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INFLUENCE OF IBA CONCENTRATIONS ON *IN VITRO* ROOTING OF CUCUMBER VARIETIES

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ABSTRACT

In vitro root induction was observed in cucumber as influenced by varieties and IBA concentrations. Four cucumber varieties viz. Shila, Green Field, Shital and Shahi-50 and three IBA concentrations viz. 0.0 (Control), 1.0, and 2.0 mg l^{-1} were used in this investigation. The Shital variety with in maximum number and the highest percentage of shoot produced roots. No root induction was observed when medium was auxin free. Higher concentration of IBA resulted in higher number (3.94) and percentage (98.65 %) of root. Half strength MS medium when fortified with 2.0 mg l^{-1} IBA took minimum time (6.88 days). Combined effect of varieties and IBA concentrations were found significant in all the parameters studied. The highest number (19.54) of roots were obtained from variety Shital when they were interacted with higher IBA concentration (2.0 mg l^{-1}) followed by the interaction of same variety with lower concentration (19.18). The highest root length (8.80 cm) was found in the interaction of Shila x 2.0 mg l^{-1} IBA and the lowest root length (6.31 cm) was found in the interaction of Shahi-50 x 1.0 mg l^{-1} IBA.

Key words
Cucumber, *In vitro* flowering, micro shoot, root induction.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) (2n = 14), a member of the family Cucurbitaceae, is one of the oldest vegetable crop supposed to have originated 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).

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The total area and production of cucumber in Bangladesh during 2003 - 04 were 13925 ha and 25215 t, respectively (BBS 2005). The production has increased upto 32000 mt during the year 2006-'07 (BBS 2008). The data indicates that total production has increased during the last few years with increased demand of cucumber. However, average yield of cucumber during 2002-'03, 2003-'04 and 2004-'05 were 4.53 t ha^{-1}, 4.53 t ha^{-1} and 4.43 t ha^{-1}, 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^{-1}), former USSR (7.57 t ha^{-1}), Japan (44.23 t ha^{-1}), USA (11.06 t ha^{-1}), Turkey (16.07 t ha^{-1}), Netherlands (192.50 t ha^{-1}), Spain (30.00 t ha^{-1}) (Nonnecki 1989).

Yield of cucumber is reducing every year due to effect of several biotic and abiotic factors. 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 abiotic stress tolerant high yielding healthy cucumber seedlings in a short period of time to meet-up this high demand. Once *in vitro* micro shoots are produced, they must be rooted. *In vitro* root induction is an important stage for micropropagation work. Without roots we shall not obtain complete plantlets. To keep this view in mind, present investigation was conducted.

MATERIAL AND METHODS

The experiment was carried out at the Biotechnology Laboratory, under the Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh during June, 2005 to July, 2009 as a part of Ph.D. research works. There were two factors in this experiment. Factor A consisted of four cucumber varieties and factor B consisted of three concentrations of indole butyric acid (IBA). Four cucumber varieties were Shila, Green Field (GF), Shital and Shahi-50 and three IBA concentrations were 0.0 (Control), 1.0, and 2.0 mg l^{-1}. So total number of treatments were 12. Each treatment consisted of four test tubes/vials and replicated three times. The experiment was laid in 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 half strength MS (Murashige and Skoog 1962) medium was used as basal medium for root initiation.

Elongated shoots (minimum 3-5 cm long) were collected from *in vitro* grown multiple shoots of four cucumber varieties. 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 (C_2H_5OH) 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. Shoot tips were prepared inside the laminar airflow cabinet using a fine sterile forcep and scalpel. The excised micro shoots were then inoculated on to the culture vials containing various concentrations of IBA for *in vitro* root induction. The physical conditions for growth and development of cultures were maintained at the temperature of 25 ± 1^0C 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%.

Data on number of shoots that produced root, percentage (%) of shoots produced root, days to root initiation, number of roots/shoot and length of root (cm) were recorded. Data were recorded after 25 days of culture and arithmetic mean was calculated. 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

Effect of variety

High rooting frequency is important for successful micropropagation of crops. Regenerated shoots produced roots when it was cultured in the rooting media (Table 1). There were significant difference among the varieties in all the parameters studied. Maximum number (2.65) of shoots of variety Shital produced root followed by GF (2.61). Minimum number (2.40) of shoots produced root in variety Shahi-50. Regarding percentage of shoots produced root, it was found that the highest percentage (66.32 %) shoots of Shital, while variety Shahi-50 produced the lowest percentage (60.07 %) rooted shoots. Wehner and Locy (1981) reported that they had obtained at least one root from thirty two lines out of eighty five cultivars and lines. In an another investigation, Shibli and Ajlouni

(1996) found the efficient rooting in 'Sahara F_1' cucumber. These findings indicated that root formation ability varied from variety to variety. Variety GF took the shortest time (4.11 days) for root initiation which was statistically identical with variety Shital (4.26 days). Shahi-50 took the highest time (5.56 days) for root initiation. Maximum number (12.91) of roots per micro shoot were produced by variety Shital and variety Shahi-50 produced the minimum number (10.27) of roots. Variety Shila and GF showed moderate performance in this regard. Shibli and Ajlouni (1996) observed the highest number (14.4) of roots in 'Sahara F_1' cucumber. Jawahar *et al*. (1997) reported that tomato cultivar PKM.1 produced the highest number (16.0) of roots per shoot after seven days of culture. The mentioned findings were similar with the present result. The length of root three weeks after initiation was the longest (5.75 cm) in variety Shila and the shortest (4.38 cm) in variety Shahi-50 (Table 1).

Effect of IBA

The higher concentration (2.0 mg l^{-1}) of IBA was statistically superior to the lower concentration (1.0 mg l^{-1}) regarding all the parameters studied (Table 1). Higher concentration of IBA resulted in higher number (3.94) and percentage (98.65 %) of root. Half strength MS medium when fortified with 2.0 mg l^{-1} IBA took minimum time (6.88 days) for root initiation.

TABLE 1. EFFECT OF VARIETY AND IBA CONCENTRATION IN HALF STRENGTH MS MEDIA ON *IN VITRO* ROOTING OF CUCUMBER

Treatments	Number of shoots produced root	% of shoots produced root	Days to root initiation	Number of roots/shoot	Length of root (cm)
Variety					
Shila	2.46bc	61.54bc	4.78b	11.95b	5.75a
GF	2.61ab	65.40ab	4.11c	11.58c	5.12b
Shital	2.65a	66.32a	4.26c	12.91a	5.15b
Shahi-50	2.40c	60.07c	5.56a	10.27d	4.38c
Concentration of IBA (mg l^{-1})					
0.00 (Control)	0.00c	00.00c	0.00b	00.00c	0.00c
1.00	3.65b	91.35b	7.14a	17.27b	7.44b
2.00	3.94a	98.65a	6.88b	17.75a	7.87a

Means in a column followed by uncommon letter (s) varied significantly at 5% level of significance

The highest number of roots per shoot (17.75) obtained from 2.0 mg l^{-1} IBA fortified half strength MS medium. Shibli and Ajlouni (1996) obtained 11.1 and 14.4 roots per microshoot from MS medium enriched with 1.0 mg l^{-1} and 2.0 mg l^{-1} IBA, respectively in cucumber. Debnath *et al.* (2000) obtained about 14 roots per shoot in pointed ground using 2.0 mg l^{-1} IBA in half strength MS medium. Above findings agreed with the present result that 2.0 mg l^{-1} IBA was suitable for efficient root initiation in cucumber. The higher root length (7.87 cm) was recorded in half strength MS medium supplemented with 2.0 mg l^{-1} IBA. Shibli and Ajluni (1996) also obtained the highest root length (11.8 cm) in cucumber using 2.0 mg l^{-1} IBA in MS medium. *In vitro* root induction of variety Shila, GF and Shital on half strength MS medium supplemented with 2.0 mg l^{-1} of IBA showed in plate 1.

FIG. 1

FIG. 2

FIG. 3

PLATE 1 : FIG. 1. ROOT INITIATION OF SHILA ON HALF STRENGTH MS MEDIUM SUPPLEMENTED WITH 2.0 MG L^{-1} IBA; FIG. 2. ROOT INITIATION IN GREEN FIELD ON HALF STRENGTH MS MEDIUM SUPPLEMENTED WITH 2.0 MG L^{-1} IBA AND FIG. 3. ROOT INITIATION OF SHITAL ON HALF STRENGTH MS MEDIUM SUPPLEMENTED WITH 2.0 MG L^{-1} IBA

Interaction between variety and IBA for rooting

Combined effects were found in all the parameters studied. No rooting response was found in the control medium (Table 2). The highest number shoots (4.00) of variety Shila, GF and Shital produced root when they were cultured in half strength MS medium supplemented with higher IBA concentration (2.0 mg l^{-1}). Hundred percent micro shoots of variety Shila, GF and Shital produced root at the higher IBA concentration (Fig. 4). In an investigation, Munshi *et al.* (2007) observed the highest percentage (98 %) micro shoots of cabbage produced root

when they were cultured in 0.5 mg l^{-1} IBA fortified half strength MS medium but at the highest (2.0 mg l^{-1}) IBA concentration only 78 % shoots were rooted. Sultana and Bari Miah (2003) reported that half strength MS media when supplemented with different concentrations of IBA, maximum (60%) micro cuttings were rooted at 0.5 mg l^{-1} IBA in bitter gourd. These findings differed with present results because the crop used in the present study was different from both investigations. Probably it was the main reason for the result obtained by Munshi *et al.* (2007) and Sultana and Bari Miah (2003). The lowest time (5.90 days) was taken by the interaction of GF x 2.0 mg l^{-1} IBA and the highest time (8.41 days) was taken by the interaction of Shahi-50 and 1.0 mg l^{-1} IBA. Interaction of each variety with higher IBA concentration (2.0 mg l^{-1}) produced higher number of roots per shoot than lower concentration. The highest number (19.54) roots were produced by variety Shital when they were interacted with higher IBA concentration followed by the interaction of same variety with lower concentration (19.18). Shibli and Ajlouni (1996) obtained maximum roots from 'Sahara F_1' cucumber in MS medium supplemented with 2.0 mg l^{-1} IBA. The highest root length (8.80 cm) was found in the interaction of Shila x 2.0 mg l^{-1} IBA and the lowest root length (6.31 cm) was found in the interaction of Shahi-50 x 1.0 mg l^{-1} IBA (Fig. 5). In the present investigation it was found that all varieties produced profuse and healthy roots except Shahi-50. This variety produced fragile and weak roots and some of those roots were damaged during transfer in polybags (Plate 2). Some shoots of Shila produced *in vitro* flowers when they were cultured in half strength MS medium supplemented with 2.0 mg l^{-1} IBA (Plate 2). Al-Juboory *et al.* (1991) observed adventitious flower development from cotylendon explants of 'Burpless hybrid' cucumber in modified MS medium supplemented with 3.0 mg l^{-1} NAA and three separate concentrations of BA (0.2, 0.3 and 0.5mg l^{-1}, respectively). Msikita *et al.* (1990) also observed *in vitro* flower formation from cotyledonary explants of 'Burpless hybrid' cucumber in modified MS medium supplemented with 2.0 mg l^{-1} BAP and 0.3 mg l^{-1} NAA. These findings support the result of present investigation that Bangladeshi cucumber varieties are able to produce *in vitro* flowers.

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TABLE 2. INTERACTION EFFECT OF VARIETY AND IBA CONCENTRATION ON *IN VITRO* ROOTING OF CUCUMBER

Variety	Concentration of IBA (mg l^{-1})	Number of shoots produced root	Days to root initiation	Number of roots/shoot
Shila	Control (0.0)	0.00 c	0.00 e	00.00 f
	1.0	3.38 b	7.24 b	17.75 b
	2.0	4.00 a	7.10 b	18.09 b
GF	Control (0.0)	0.00 c	0.00 e	00.00 f
	1.0	3.84 a	6.41 cd	17.06 c
	2.0	4.00 a	5.90 d	17.67 b
Shital	Control (0.0)	0.00 c	0.00 e	00.00 f
	1.0	3.95 a	6.51 c	19.18 a
	2.0	4.00 a	6.27 cd	19.54 a
Shahi-50	Control (0.0)	0.00 c	0.00 e	00.00 f
	1.0	3.42 b	8.41 a	15.09 e
	2.0	3.78 a	8.26 a	15.71 d

Means in a column followed by uncommon letter (s) varied significantly at 5% level of significance

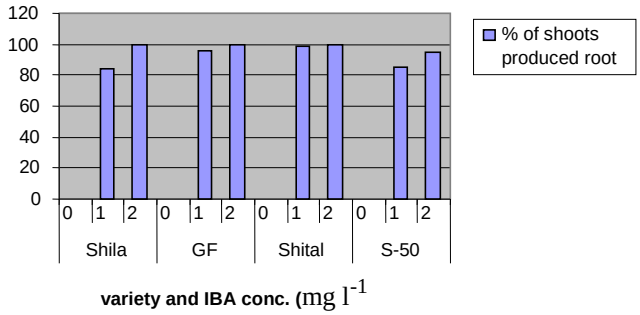

FIG. 4. COMBINED EFFECT OF VARIETY AND IBA CONC. (MG L^{-1}) ON % OF SHOOTS PRODUCED ROOT

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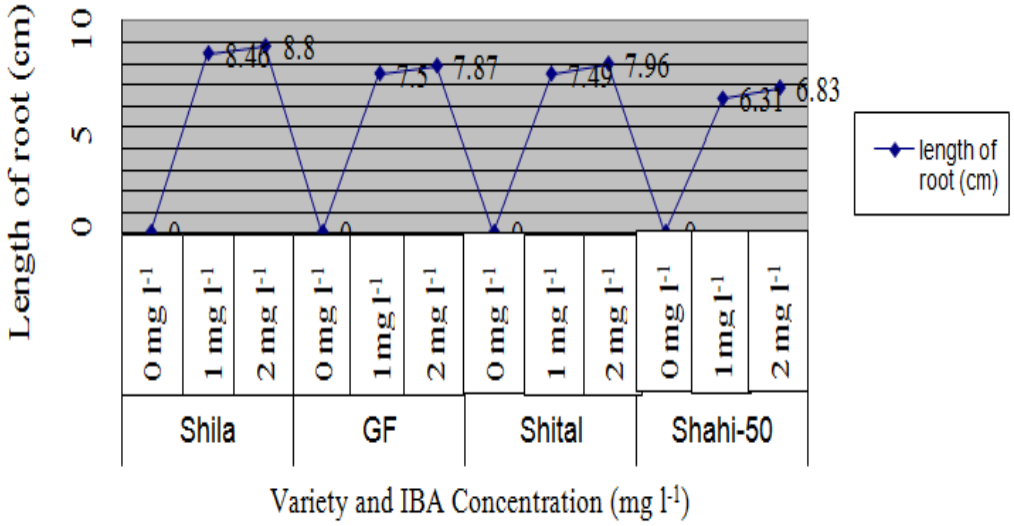

FIG.5. COMBINED EFFECT OF VARIETY AND IBA CONCENTRATION (MG L^{-1}) ON LENGTH OF ROOT

PLATE 2. FIG. 6. ROOT INITIATION OF SHAHI-50 ON HALF STRENGTH MS MEDIUM SUPPLEMENTED WITH 2.0 MG L^{-1} IBA AND FIG. 7. *IN VITRO* FLOWERING OF SHILA ON HALF STRENGTH MS MEDIUM SUPPLEMENTED WITH 2.0 MG L^{-1} IBA

CONCLUSION

From the above findings it was revealed that MS medium supplemented with 2.0 mg l^{-1} IBA was suitable for profuse *in vitro* root induction in cucumber.

These findings may be helpful for researchers/growers who are working on cucumber.

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