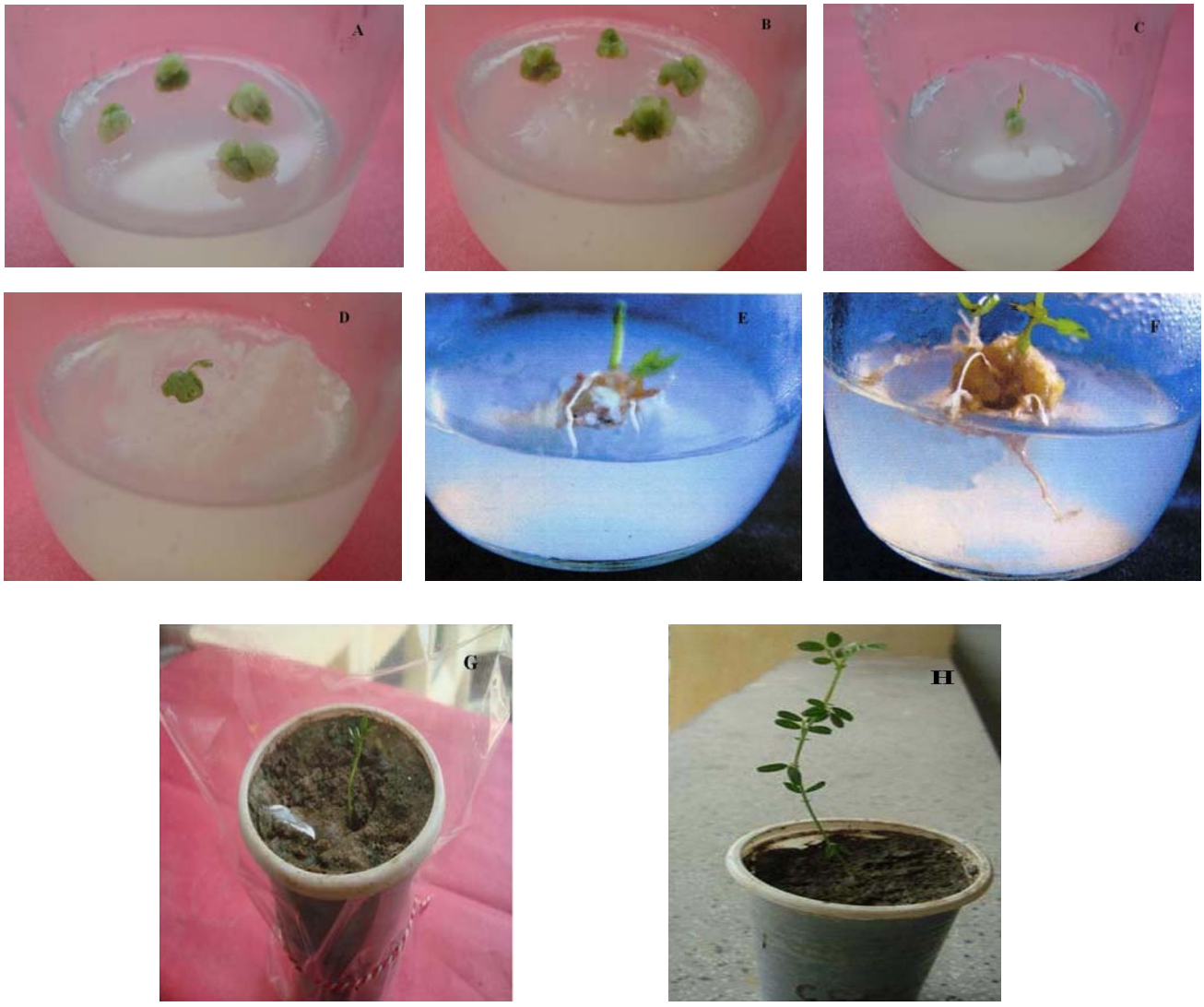

Fig. 1. A: Development of callus with MS + 1.5 mg/L 2, 4-D + 0.5 mg/L BAP using leaf**, B:** Development of callus with MS + 2 mg/L 2, 4-D + 0.5 mg/L NAA using nodal segment, **C:** Initiation of shoot from the callus with MS + 2.0 mg/L Kn + 0.2 mg/L NAA using leaf**, D:** Initiation of shoot from the callus with MS + 0.5 mg/L BAP + 0.25 mg/L Kn. using nodal segment, **E:** Root initiation from regenerated shoot on MS + 10 mg/L IAA using leaf**, F:** Root initiation from regenerated shoot on MS + 15 mg/L IBA using nodal segment**, G:** Hardening of regenerated plant **H:** Survival of plant

CONCLUSION

In conclusion, the present study it can be concluded that among three varieties, BARI Masur-4 showed best performance followed by BARI Masur-5 and BARI Masur-3. When the combined effect of explant was considered, leaf showed better performance than nodal segment. Incase of callus induction MS + 1.5 mg/L 2, 4-D + 0.5 mg/L BAP and MS + 2 mg/L 2, 4-D + 0.5 mg/L NAA, for shoot induction MS + 2.0 mg/L Kn + 0.2 mg/L NAA and MS medium + 0.5 mg/L BAP + 0.25 mg/L Kn, for root initiation MS medium + 10 mg/L IAA and MS medium containing 15 mg/L IBA were best with leaf disc and nodal segment explant respectively.

REFERENCES

Anju G, Pawan KJ (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.

BBS (Bangladesh Bureau of Stastistics) (2008) Stastical Year Book of Bangladesh; Bangladesh Bur. Stat., Stat. Div., Min. Plan. Govt. People's Repub. Bangladesh. p.2.

Carlson PS (1975) Crop improvement through technique of plant cells and tissue cultures. *Biol. Sci*. 25, 247-749.

Cubero JI (1984) Texonomy, distribution and evaluation of lentil and its relatives. The Hugue . The Netherland. p. 187-203.

Das DK, Roy M, Mandal N (2002) *In vitro* organogenesis from shoot tip in blackgram. *Indian J. Genet. Plant Breed.*62(1), 91-92.

Dimitrove DG (1973) Effect of growth condition on protein in lentil. Field Crop Abst. 28(1), 33.

FAO (Food and Agricultural Organization) (2009) STASTICS DIVITION FAO 2009. FAO Stastical Year Book 2009. Rome, Italy.

Geetha N, Venkatachalam P, Rao GM (1997) *In vitro* plant regeneration from different seedling explants of blackgram (*Vigna mungo* (L.) Hepper) via organogenesis. Breed. Sci. 47(4), 311-315, 389.

Gowda CLL, Kaul AK (1992) Pulses in Bangladesh, BARI and FAO publication. 6(1), 27-29.

Khanam R (1994) Study of *in vitro* morphogenesis in lentil (*Lens culinaris* Medik.). M. Sc. thesis, Department of Botany, University of Dhaka, Bangladesh.

Khanam R, Sarker RH, Hoque MI, Haque MM (1995) *In vitro* root morphogenesis in lentil (*Lens culinaris* Medik.). Plant Tissue Cult. 5(1), 35-41.

Ladizinsky G (1979) The origin of lentil and its wild gene pool. Euphytica, 28, 179-187.

Mathur VL, Om Prakash (1997) *In Vitro* studies in *Vigna mungo* L. Hepper. Legume Res. 20(3/4), 203-206.

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

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

Polanco MC, Pelaez MI, Ruiz ML (1988) Factors affecting callus and shoot formation from *in vitro* cultures of *Lens culinaris* Medik. Plant Cell, Tissue and Organ Cult. 15(2), 175-182.

Rao PS (1985) Plant protoplast: a new tool in plant biotechnology. Current Science. 54(7), 335-336.

Rao PS, Chadha MS (1986) Protoplast culture of some economically important plant. Studies on plant regeneration. Proceedings of a symposium organized jointly by International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations, 19-23 August, Vienna. pp. 493-496.

Razdan MK, Cocking EC (1981) Improvement of legumes by exploiting extra specific genetic variations. Euphytica 30, 819-833.

Raghuvanshi SS, Singh RK (1989) Plantlet regeneration from nodal segment and shoot tip derived expants of lentil. Lens Newsl. 16: 1, 33-35.