INFLUENCE OF EXPLANTS, NAA AND BAP CONCENTRATIONS ON IN-VITRO CALLUS INDUCTION IN CUCUMBER VARIETIES

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(Received 25th June 2022; accepted 05th September 2022)

Abstract. In vitro callus induction in cucumber varieties as influenced by explants and different concentrations and combinations of NAA and BAP was studied. Three cucumber varieties viz. Shila, Green Field (GF) and Shital; three types of explant viz. leaf, node and internode; three NAA concentrations viz. 1.0, 2.0 and 3.0 mg/l and three BAP concentrations viz. 0.0, 1.0 and 2.0 mg/l were used in this investigation. Maximum number of callus was obtained from variety Shital (5.59) followed by Green Field (5.01) and Shila (4.19), respectively. Different explants showed significant difference in all the parameters studied but internodal explants were superior over other two explants. Internodal explants took minimum time (10.53 days) for callus induction whereas leaf segments took maximum time (15.38 days). The internodonal explants of variety GF produced the highest weighted (3.49 gm) callus when they were interacted with 3.0 mg/l NAA and 2.0 mg/l BAP in MS medium and the same variety also produced the lowest weighted (0.99 gm) callus when it's nodal explants cultured in MS medium supplemented only with 3.0 mg/l NAA.
Keywords: *callus weight, cytokinin, auxin, explant*

Introduction

Cucumber (Cucumis sativus L.) (2n = 14), a member of the family Cucurbitaceae, is one of the oldest vegetable crop supposed to be originate in India, between the Bay of Bengal and the Himalayas (Peirce, 1987). Cucumis sativus L. is a cucumber species which has commercial importance (Nonnecki, 1989). In Bangladesh average yield of cucumber during 2002-'03, 2003-'04 and 2004-'05 were 4.45, 4.45 and 4.37 mt/ha, respectively (BBS, 2006) which indicate that the yield has declined slightly. Yield of cucumber is very low in our country compared to leading cucumber producing countries like China (12.24 t/ha), former USSR (7.57 t/ha), Japan (44.23 t/ha), USA (11.06 t/ha), Turkey (16.07 t/ha), Netherlands (192.50 t/ha), Spain (30.00 t/ha) (Nonnecki, 1989).

Different biotic and abiotic factors are responsible for low yield of cucumber in our country. Abiotic stresses include drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural states of environment (Wang et al., 2003). Without these, the population of our country is increasing day by day but land is decreasing. Therefore, we need to utilize the lands which are not under cultivation at present, such as, coastal zone which have high saline properties. That's why we need millions of healthy cucumber seedlings in a short period of time. In the past, conventional breeding methods developed various crop species with improved environmental stress tolerance (Cullins, 1991) but they are time consuming and laborious. Efficient plant regeneration from cultured cells and tissues/calli is a pre-requisite for the successful application of biotechnology in crop improvement. We can

transfer desire gene(s) into callus through genetic transformation system. The use of genetic transformation may allow the production of abiotic stress tolerant/resistant plants in a significantly shorter period of time than using conventional breeding, especially if several traits are introduced at the same time. Keeping in mind the above facts, the present investigation was conducted.

Materials and Methods

The experiment was carried out at the Biotechnology Laboratory, under the Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh. There were 4 factors in this experiment. Factor A consisted of three cucumber varieties viz. Shila, Green Field (GF) and Shital; factor B consisted of three types of explant viz. leaf, node and internode; factor C consisted of three NAA concentrations viz. 1.0, 2.0 and 3.0 mg/l and factor D consisted of three BAP concentrations viz. 0.0, 1.0 and 2.0 mg/l were used in this investigation. So, total number of treatments was 81 (3x3x3x3). Each treatment consisted of 2 test tubes/vials and replicated 3 times. The experiment was laid under Completely Randomized Design (CRD). A nutrient medium for plant regeneration usually consists of organic and inorganic salts, irons, a carbon source, some vitamins and growth regulators. In this study MS (Murashige and Skoog, 1962) medium was used as basal medium.

Leaf segments having 0.5-1.0 cm length, 0.50-0.75 cm width and nodes and internodes having 0.75 -1.25 cm lengths were prepared. The explants were collected from three in vitro grown cucumber plantlets. To maintain aseptic condition, precautions were taken in every step of works. All inoculations and aseptic manipulations were carried out in a laminar airflow cabinet. It is usually started half an hour before use. The cabinet was wiped with 70% ethyl alcohol (C2H5OH) to reduce the chances of contamination. The inoculating instruments like scalpels, forceps etc. were sterilized. Other required materials like distilled water, hard papers etc. were sterilized by autoclave. Hands were properly washed with soap before starting work in laminar airflow cabinet. During operation, hands and cabinet base were rubbed with 70% ethyl alcohol frequently for maintaining clean condition. To obtain possible contamination free condition in clean bench proper care was taken during explant preparation. Leaf, nodal and internodal segments were prepared in the laminar airflow cabinet using a fine sterile forcep and scalpel. The excised explants were then inoculated on to the culture test tubes/vials containing MS medium supplemented with various concentrations and combinations of NAA (1.0, 2.0 and 3.0 mg/l) and BAP (0.0, 1.0 and 2.0 mg/l) for in vitro calli formation. The physical conditions for growth and development of cultures were maintained at the temperature of 25 ± 10C and a light intensity of 2000-3000 lux provided by fluorescent tube. The photoperiod was maintained at 16 hours light and 8 hours dark (16L/8D) and the relative humidity was 60-70%. First subculture was carried out at 25 days after explant inoculation. Second and third subculture was carried out at 50 and 75 days after explant inoculation, respectively. During subculture callus were transferred onto fresh medium containing the same hormonal concentrations for further proliferation and development. Weight of callus was measured at second subculture.

Data on number of explants produced callus, percentage of explants produced callus, days to callus initiation and weight of callus at second subculture (gm) were recorded. The data were analyzed using MSTAT-C statistical software. Differences among the

means were compared following Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Results and Discussion

Influence of variety

All the varieties produced callus after ten to fifteen days of explant inoculation. The varieties showed significant difference in all the parameters studied (*Table 1*). Regarding number of explants producing callus, maximum number of callus was produced by Shital (5.59) followed by Green Field (5.01) and Shila (4.19), respectively. In a study with seven cultivars of cucumber, Lou and Kako (1994) observed that all cultivars formed callus from first leaves and internode explants except 'Aonagajibai'. The highest percentage (93.17 %) of calli was obtained from variety Shital which was followed by Green Field (83.62 %) (*Figure 1*). Shila produced the lowest percentage (69.94 %) of calli. Lou and Kako (1994) also observed cent percent (100%) callus formation in 'Fushinarimidori' cultivar. They obtained this result by using 6 % sucrose (W/V) in MS media. Shital took minimum time (12.12 days) for callus induction which was followed by Shila (12.45 days) and Green Field (12.76 days), respectively. Lou and Kako (1995) reported that calli were formed after 3 weeks of explantation in 'Fushinarimidori' cultivar. Green Field produced the highest (1.48 gm) weighted callus which was significantly different from Shital (1.34 gm) and Shila (1.31 gm), respectively. It was apparent from the result that variety Shital was the best for obtaining best callus in cucumber, than other tested varieties.

Table 1. *Influence of variety, explant, NAA concentration and BAP concentration on callus induction ability of cucumber.*

Treatments	Number of explants produced callus	Days to callus induction	Wt. of callus at second subculture (gm)
Variety			
Shila	4.19c	12.45b	1.31b
Green field	5.01b	12.76a	1.48a
Shital	5.59a	12.12c	1.34b
Explant			
Leaf	5.11b	15.38a	1.48a
Node	4.50c	11.41b	1.13b
Internode	5.18a	10.53c	1.51a
Concentration of NAA (mg/l)			
1.0	4.96b	12.42a	1.34b
2.0	5.03a	12.47a	1.35b
3.0	4.80c	12.44a	1.44a
Concentration of BAP (mg/l)			
0.0	4.88c	12.45a	1.27c
1.0	4.98a	12.49a	1.34b
2.0	4.93b	12.39b	1.51a

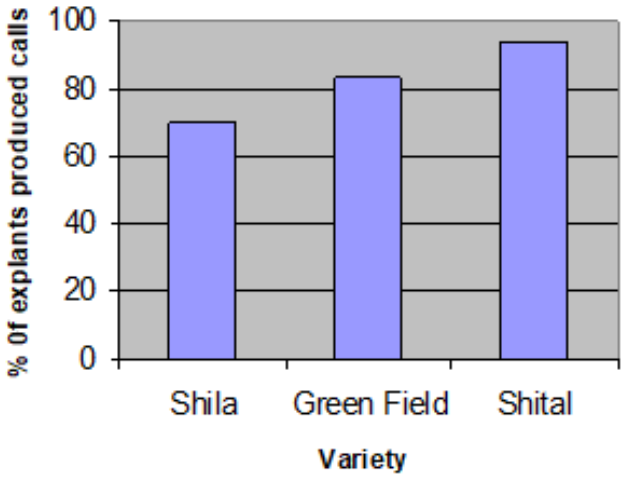

Figure 1. *Effect of variety on percentage of explants produced callus.*

Influence of explant

Influence of explant showed significant difference in all the parameters studied (*Table 1*). Internodal explants were superior to all other tested explants in all parameters. Internodal explants produced the highest number (5.18) of calli, which was closely followed by leaf explants (5.11). The lowest number of calli was produced from nodal explants (4.50). This result was in contrast to that reported by Lou and Kako (1994). They found better callus was formed from young first leaves and cotylendonary explants than internodal explants. Shibli and Ajlouni (1996) obtained callus using only one node as explant in cucumber, but they didn't mentioned neither the number nor percentage of callus produced. Regarding percentage of explants produced callus internodal explants produced higher (86.40 %) percentage of callus than leaf (85.31 %) and nodal (75.01 %) explant, respectively (*Figure 2*).

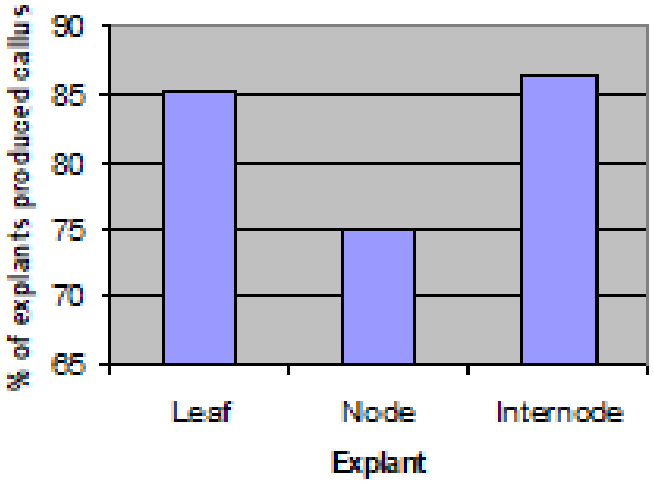

Figure 2. *Effect of explant on percentage of explants produced callus.*

Internodal explants took minimum time (10.53 days) for callus induction whereas leaf segments took maximum time (15.38 days). Different explants took different time

for callus induction. Because different explant tissues generally show distinct planes of cell division, cell proliferation and organization into specialized structures such as vascular systems (Chawla, 2002). Besides, the genetic makeup, endogenous level of growth hormones and metabolic status of explant and certain other factors which are crucial and important for obtaining a particular morphogenic response, such as season and time of collection of explant, the manner of its plantation on the nutrient agar (polarity), physical state of the medium (liquid or semi-solid) and its PH, light intensity, quality, temperature, humidity, even the type and size of the culture vessels, kinds of closing devices like cotton plugs, polypropylene caps etc. (Chandra, 2003).

In case of weight of callus at second subculture the internodal explants showed the best performance. The internodal explants produced the highest (1.51 gm) callus weight which was statistically identical (1.48 gm) with leaf callus but significantly different (1.13 gm) from nodal explants. In this investigation fresh weight of callus were taken because callus growth can be monitored by fresh weight measurements, which are convenient for observing the growth of cultures over time in a non-destructive manner (Chawla, 2002).

Influence of NAA concentration

Auxins are generally added in a culture medium to stimulate callus production and cell growth, to initiate shoots and particularly roots and to induce somatic embryogenesis and stimulate growth from shoot apices and shoot tip cultures (Torres, 1989). Among three tested concentrations, the second highest concentration (2 mg/l) of NAA was the best regarding number and percentage of explants produced callus (*Table 1* and *Figure 3*). The highest concentration (3 mg/l) produced the lowest number (4.80) of callus. There were significant differences among three NAA concentrations in case of percentage of explants produced callus. No significant differences were found among NAA concentrations in case of days to callus induction. Regarding weight of callus at second subculture, it was found that the highest concentration (3.0 mg/l) of NAA produced the highest weighted (1.44 gm) callus and the lowest concentration (1.0 mg/l) produced the minimum weighted (1.34 gm) callus. In this investigation, emphasis was given to produce callus using different concentrations of NAA. Many researchers obtained callus in cucumber using NAA singly or with other plant growth regulators (Lou and Kako, 1995; Lou and Kako, 1994; Kim et al., 1988).

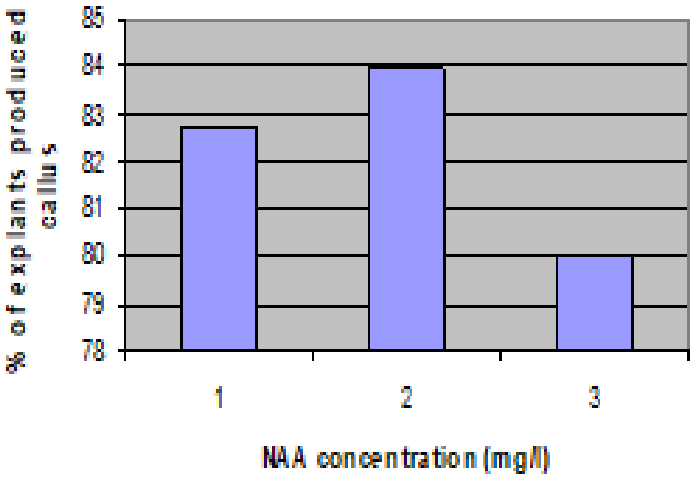

Figure 3. *Effect of NAA concentration on percentage of explants produced callus.*

Influence of BAP concentration

BAP concentrations showed significant difference in all the parameters studied (*Table 1*). BAP at 1.0 mg/l was superior to other two concentrations both at number and percentage of explants produced callus. Maximum number (4.98) of explants produced callus when MS medium fortified with 1.0 mg/l BAP and minimum number (4.88) was observed in control (0.0 mg/l) medium. Actually cytokinin could not produce callus if added singly in a medium. They can promote cell division if added together with an auxin (Chawla, 2002). *Table 1* showed that callus formed even in control medium. In case of percentage of explants produced callus, the highest (83.02 %) percentage of explants produced callus when MS medium was supplemented with 1.0 mg/l of BAP (*Figure 4*). The lowest time (12.39 days) was taken when MS medium supplemented with the highest concentration (2.0 mg/l). The highest concentration also produced the highest weighted (1.51 gm) callus followed by the second highest concentration (1.34 gm) and the control medium (1.27 gm), respectively.

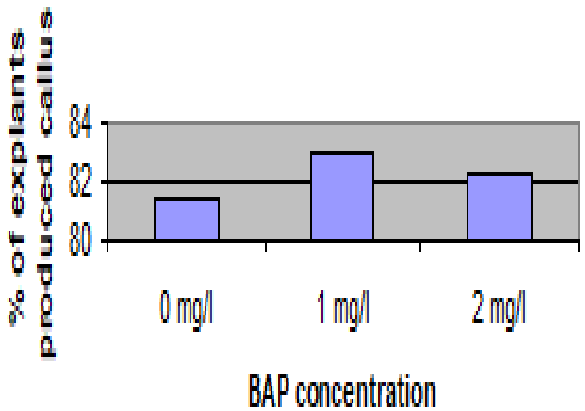

Figure 4. *Effect of BAP concentration on percentage of explants produced callus.*

Interaction of variety, explant, NAA and BAP cincentration

Combined effects of variety, explant, NAA and BAP concentration were found significant in all the parameters studied (*Table 2*). Variety Shital produced the highest number (6.00) of callus when either its leaf explants interacted in MS medium only with 1.0 mg/l of NAA or internodal explants interacted in MS medium with 1.0 mg/l each of NAA and BAP. Nodal explants of variety Shila produced the lowest (3.12) number of callus when they were interacted with 3.0 mg/l NAA and 1.0 mg/l BAP. In an investigation, Nabi et al. (2002) found that the combination of 1.0 mg/l of BAP and 0.1 mg/l or 0.2 mg/l NAA was the most suitable for callus induction in teasle gourd. This finding supports the present result from the hormonal point of view that they were used same hormones (eg. NAA and BAP) for callus induction although the concentrations and combinations were different from Nabi et al. (2002) investigation. The same trend was also observed in case of percentage of explants produced callus. *Figures 5*, *Figure 6* and *Figure 7* showed the leaf callus of three varieties.

Table 2*. Integrated influence of variety, explant, NAA and BAP concentration on different callus induction parameters in cucumber.*

Variety	Explant	Concentration of NAA (mg/l)	Concentrtion of BAP (mg/l)	Number of explants produced callus	% of explants produced callus	Days to callus induction	Wt. of callus at second subculture (gm)
Shila	Leaf	1.0	0.0	4.18 r-u	69.73 r-u	15.17 e	1.30 k-p
			1.0	4.20 r-u	70.14 r-u	15.32 e	1.34 k-p
			2.0	4.04 v-x	67.37 v-x	15.21 e	1.39 j-o
		2.0	0.0	4.29 qr	71.56 qr	15.28 e	1.21 n-s
			1.0	4.49 op	74.90 op	15.69 d	1.35 k-p
			2.0	4.38 pq	73.11 pq	15.27 e	1.39 k-o
		3.0	0.0	4.52 no	75.37 no	15.60 d	1.31 k-p
			1.0	4.08 u-x	68.03 u-x	15.44 de	1.34 k-p
			2.0	4.17 r-v	69.52 r-v	15.21 e	1.39 k-o
	Node	1.0	0.0	3.80 z	63.35 z	11.41 lm	1.09 q-t
			1.0	4.10 t-x	68.48 t-x	11.17 m-o	1.09 q-t
			2.0	4.44 op	74.04 op	11.32 m-o	1.10 q-t
		2.0	0.0	4.50 op	75.09 op	11.73 jk	1.02 st
			1.0	4.62 n	77.14 n	11.26 m-o	1.05 q-t
			2.0	3.88 yz	64.78 yz	11.17 m-o	1.17 p-t
		3.0	0.0	3.60 [	60.14 [	11.90 ij	1.05 r-t
			1.0	3.12 \	52.08 \	11.62 kl	1.09 q-t
			2.0	3.20 \	53.48 \	11.37 l-o	1.10 q-t
	Internode	1.0	0.0	4.22 r-t	70.41 r-t	10.80 p	1.10 q-t
			1.0	4.10 t-x	68.44 t-x	10.58 p-r	1.24 m-r
			2.0	4.92 kl	82.04 kl	10.79 p	1.62 c-h
		2.0	0.0	4.00 xy	66.74 xy	10.34 r-t	1.34 k-p
			1.0	4.10 t-x	68.36 t-x	11.16 m-o	1.47 f-k
			2.0	4.76 m	79.46 m	10.17 t	1.87 b
		3.0	0.0	4.43 op	73.94 op	10.43 r-t	1.43 i-m
			1.0	4.78 m	79.81 m	10.60 p-r	1.59 c-j
			2.0	4.24 rs	70.76 rs	10.17 t	2.02 b
Green field	Leaf	1.0	0.0	5.20 g-i	86.78 g-i	16.30 c	1.64 c-g
			1.0	5.19 g-i	86.63 g-i	16.20 c	1.67 c-e
			2.0	5.00 jk	83.44 jk	16.60 ab	1.69 cd
		2.0	0.0	5.52 f	92.08 f	16.27 c	1.62 c-i
			1.0	5.00 jk	83.44 jk	16.59 ab	1.64 c-f
			2.0	5.99 a	99.84 a	16.45 bc	1.70 c
		3.0	0.0	5.20 g-i	86.78 g-i	16.72 a	1.63 c-g
			1.0	5.10 h-j	85.13 h-j	16.41 bc	1.67 c-e
			2.0	5.00 jk	83.39 jk	16.27 c	1.70 c
	Node	1.0	0.0	4.00 xy	66.78 xy	11.40 l-n	1.10 q-t
			1.0	4.82 lm	80.46 lm	11.25 m-o	1.21 n-s
			2.0	4.15 s-w	69.21 s-w	11.17 m-o	1.31 k-p
		2.0	0.0	4.00 xy	66.78 xy	11.29 m-o	1.09 q-t
			1.0	4.86 lm	81.13 lm	11.69 jk	1.06 q-t
			2.0	4.99 jk	83.31 jk	11.41 lm	1.25 l-r
		3.0	0.0	4.03 wx	67.24 wx	11.17 m-o	0.99 t
			1.0	4.92 kl	82.04 kl	11.35 l-o	1.09 q-t

			2.0	4.50 op	75.08 op	11.17 m-o	1.18 p-t
	Internode	1.0	0.0	5.72 e	95.38 e	10.73 pq	1.38 k-o
			1.0	5.73 de	95.64 de	11.06 o	1.41 j-n
			2.0	5.00 jk	83.41 jk	10.28 st	1.45 f-l
		2.0	0.0	5.31 g	88.56 g	10.17 t	1.31 k-p
			1.0	5.32 g	88.81 g	10.71 pq	1.38 k-o
			2.0	5.10 ij	85.06 ij	10.41 r-t	1.40 j-o
		3.0	0.0	5.00 jk	83.46 jk	11.07 o	1.34 k-p
			1.0	5.51 f	91.91 f	10.28 st	1.47 f-k
			2.0	5.15 hi	85.86 hi	10.17 t	3.49 a
Shital	Leaf	1.0	0.0	0.0	6.00 a	99.99 a	14.17 g
			1.0	1.0	5.89 a-c	98.19 a-c	14.21 g
			2.0	2.0	5.99 a	99.84 a	14.60 f
		2.0	0.0	0.0	5.87 a-c	97.86 a-c	14.59 f
			1.0	1.0	5.99 a	99.98 a	14.33 g
			2.0	2.0	5.99 a	99.94 a	14.22 g
		3.0	0.0	0.0	5.82 b-e	97.04 b-e	14.29 g
			1.0	1.0	5.99 a	99.84 a	14.74 f
			2.0	2.0	5.00 jk	83.45 jk	14.17 g
	Node	1.0	0.0	5.00 jk	83.36 jk	11.27 m-o	1.10 q-t
			1.0	5.17 hi	86.29 hi	11.41 lm	1.20 o-s
			2.0	5.21 g-i	86.89 g-i	11.43 lm	1.25 l-q
		2.0	0.0	5.00 jk	83.36 jk	12.16 h	1.10 q-t
			1.0	5.23 g-i	87.19 g-i	11.17 m-o	1.21 n-s
			2.0	5.00 jk	83.39 jk	11.31 m-o	1.24 m-r
		3.0	0.0	5.01 jk	83.57 jk	11.17 m-o	1.10 q-t
			1.0	5.02 jk	83.66 jk	11.29 m-o	1.20 o-s
			2.0	5.21 g-i	86.86 g-i	12.11 hi	1.21 n-s
	Internode	1.0	0.0	5.87 a-c	97.88 a-c	10.27 st	1.37 k-p
			1.0	6.00 a	99.99 a	11.10 no	1.41 j-n
			2.0	5.92 ab	98.68 ab	10.24 st	1.44 g-m
		2.0	0.0	5.94 ab	99.04 ab	10.18 t	1.32 k-p
			1.0	5.78 c-e	96.36 c-e	10.49 q-s	1.36 k-p
			2.0	5.99 a	99.98 a	11.14 m-o	1.48 e-k
		3.0	0.0	5.85 b-d	97.54 b-d	10.28 st	1.21 n-s
			1.0	5.24 gh	87.38 gh	10.20 t	1.40 j-o
			2.0	5.87 a-c	97.98 a-c	10.62 p-r	1.50 d-k

Figure 5. *Leaf calli of cucumber (var. Shila) in MS medium.*

Figure 6. *Leaf calli of cucumber (var. GF) in MS medium.*

Figure 7. *Leaf calli of cucumber (var. Shital) in MS medium.*

Variety GF took the longest duration (16.72 days) for callus induction when its leaf explants interacted only with 3.0 mg/l of NAA is MS medium. Both Shila and GF took the shortest time (10.17 days) for callus induction. The internodal explants of Shila when cultured in MS medium supplemented with either 2.0 mg/l each of NAA and BAP or 3.0 mg/l of NAA and 2.0 mg/l of BAP and the same explants of GF when cultured in MS medium enriched with only 2.0 mg/l NAA or 3.0 mg/l of NAA and 2.0 mg/l of BAP took the lowest (10.17 days) time followed by (10.18 days) variety Shital when it's internodal explants interacted only with 2.0 mg/l NAA in MS medium. Lou and Kako (1994) reported that they obtained callus from young first leaf, cotyledon and internode explant of seven cucumber cultivars after three weeks of inoculation in the medium. Islam et al. (1994) obtained callus within two weeks of culture in bitter gourd. Huda et al. (2003) found the callus formation from cotyledon explants of chickpea within 8-14 days of incubation. These findings agreed with present investigation, since in present investigation callus was formed within 10-17 days of explant incubation in all cucumber varieties.

The internodes explants of variety GF produced the highest weighted (3.49 gm) callus when they were interacted with 3.0 mg/l NAA and 2.0 mg/l BAP in MS medium and the same variety produced the lowest weighted (0.99 gm) callus when it's nodal explants cultured in MS medium supplemented only with 3.0 mg/l NAA (*Table 2*). Kim et al. (1988) observed the lowest callus weight (1.0 gm) of cucumber in 'Karak'

cultivar and the highest callus weight (7.7 gm) in 'Manchoonchoungjang' cultivar after five weeks of culture in MS medium supplemented with 0.5 µM 2,4-D and 5 µM BAP. But in present investigation callus weight were measured 7 weeks after incubation. The fresh weight of callus tissue was almost same with present result. So, Kim et al. (1988) findings support the present investigation in this regard but their findings differed regarding selection of auxin and its concentrations. The use of NAA instead of 2,4-D and it's concentrations were completely different from Kim et al. (1988) investigation.

Conclusion

To obtain higher percentage of callus within the shortest period of time an efficient callus production protocol in cucumber was developed. For obtaining large size, friable callus researchers can use this protocol. This protocol will help the researchers who want to work on genetic tranformation in cucumber. Agronomically and economically important traits could be transfer to the locally grown cucumber varieties using this protocol.

Acknowledgement

We acknowledge Horticulture Research Centre of Bangladesh Agricultural Research institute (BARI) and the ministry of Science and Information and Communication technology of the Government of Bangladesh for financial support.

Conflict of interest

The authors declare that the investigation was carried out in absence of any conflict of interest.

REFERENCES

[1] Bangladesh Bureau Statistics (BBS) (2006): Monthly statistical bulletin-Bangladesh. – Bangladesh Bureau of statistics, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh 55p.
[2] Chandra, R. (2003): Comprehensive micropropagation of horticultural crops. – IBDC 344p.
[3] Chawla, H.S. (2002): Introduction to Plant Biotechnology. – CRC Press 760p.
[4] Cullins, C.A. (1991): Breeding for resistance to physiological stress. In: advanced methods in plant breeding and biotechnology. – CAB International Redwood Press 11p.
[5] Huda, S., Islam, R., Bari, M.A., Asaduzzaman, M. (2003): Shoot differentiation from cotyledon derived callus of chickpea (Cicer arietinum L.). – Plant Tissue Cult. 13 (1): 53-59.
[6] Islam, R., Sarkar, K.P., Naderuzzaman, A.T.M., Joarder, O.I. (1994): In vitro regeneration of plants from cotyledons of Momordica charantia L. – Plant Tissue Cult. 4(2): 105-109.
[7] Kim, S.G., Chang, J.R., Cha, H.C., Lee, K.W. (1988): Callus growth and plant regeneration in diverse cultivars of cucumber (Cucumis sativus L.). – Plant Cell, Tissue and Organ Culture 12(1): 67-74.

[8] Lou, H., Kako, S. (1995): Effects of auxin combinations on somatic embryogenesis in Cucumis sativus L. – Journal of the Japanese Society for Horticultural Science 64(3): 571-579
[9] Lou, H., Kako, S. (1994): Somatic embryogenesis and plant regeneration in cucumber. – HortScience 29(8): 906-909.
[10] Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bio assays with tobacco tissue cultures. – Physiologia Plantarum 15(3): 473-497.
[11] Nabi, S.A., Rashid, M.M., Al-Amin, M., Rasul, M.G. (2002): Organogenesis in teasle gourd (Momordica dioica Roxb.). – Plant Tissue Cult. 12 (2): 173-180.
[12] Nonnecki, I.L. (1989): Vegetable Production. – Van Nostrand Reinhold, New York 670p.
[13] Peirce, L.C. (1987): Vegetables. Characteristics, production, and marketing. – John Wiley and Sons 448p.
[14] Shibli, R.A., Ajlouni, M.A. (1996): In vitro propagation and Ex vitro acclimatization of 'Sahara F1'cucumber (Cucumis sativus L.). – Plant Tissue Cult. 6(2): 95-98.
[15] Torres, K.C. (1989): Tissue Culture Techniques for Horticultural Crops. – Van Nostrand Reinhold 224p.
[16] Wang, W., Vinocur, B., Altman, A. (2003): Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. – Planta 218(1): 1-14.