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Effect of NAA and IBA on rooting of *Stevia (Stevia rebundiana*)

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ABSTRACT

The experiment was conducted in the Tissue Culture Laboratory of the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during June, 2007 to April, 2008 for effect of NAA and IBA on rooting of *stevia* (*Stevia rebundiana*). In this experiment, five levels of NAA (0.0, 0.2, 0.5, 1.0 and 2.0 mg/l) and six levels of IBA (0.0, 0.2, 0.5, 1.0, 2.0 and 4.0 mg/l) were used as explants in the experiment. The treatment effects were found significant in most the characters studied. The highest number of roots per shoot (8.69) was recoded from 1mg/l NAA at 45 days and the longest root (1.72cm) was recorded from 0.2 mg/l NAA. The concentration of 0.2mg/l IBA was proved to the best regarding the number of roots/shoot (9.51) and the earliest rooting (14.08 days) while 0.5 mg/l IBA produced the highest percentage of root inducing shoots (88.84). The treatment combination N_5I_2 produced the highest number of root initiation at minimum days and the highest percentage of root inducing shoot and root length were recorded from control treatment (N_1I_1).

Keywards: NAA, IBA, explants, *stevia*

Introduction

Stevia is an important medicinal perennial herb native to subtropical and tropical South America and Central America. *Stevia* is a genus of about 150 species of herb and shrubs in the sunflower family (Asteraceae). The species *Stevia rebaudiana* Bertoni 60-70 cm long perennial herb commonly known as sweet leaf, sugar leaf, or simply *stevia*, is widely grown for its sweet leaves. As a sugar substitute, *stevia* taste has slower onset and longer duration than that of sugar, although some of its extracts may a bitter taste or liquorice – like after at high concentrations.

In 1931, two France chemists isolated the glycosides that give *stevia* its sweet taste. These compounds were named stevioside and rebaudioside and are 250 – 300 times sweeter than sucrose (ordinary table sugar), but noncaloric (Chalapathi *et al*., 1999) and non cariogenic (Gardana *et al*., 2003), heat stable, pH stable and non-fermentable. With its extracts having 300 times as sweet as sucrose (Small and Catling, 2001), *stevia* has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives. *Stevia* also has shown promise in medical research for treating such conditions as obesity and high blood pressure. *Stevia* has a negligible effect on blood glucose, even enhancing glucose tolerance; therefore, it is attractive as a natural sweetener to diabetics and others on carbohydrate-controlled diets. In the early 1970s, Japan began cultivating *stevia* as an alternative to artificial sweeteners such as cyclamate and saccharin, suspected carcinogens. The plant's leaves, the aqueous

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extract of the leaves, and purified steviosides are used as sweeteners. Since the Japanese firm Morita Kagaku Kogyo Co., Ltd. produced the first commercial *stevia* sweetener in Japan in 1971, the Japanese have been using *stevia* in food products, soft drinks (including Coca Cola), and for table use. Japan currently consumes more *stevia* than any other country; it accounts for 40% of the sweetener market. In Bangladesh, *stevia* cultivation and is very limited.

Seed production capacity of *stevia* is low (Andolfi *et al*., 2002) and only a small percentage of them germinate. Vegetative propagation such as the direct planting of stem cutting in the field had a limited success (Chalapathi *et al*., 1999). Planting of cloned *stevia* is a more effective method of reproduction. Different growth hormones (BA, NAA, Kinetin) were evaluated for shoot formation (Kornilova and Kalashnikove, 1996) and IBA, IAA and NAA tested for rooting (Toruan Mathius *et al*., 1995; Kornilova and Kalashnikove, 1996 and Swanson *et al*., 1992). There are some discrepancies among the concentrations and types of growth regulators. Therefore, the present experiment was undertaken to determine the optimum concentration of NAA and IBA for better proliferation and development of root.

Materials and Methods

Two experiments were conducted in the tissue culture laboratory of Horticulture Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, during June 2007 to April 2008. The experiment consists two factors a) α - Napthalene Acetic Acid (NAA) with five levels Viz. N_1- 0.0 (control), N_2- 0.2, N_3-0.5, N_4- 1.0 and N_5- 2.0 mg/l along with Indole -3- Butyric Acid (IBA) with six levels Viz. I_1- 0.0 (control), I_2- 0.2, I_3- 0.5, I_4- 1.0, I_5- 2.0 and I_6- 4.0 mg/l. The experiment was laid out in two factors Completely Randomized Design (CRD) with three replications. *Stevia* plants were collected from the BRAC Plant Biotechnology Laboratory, Gazipur, and grown in the earthen pot in the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706. Finally, it was grown up to adult. The tender and actively growing green nodal segments with two nodes (about 2 cm long) were used as explants for establishing the plant in the media.

Preparation of culture media

To prepare one liter of half strength MS medium, the following steps were taken:

i. Thirty gm sucrose (3%) was dissolved in 750 ml of distilled water in a one-liter beaker.
ii. Exact 50 ml stock solution of macro-nutrients (MS - I), 5 ml stock solution of micro-nutrients (MS - II), 5 ml stock solution of Fe - EDTA (MS - III) and 5 ml stock solution of organic nutrients (MS - IV) were added to this sucrose containing 750 ml distilled water and mixed properly by using magnetic stirrer. The volume was made up to 900 ml and then it was transferred in to 10 beakers (100 ml) each containing 90 ml media.
iii. Media were supplemented with different concentrations of NAA and IBA as per treatments combinations.
iv. The pH of the medium was adjusted to 5.7 ± 1 with a pH meter by mixing 0.1N sodium hydroxide (NaOH) or 0.1 N HCl.
v. Finally, the volume of the solution was made up to 100 ml by adding distilled water.

vi. In order to solidify the media, high brand gelrite 0.3 gm (3%) of was added to the salt solution, thoroughly mixed with stirrer and then gently boiled in a microwave oven to completely dissolve the gelrite.
vii. In such way 3.0 liter media were prepared and transferred in to 30 beakers for making 30 treatment combinations.
viii. About 10 ml, prepared melted medium was dispense into culture tubes. The culture tubes were sealed with aluminum foil and marked with glass marker pen to indicate the different concentrations of hormone.
ix. The culture tubes were sterilized at 1.06 kg/cm^2 pressure at 121 ^{o}C for 15 minutes in an autoclave.
x. After autoclaving, the culture media were taken out and allowed to cool and solidify. The solidified media were stored at ambient condition.
xi. Inoculation was done after 3 - 4 days to the media free from contamination.

Preparation of growth substances

The growth hormone NAA and IBA were used in the study. For preparing the separate stock solutions of each growth hormone, 25 mg of solid growth hormone were placed on a clean beaker and then dissolved in solvent (0.1 N NaOH). The solution poured into a measuring cylinder and made the volume up to 250 ml marked level by adding distilled water. The prepared solution of growth hormones was stored in a refrigerator at 9 ± 1º c temperature for a specific time to use as stock solution. The shoots were used as explants. After multiplication, the shoots were cut at base to transfer into rooting media. The shoots were cut about 2.5 cm long for transfer into the rooting media. The recorded data were days to root initiation, percentage of root induced shoot, number of roots per shoot and length of root. Results were analyzed using MSTAT-C statistical package. Differences among the means were compared following DMRT test at 1% level of significance. The regenerated healthy rooted plant lets when attained at 6 – 8 leaf stage were transferred from culture room and kept in room temperature (20 – 25 º c) for 7 days. The plant lets were removed from the culture tubes and washed out all the adhering media carefully so that the root damage was least.

Results and Discussion

Response of NAA

Number of roots

Various concentration of NAA was used for the root initiation and its significant effects on root initiation at different days have shown in Fig. 1. The highest number (5.09) of root per transplanted shoot was found in 1.0 mg/l NAA at 25 days followed by 0.2 mg/l (4.79), 0.0 mg/l (4.77) and 2.0 mg/l (4.05). The lowest (3.89) root initiation was observed in 0.5 mg/l NAA which was statistically similar with 2.0 mg/l (4.05). At 35 days, the highest number (7.00) of root was observed in 1.0 mg/l of NAA followed by 0.2 mg/l (6.69) which were statistically different from each other. The lowest number (5.68) of root was observed in 2.0 mg/l of NAA. Similar trend in root initiation with different concentrations of NAA was found at 45 days (Fig. 1). At 45 days, the highest number (8.69) of root per shoot was found in 1.0 mg/l followed by 0.2 mg/l (8.01), control (7.87) and 0.5 mg/l (7.87). The lowest number (7.53) of root was found in 2.0 mg/l of NAA. At all days of culture 1.0 mg/l NAA produced highest number of root/explants and 2.0 mg/l NAA produced

lowest number of root/explants at 35 and 45 days of culture. The results were agreed with the findings of Bespalhok *et al*. (1992) who reported that 1.0 mg/l NAA had a beneficial effect on root induction in *Stevia*.

Percentage of root inducing shoot

Percentage of root inducing shoot varied significantly among the concentration of NAA (Table 1). The highest parentage (87.92) of root inducing shoot was found with control (0.0 mg/l NAA) followed by 1.0 mg/l (87.17) which was statistically similar with control. The lowest percentage of root inducing shoot (76.08) was found in 0.2 mg/l of NAA. The results were in agreement with the findings of Swanson *et al*. (1992) who reported that supplementation of the shoot media with NAA (1.0 mg/l) induced shoot culture to grow roots.

Days to root initiation

Significant variation on days to root initiation was observed among the concentrations of NAA (Table 1). The days required for root initiation was minimum in control (14.48 days) followed by 0.2 mg/l NAA (16.87) and 0.5 mg/l (17.33) while it was most delayed in 2.0 mg/l NAA (19.19)

Root length

The longest root (1.72 cm) was found in the concentration of 0.2 mg/l NAA which performed statistically superior from others. Second highest root length (1.54 cm) was produced with control (0.0 mg/l) followed by 1.0 mg/l (1.50 cm), 2.0 mg/l (1.39 cm). The concentration of 1.0 mg/l NAA performed statistically identical to control. Lowest root length (1.20 cm) was found in 0.5 mg/l of NAA.

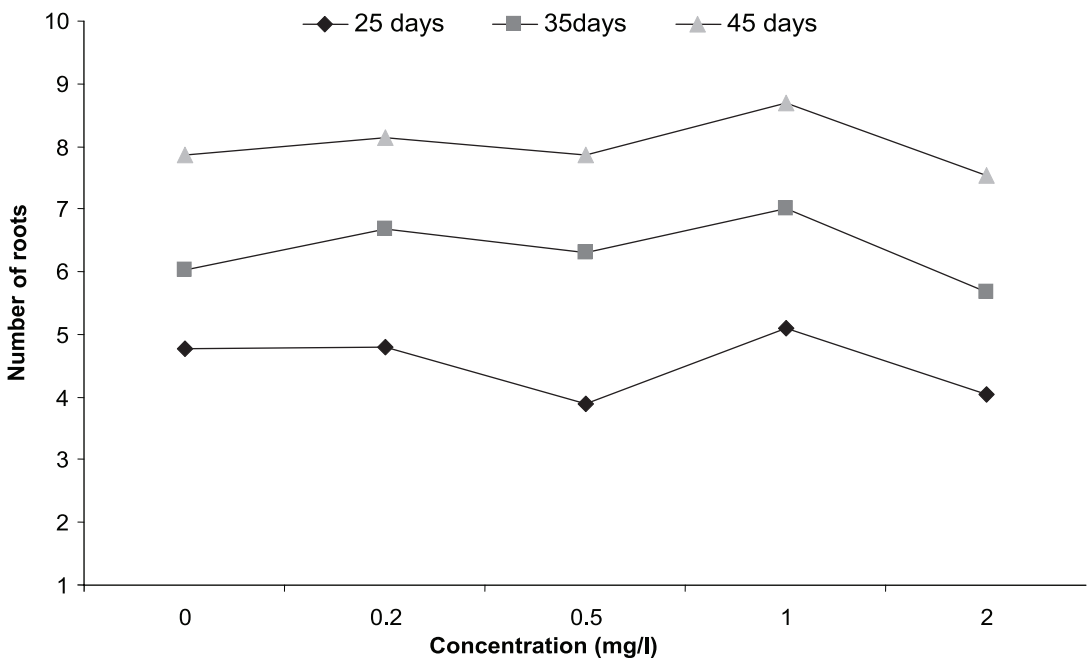

Fig. 1. Response of NAA on root initiation of stevia at different days of culture

Table 1. Response of NAA on percentage of root inducing shoot, days to root initiation and length of root of *stevia*

NAA (mg/l)	Percentage of root inducing shoot	Days to root initiation	Root length(cm)
0	87.92 a	14.48 c	1.54 b
0.2	76.08 d	16.87 b	1.72 a
0.5	83.29 bc	17.33 b	1.20 d
1.0	87.17 ab	17.43 b	1.50 b
2.0	79.40 cd	19.19 a	1.39 c
Level of significance	**	**	**
CV (%)	2.27	1.6	2.55

Means bearing same letter (s) do not differ significantly at 1% level of probability

Response of IBA

Number of roots

Number of roots increased with increasing the days of culture among the concentrations of IBA (Fig. 2). At 25 days of culture the highest number (5.82) of root per transplanted shoot initiated with the concentration of 0.5 mg/l IBA followed by the concentration of 0.2 mg/l (5.23), control (4.76) and 1.0 mg/l (4.18). It was also observed that the number of root increased with the increase of concentration of IBA up to 0.5 mg/l and onwards it was decreased with the increase of the concentration of IBA.

Significant variation on the number of roots ranging from 5.64 to 7.37 was observed among the different concentrations of IBA at 35 days (Fig. 2). Number of roots increased with increasing the concentrations of IBA up to 0.5 mg/l and onwards it was decreased. The highest number (7.37) of root was found in 0.5 mg/l followed by 0.2 mg/l (7.34), control (6.21), 1.0 mg/l (5.81) and 4.0 mg/l (5.68). The concentration of 0.5 mg/l and 0.2 mg/l were statistically similar. The lowest number (5.64) of root was found in 2.0 mg/l which was statistically similar with 1.0 mg/l (5.81) and 4.0 mg/l (5.68) of IBA.

Similar trend on root initiation of *Steva* shoot with different concentrations of IBA was found at 45 days of culture (Fig. 2). The highest number (9.51) of root was found in 0.2 mg/l closely followed by 0.5mg/l (9.15) and they were statistically similar. The lowest number (6.89) of root was found in 4.0 mg/l which was statistically similar with 1.0 mg/l (7.21) and control (7.25). Torun *et al.* (1995) reported that root induction of *Stevia* shoots was achieved in medium with 0.5 mg/l IBA which corroborates the present finding.

Percentage of root inducing shoot

Results presented on Table 2 showed that the highest percentage (88.84%) of root inducing shoot was found in concentration of 0.5 mg/l followed by the control (88.40%), 0.2 mg/l (84.85%) and 2.0 mg/l (81.52%) of IBA. The concentration of IBA 0.5 mg/l was very close to control and statistically similar with control and 0.2 mg/l. The lowest percentage (74.18) of root was found in 4.0 mg/l which was statistically dissimilar with others. Control (growth regulator free) performed best, the result corroborate the findings of Constantinovici and Cachita (1997). They reported that roots emerged from the internodes of *Stevia* either of the rooting media growth regulator free or containing kinetin.

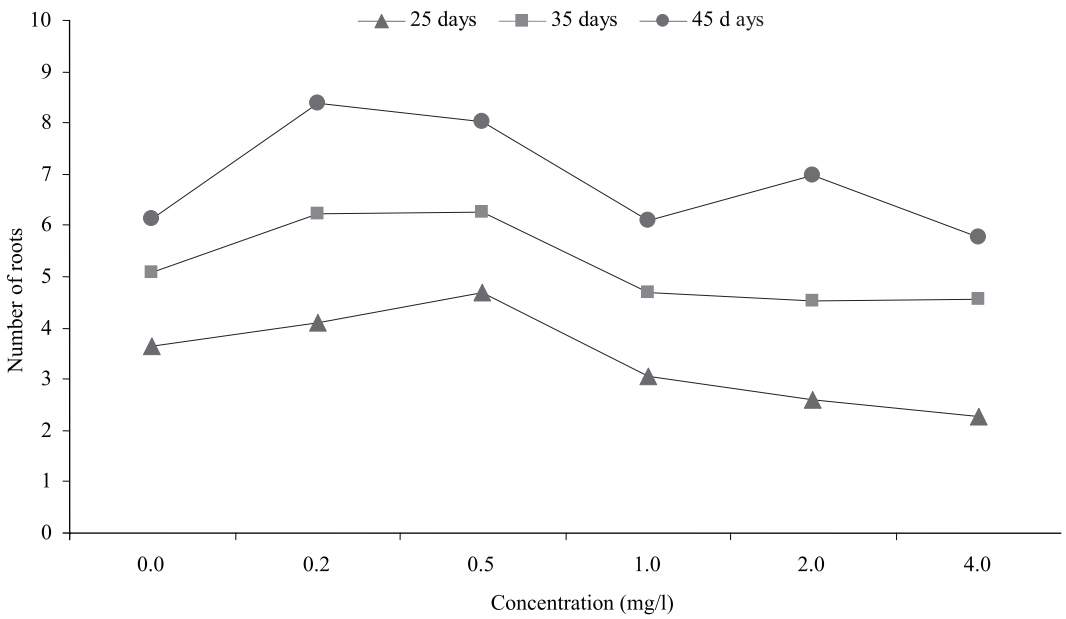

Fig. 2. Response of IBA on root initiation of stevia at different days of culture

Days to root initiation

Significant variation was observed on days to root initiation among the concentrations of IBA (Table 2). Root initiation was the earliest (14.08 days) in 0.2 mg/l IBA followed by the control (14.27 days), 0.5 mg/l (15.68 days), 1.0 mg/l (17.45 days), 2.0 mg/l (20.22 days) and 4.0 mg/l (20.66). The concentration of 0.2 mg/l and control performed statistically similar.

Root length

Root length varied significantly among the concentrations of IBA (Table 2). The longest root (2.14 cm) was found in the control which was statistically different from others followed by 0.5 mg/l (1.79 cm), 0.2 mg/l (1.79 cm), 2.0 mg/l (1.18) and 1.0 mg/l (1.16). The lowest root length (0.74 cm) was produced by 4.0 mg/l IBA. It was observed that the higher concentration of IBA decreased the root length.

Interaction effect of NAA and IBA

Number of roots

The number of roots increased with increasing the days of culture in most of the treatment combinations (Table 3, Fig. 6A, 6B and 6C). At 25 days the treatment combination N_5I_2 (2.0 mg/l NAA + 0.2 mg/l IBA) produced the maximum number (6.18) of root and N_4I_2 (1.0 mg/l NAA + 0.2 mg/l IBA) produced second highest number (6.03) of root. Treatment combination N_5I_2 was statistically similar with N_4I_2, N_4I_3 (0.0 mg/l NAA + 0.0 mg/l IBA) (6.00), N_2I_1 (0.2 mg/l NAA + 0.0 mg/l IBA) (5.99), N_2I_3 (0.2 mg/l NAA + 0.5 mg/l IBA) (5.99) and N_1I_2 (0.0 mg/l NAA + 0.2 mg/l IBA) (5.97). The lowest numbers of root (2.21) observed with N_5I_4 (2.0 mg/l NAA + 1.0 mg/l IBA) which was statistically similar with N_3I_6 (0.5 mg/l NAA + 4.0 mg/l IBA) (2.29) and N_5I_6 (2.0 mg/l NAA + 4.0 mg/l IBA) (2.45). At 35 days the treatment combination N_4I_3 (1.0 mg/l NAA + 0.5 mg/l IBA) produced the

Table 2. Response of IBA on percentage of root inducing shoot, days to root initiation and length of root of stevia

IBA (mg/l)	*Percentage of root inducing shoot	Days to root initiation	Root length (cm)
0	88.40 a	14.27 d	2.14 a
0.2	84.85 ab	14.08 d	1.79 b
0.5	88.84 a	15.68 c	1.79 b
1.0	78.84 c	17.45 b	1.16 c
2.0	81.52 bc	20.22 a	1.18 c
4.0	74.18 d	20.66 a	0.74
Level of significance	**	**	**
CV (1%)	2.27	1.67	2.55

Means bearing same letter (s) do not differ significantly at 1% level of probability

*Data were analyzed by transforming it in Arc Sine in case of Percentage of shoot inducing explants

maximum number (9.11) of root which was statistically different from others and N_1I_2 (0.0 mg/l NAA + 0.2 mg/l IBA) produced second highest number (8.50) of root. The lowest numbers of root (3.41) observed with N_3I_6 (0.5 mg/l NAA + 4.0mg/l IBA) which was statistically similar with N_5I_4 (2.0 mg/l NAA + 4.0 mg/l IBA) (3.60). At 45 days the treatment combination N_1I_2 (0.0 mg/l NAA + 0.2 mg/l IBA) produced the highest number (10.95) of root and N_5I_2 (2.0 mg/l NAA + 0.2 mg/l IBA) produced second highest number (10.56) of root which was statistically similar with N_1I_2. In case of lowest number (4.60) of root similar trend was observer as at 35 days. The treatment combination N_1I_2 (0.0 mg/l NAA + 0.2 mg/l IBA), N_5I_2 (2.0 mg/l NAA + 0.2 mg/l IBA) and N_5I_3 (1.0 mg/l NAA + 0.5 mg/l IBA) performed best in all days of culture. N_3I_6 (0.5 mg/l NAA + 4.0mg/l IBA) was statistically similar with N_5I_6 (2.0 mg/l NAA + 4.0 mg/l IBA) and N_5I_4 (2.0 mg/l NAA + 1.0 mg/l IBA) and performed poor in all days of culture.

Percentage of root inducing shoot

Percentage of root inducing shoots varied significantly among the treatment combinations (Table 4). The highest percentage (96.60) of root inducing shoot was found in control (N_1I_1) whereas 2nd highest percentage (95.50) of root produced by N_1I_3, N_3I_3, N_4I_2, N_4I_5, N_4I_6, N_5I_1 and N_5I_3. The lowest percentage (62.20) of root inducing shoot was found with the treatment combination of N_2I_6 (2.0 mg/l NAA + 4.0 mg/l IBA) which was statistically similar with the treatment combination of N_5I_5 (62.20) and N_3I_6 (65.50),

Days to root initiation

Interaction effect of NAA and IBA on days to root initiation was found significant (Table 4). The days required for root initiation was minimum (9.59 days) in (control) followed by N_1I_2 (11.44 days), N_2I_1 (13.17 days), N_2I_2 (13.57 days), N_2I_3 (13.91 days). Rooting was delayed in N_5I_6 (23.28 days) which was statistically similar to N_5I_4 (23.07 days).

Root length

Interaction effect of NAA and IBA on the root length was also found significant (Table 4). The longest root (3.50 cm) was found in N_1I_1

Table 3. Interaction effect of NAA and IBA on root development of *stevia* at different days of culture

Treatment combinations	Number of roots at different days of culture		
	25 days	35 days	45 days
N_1I_1	5.77 bc	6.08 j-k	8.45 ef
N_1I_2	5.97 ab	8.50 b	10.95 a
N_1I_3	5.75 bc	6.02 kl	9.10 d
N_1I_4	2.80 i	4.81 r	5.64 no
N_1I_5	3.43 gh	4.34 s	5.40 op
N_1I_6	4.90 d	6.50 i	7.70 hi
N_2I_1	5.99 ab	7.82 d	8.90 de
N_2I_2	4.22 e	5.91 lm	8.20 fg
N_2I_3	5.99 ab	7.62 e	7.93 gh
N_2I_4	5.62 c	6.03 kl	7.12 jk
N_2I_5	3.41 h	5.73 mn	8.74 de
N_2I_6	3.50 gh	7.04 g	7.95 gh
N_3I_1	2.90 i	6.11 jk	6.42 lm
N_3I_2	3.74 fg	6.62 i	8.75 de
N_3I_3	5.56 c	8.03 c	9.61 c
N_3I_4	4.65 d	7.36 f	9.07 d
N_3I_5	4.25 e	6.26 j	8.77 de
N_4I_6	2.29 k	3.41 t	4.60 q
N_4I_1	4.22 e	5.62 no	6.34 lm
N_4I_2	6.03 ab	7.24 f	9.10 d
N_4I_3	6.00ab	9.11 a	10.40 b
N_4I_4	5.64 c	7.25 f	9.17 cd
N_4I_5	4.79 d	6.83 h	10.33 b
N_4I_6	3.84 f	5.96 kl	6.78 kl
N_5I_1	4.92 d	5.40 p	6.11 mn
N_5I_2	6.18 a	8.45 b	10.56 ab
N_5I_3	5.79 bc	6.93 j-l	8.73 de
N_5I_4	2.21 k	3.60 t	5.03 oq
N_5I_5	2.73 ij	5.05 q	7.34 ij
N_5I_6	2.45 jk	5.50 op	7.41 ij
Level of significance	**	**	**
CV (%)	3.14	1.42	2.84

Means bearing same letter (s) do not differ significantly at 1% level of probability

Five levels of NAA: $N_1 = 0.0$, $N_2 = 0.2$, $N_3 = 0.5$, $N_4 = 1.0$, $N_5 = 2.0$

Six levels of IBA: $I_1 = 0.0$, $I_2 = 0.2$, $I_3 = 0.5$, $I_4 = 1.0$, $I_5 = 2.0$, $I_6 = 4.0$

Table 4. Interaction effects of NAA and IBA on root inducing shoot, days to root initiation and length of root of *stevia*

Treatment combinations	Percentage of root inducing shoot	Days of root initiation	Root length (cm)
N_1I_1	96.60 a	9.59 r	3.50 a
N_1I_2	92.20 bc	11.44 q	1.97 f
N_1I_3	95.50 ab	15.25 mn	1.42 j
N_1I_4	85.50 de	16.00 j-l	0.93 o
N_1I_5	85.50 de	17.23 fg	0.71 p
N_1I_6	72.20 f	17.39 fg	0.73 p
N_2I_1	82.20 e	13.17 p	2.40 c
N_2I_2	72.20 f	13.57 op	2.13 e
N_2I_3	72.20 f	13.91 o	2.25 d
N_2I_4	75.50 f	16.45 h-j	1.14 m
N_2I_5	92.20 bc	22.43 b	1.64 g
N_2I_6	62.20 g	21.70 c	0.75 p
N_3I_1	92.20 bc	16.11 j-l	1.96 f
N_3I_2	88.87 cd	15.53 lm	1.91 f
N_3I_3	95.50 ab	17.70 f	1.05 n
N_3I_4	85.50 de	16.22 i-k	0.93 o
N_3I_5	72.20 f	19.76 d	0.70 p
N_4I_6	65.50 g	18.66 e	0.62 q
N_4I_1	75.50 f	15.68 k-m	1.34 k
N_4I_2	95.50 ab	14.72 n	1.39 jk
N_4I_3	85.50 de	16.89 gh	2.67 b
N_4I_4	75.50 f	15.53 lm	1.56 hi
N_4I_5	95.50 ab	19.46 d	1.36 jk
N_4I_6	95.50 ab	22.28 bc	0.72 p
N_5I_1	95.50 ab	16.80 g-i	1.53 hi
N_5I_2	75.50 f	15.14 mn	1.57 hi
N_5I_3	95.50 ab	14.66 n	1.58 gh
N_5I_4	72.20 f	23.07 a	1.27 l
N_5I_5	62.20 g	22.22 bc	1.50 i
N_5I_6	75.50 f	23.28a	0.87 o
Level of significance	**	**	**
CV (%)	2.27	1.67	2.55

Means bearing same letter (s) do not differ significantly at 1% level of probability

Five levels of NAA: $N_1 = 0.0$, $N_2 = 0.2$, $N_3 = 0.5$, $N_4 = 1.0$, $N_5 = 2.0$

Six levels of IBA: $I_1 = 0.0$, $I_2 = 0.2$, $I_3 = 0.5$, $I_4 = 1.0$, $I_5 = 2.0$, $I_6 = 4.0$

Data were analyzed by transforming it in aresine in case of Percentage of shoot inducing explants

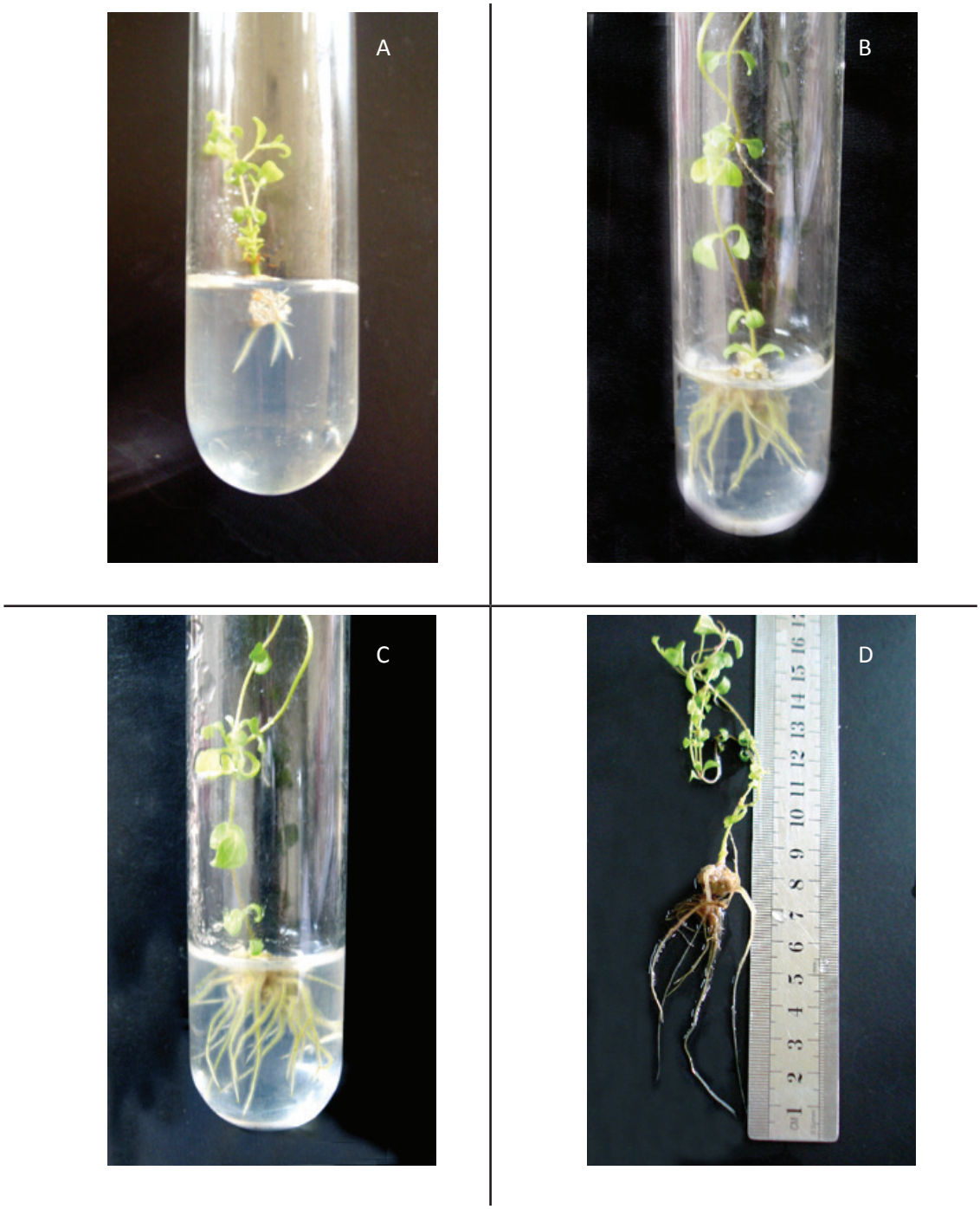

Fig. 3. Response of NAA and IBA on root development of stevia at different days of culture (A. 30 days, B. 45 days, C. 60 days and D. highest root length in cm)

(control) which was statistically different from other treatment combination. Second highest root length (2.67 cm) was observed in the treatment combination N_4I_3 (1.0 mg/l NAA + 4.0 mg/l IBA). The shortest root (0.62 cm) was found in N_3I_6 (0.5mg/l NAA + 4.0 mg/l IBA) which was statistically dissimilar from other treatment combination followed by N_3I_5 (0.70).

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