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Effects of Auxin on Root Development in Sugarbeet

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ABSTRACT

This study was carried out in Biotechnology Laboratory of Bangladesh Sugarcrop Research Institute to compare different tropical sugarbeet cultivars with respect to different concentration of auxins (IBA, IAA and NAA) for root development under *in vitro* condition. The regenerated shoots were transferred to rooting medium for development of adventitious root to raise full-fledged plantlets. The proliferated shoots were separated aseptically and culture in full and half strength MS medium fortified with five different concentrations (viz., 0, 1, 2, 3 and 4 mgl^{-1}) of auxins (IBA, IAA and NAA) for root induction. Half strength MS medium containing 2.0 mgl^{-1} auxin produced the highest percentage rooted plants. Among the three auxins, NAA 2.0 mgl^{-1} produced the highest percentage of rooted plants and root per plant and root length was also higher in this treatment. On the other hand, the highest number of rooted plant was obtained from cultivar HI 0044 and lowest from cultivar CS 0328.

Key words: Auxins, root formation, sugarbeet

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is the only species of agricultural importance crop belongs to Chenopodiaceae family. Sugarbeet is the major sucrose-producing crop grown in temperate zones, and contributes approximately 37% of the world's supply, with the rest derived from sugarcane. In addition to sugar from sugarbeet, it is possible to obtain a considerable amount of leaves, slices from roots and molasses, which are valuable as animal feed. Sugarbeet is the second crop to producing the world's sugar and recently sugarbeet becoming an important biofuel alternative to fossil fuel energy (Zhang *et al.*, 2008). Sugarbeet is low delta crop of short duration (4 to 6 months) with low irrigation requirements of 5-6 times compared to 25-30 irrigations required by sugar cane high delta crop. Sugarbeet has a fairly wide adaptability and is relatively resistant to cold, withstand drought, and are not overly sensitive to salinity (Ahmad and Rasool, 2011; Ahmad *et al.*, 2012). It is also high tolerance to alkaline conditions.

Cultivation of sugarbeet in Bangladesh faces several field problems. Beside the impossibility of flowering and seed production in the free living conditions. However, in view of the fact that sugarbeet is a biannual plant and the modern cultivars are highly heterozygous nature due to out crossing. The developing of new varieties by

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conventional breeding is very difficult. Developing new sugarbeet varieties with conventional plant breeding methods is slow and labor intensive. Tissue culture methods integrated with conventional breeding programs are playing an increasingly significant role in the improvement of sugarbeet (Gurel, 2000; Hisano *et al.*, 2004). Advanced *in vitro* culture and genetic transformation technologies have been incorporated with classical breeding programs of sugarbeet, aiming at the production of herbicide and salt-tolerant, disease and pest-resistant cultivars (Gurel *et al.*, 2001; Gurel *et al.*, 2008). Therefore a tissue culture strategy for sugarbeet may be expected to aid the breeders in introducing specific traits into commercially valuable genotypes. Development of an *in vitro* regeneration protocol and a micropropagation system for sugarbeet is considered a very important step for its genetic manipulation by modern biotechnology methods. The present study aims to develop an efficient protocol for root development of *in vitro* grown shoot of sugarbeet.

MATERIALS AND METHODS

The experiments were conducted at Biotechnology Laboratory of Biotechnology Division, Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during 2014-2015. Six sugarbeet (*Beta vulgaris* L.) genotypes viz., CS 0327, CS 0328, HI 0044, HI 473, Shubrha and Cauvery were used as plant materials. All genotypes were obtained from the Bangladesh Sugarcrop Research Institute (BSRI) Ishurdi, Pabna. The experiment was laid out in completely randomized design with three replications (ten test tubes represent a replication). Well developed healthy shoot were aseptically transferred into the full and half strength MS medium supplemented with IBA (0, 1, 2, 3 & 4 mgl^{-1}), IAA (0, 1, 2, 3 & 4 mgl^{-1}) and NAA (0, 1, 2, 3 & 4 mgl^{-1}) for root development. After inoculation of explants 5 weeks were incubated for root development. All cultures were incubated under a cool white fluorescent light (27 µmol m^{-2} s^{-1}) with a 16 h light/8 h dark photoperiod in a growth chamber at 25±1°C. Days required for root initiation were recorded from beginning of root formation. Data on root induction (%), root/plant and root length were measured after five weeks of transferred.

RESULTS AND DISCUSSION

Effects of different concentrations of IBA on root development in sugarbeet

Different concentrations of IBA and strength of media had a significant effect on root growing percentage of *in vitro* grown shoot of sugarbeet (Table 1). Among different treatment combinations, 2.0 mgl^{-1} IBA on full strength MS media produced the highest rooted plant (25.00%) followed by 3.0 mgl^{-1} IBA (21.00%) and 1.0 mgl^{-1} IBA (19.00%) while, the lowest rooted plant (9.00%) was obtained from IBA 4.0 mgl^{-1}, which was statistically different from other treatments. In case of half strength MS media maximum rooted plant (31.67%) was noted from the similar concentration of IBA (2.0 mgl^{-1}) and the second highest rooted plant (25.67%) was calculated from treatment 1.0 mgl^{-1} IBA. Significantly lower number of rooted plant was found in treatment IBA 4.0 mgl^{-1}, while without IBA shoot did not produce any root in this study.

Table 1. Effects of different concentrations of IBA and strength of MS media on per cent of root induction in sugarbeet

Concentrations of IBA (mgl^{-1})	Rooting percentage	
	Full strength MS	½ strength MS
0.0	0.00 c	0.00 d
1.0	19.00 a	25.67 ab
2.0	25.00 a	31.67 a
3.0	21.00 a	19.00 bc
4.0	9.00 b	11.67 c
LSD (0.05)	6.9036	9.8308

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Effects of different concentrations of IBA on root morphology

The effects of different concentrations of IBA on number of root per shoot and root length showed significant variations (Table 2,Figure1). MS media containing 2.0 mgl^{-1} IBA produced the maximum root per shoot (15.00) followed by 3.0 mgl^{-1} IBA (13.67) and 1.0 mgl^{-1} IBA (11.00). The lowest number of root per shoot (7.33) was found in treatment 4.0 mgl^{-1} IBA, which was statistically different from other treatments. In the same way, MS media supplemented by 2.0 mgl^{-1} IBA produced the highest length of root (2.37 cm) and the second highest length of root (2.17 cm) was obtained from IBA 3.0 mgl^{-1}. Significantly shortest root (1.33 cm) was found in treatment 1.0 mgl^{-1} IBA. However, without supplementation of IBA on MS medium was not produced any root during the study.

Table 2. Effects of different concentrations of IBA on root characters of *in vitro* grown shoot

Concentrations of IBA (mgl^{-1})	No. of root/plant	Root length (cm)
0.0	0.00 d	0.00 d
1.0	11.00 bc	1.33 c
2.0	15.00 a	2.37 a
3.0	13.67 ab	2.17 ab
4.0	7.33 c	1.90 b
LSD (0.05)	3.8735	0.3986

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Effects of different concentrations of IAA and strength of MS media on root induction potentiality

Different concentrations of IAA and strength of media had a significant effect on root growing percentage of *in vitro* grown shoot of sugarbeet (Table 3). Among different treatment combinations, 3.0 mgl^{-1} IAA on full strength MS media produced the highest

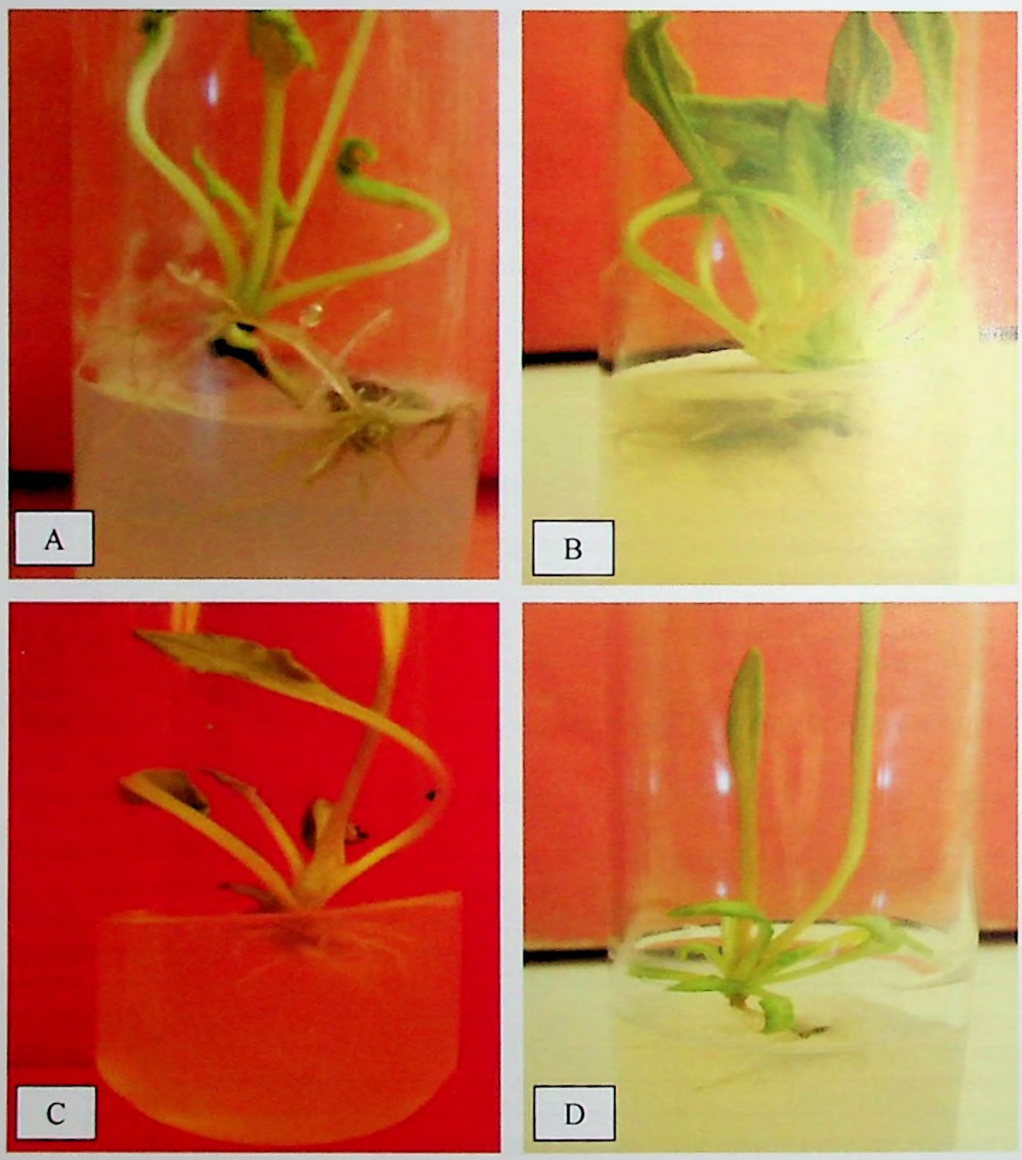

Figure 1. Effect of different concentration of IBA on rooting of *in vitro* grown shoot of sugarbeet (A) 1.0 mgl^{-1} (B) 2.0 mgl^{-1} (C) 3.0 mgl^{-1} (D) 4.0 mgl^{-1}

rooted plant (27.00%) followed by 2.0 mgl^{-1} IAA (22.00%) and 1.0 mgl^{-1} IAA (20.00%), while the lowest rooted plant (11.00%) was obtained from IAA 4.0 mgl^{-1} which is statistically different from other treatments. In case of half strength MS media maximum rooted plant (33.33%) was noted from the similar concentration of IAA (3.0 mgl^{-1}) and the second highest rooted plant (30.00%) was calculated from treatment 2.0 mgl^{-1} IAA. Significantly lower number of rooted shoot was found in treatment IAA 4.0 mgl^{-1} (10.00%), while without IAA shoot did not produce any root in this study.

Table 3. Effects of different concentrations of IAA and strength of MS media on root induction potentiality in sugarbeet

Concentrations of IAA (mgl^{-1})	Root growing (%)	
	Full strength	½ strength
0.0	0.00 d	0.00 b
1.0	20.00 b	27.00 b
2.0	22.00 ab	30.00 a
3.0	27.00 a	33.33 a
4.0	11.00 c	10.00 b
LSD (0.05)	5.3351	16.054

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Effects of different concentrations of IAA on root characters

The effects of different concentrations of IAA on number of root per shoot and root length showed significant variation (Table 4). MS media containing 2.0 mgl^{-1} IAA produced the maximum root per shoot (14.00) followed by 3.0 mgl^{-1} IAA (11.67) and 1.0 mgl^{-1} IAA (9.67). The lowest number of root per shoot (7.33) was found in treatment 4.0 mgl^{-1} IAA (Figure 2) which was statistically different from control treatment. In the same way, MS media supplemented by 2.0 mgl^{-1} IAA produced the highest length of root (2.43 cm) and the second highest length of root (2.03 cm) was obtained from IAA 3.0 mgl^{-1}. Significantly shortest root (1.10 cm) was found in treatment 1.0 mgl^{-1} IAA. However, without supplementation of IAA on MS medium shoot did not produce any root during the study.

Table 4. Effects of different concentrations of IAA on root characters of *in vitro* grown shoot

Concentrations of IAA (mgl^{-1})	No. of root/plant	Root length (cm)
0.0	0.00 c	0.00 d
1.0	9.67 ab	1.10 c
2.0	14.00 a	2.43 a
3.0	11.67 ab	2.03 ab
4.0	6.67 b	1.40 bc
LSD (0.05)	**5.1457**	**0.8122**

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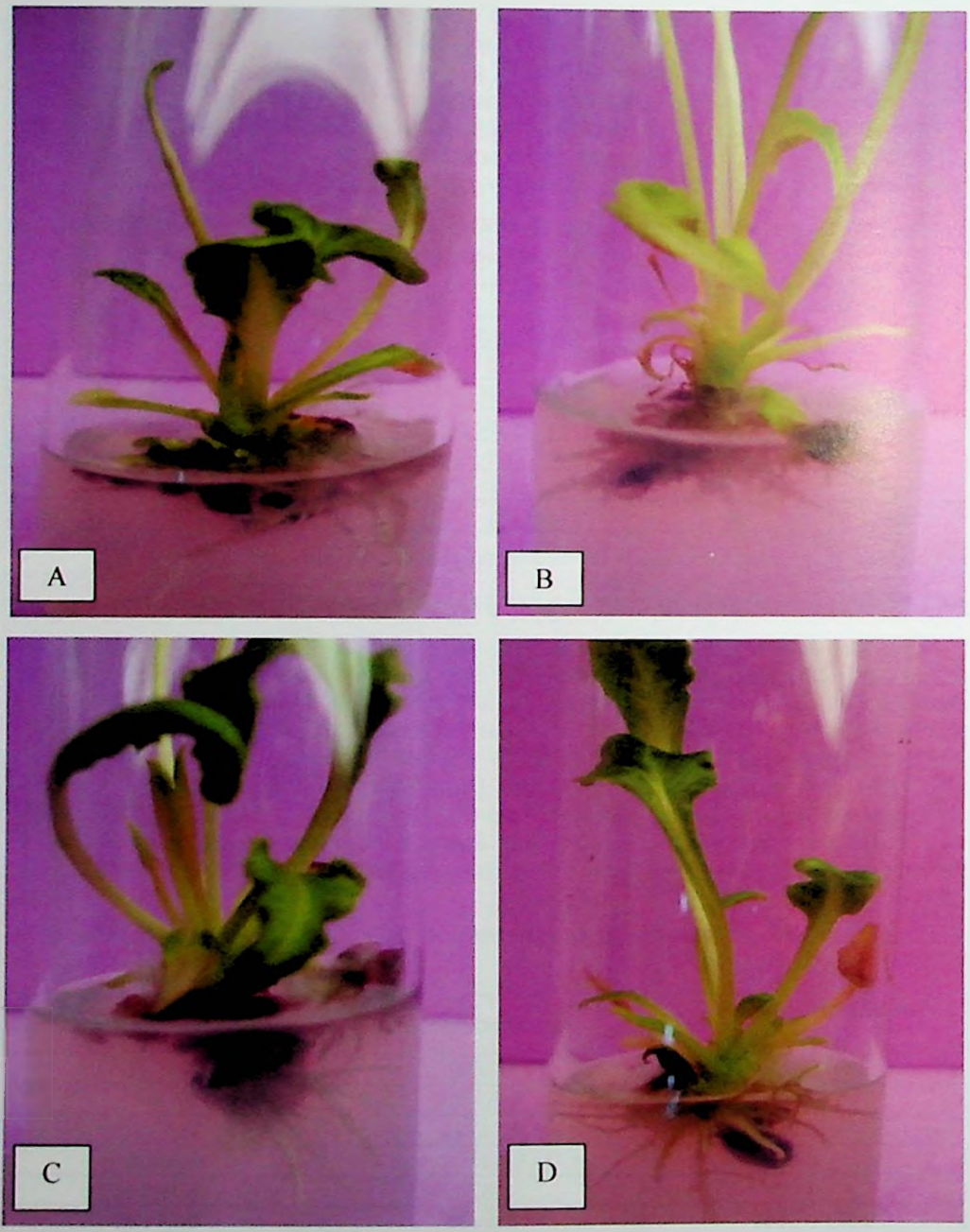

Figure 2. Effect of different concentration of IAA on rooting of *in vitro* grown shoot of sugarbeet (A) 1.0 mgl^{-1} (B) 2.0 mgl^{-1} (C) 3.0 mgl^{-1} (D) 4.0 mgl^{-1}

Effects of different concentrations of NAA and strength of MS media on root growing percentage

Different concentrations of NAA and strength of media had a significant effect on root growing percentage of *in vitro* grown shoot of sugarbeet (Table 5). Among different NAA concentrations, full strength MS media containing 2.0 mgl^{-1} NAA produced the highest rooted plant (64.00%) followed by 1.0 mgl^{-1} NAA (52.00%) and 3.0 mgl^{-1} NAA (47.00%) while, the lowest rooted plant (41.00%) was obtained from NAA 4.0 mgl^{-1}. In case of half strength MS media maximum rooted plant (73.33%) was found in the similar concentration of NAA (2.0 mgl^{-1}) and the second highest rooted plant (56.67%) was produced by treatment 1.0 mgl^{-1} NAA. Significantly lower number of rooted shoot (35.00%) was found in treatment NAA 4.0 mgl^{-1}, while without NAA shoot did not produce any root in this study.

Table 5. Effects of different concentrations of NAA and strength of MS media on root growing percentage of *in vitro* grown shoot

Concentrations of IAA (mgl^{-1})	Root growing (%)	
	Full strength	½ strength
0.0	0.00 c	0.00 d
1.0	52.00 ab	56.67 b
2.0	64.00 a	73.33 a
3.0	47.00 b	51.00 b
4.0	41.00 b	35.00 c
LSD (0.05)	16.005	13.361

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Effects of different concentrations of NAA on root characters

The effects of different concentrations of IAA on number of root per shoot and root length showed significant variation (Table 6, Figure 3). MS media containing 2.0 mgl^{-1} NAA produced the height root per shoot (18.00) followed by 3.0 mgl^{-1} NAA (16.00) and 1.0 mgl^{-1} NAA (13.67). The lowest number of root per shoot (11.00) was found in treatment 4.0 mgl^{-1} NAA which was statistically different from other treatments. Correspondingly, MS media supplemented by 2.0 mgl^{-1} NAA produced the highest length of root (3.10 cm) and the second highest length of root (2.43 cm) was found in NAA 3.0 mgl^{-1}. Shortest root (2.00 cm) was observed in treatment 4.0 mgl^{-1} NAA. However, without supplementation of NAA on MS medium shoot did not produce any root during the study. Similar findings were also reported by Tripathi and Kumar (2010).

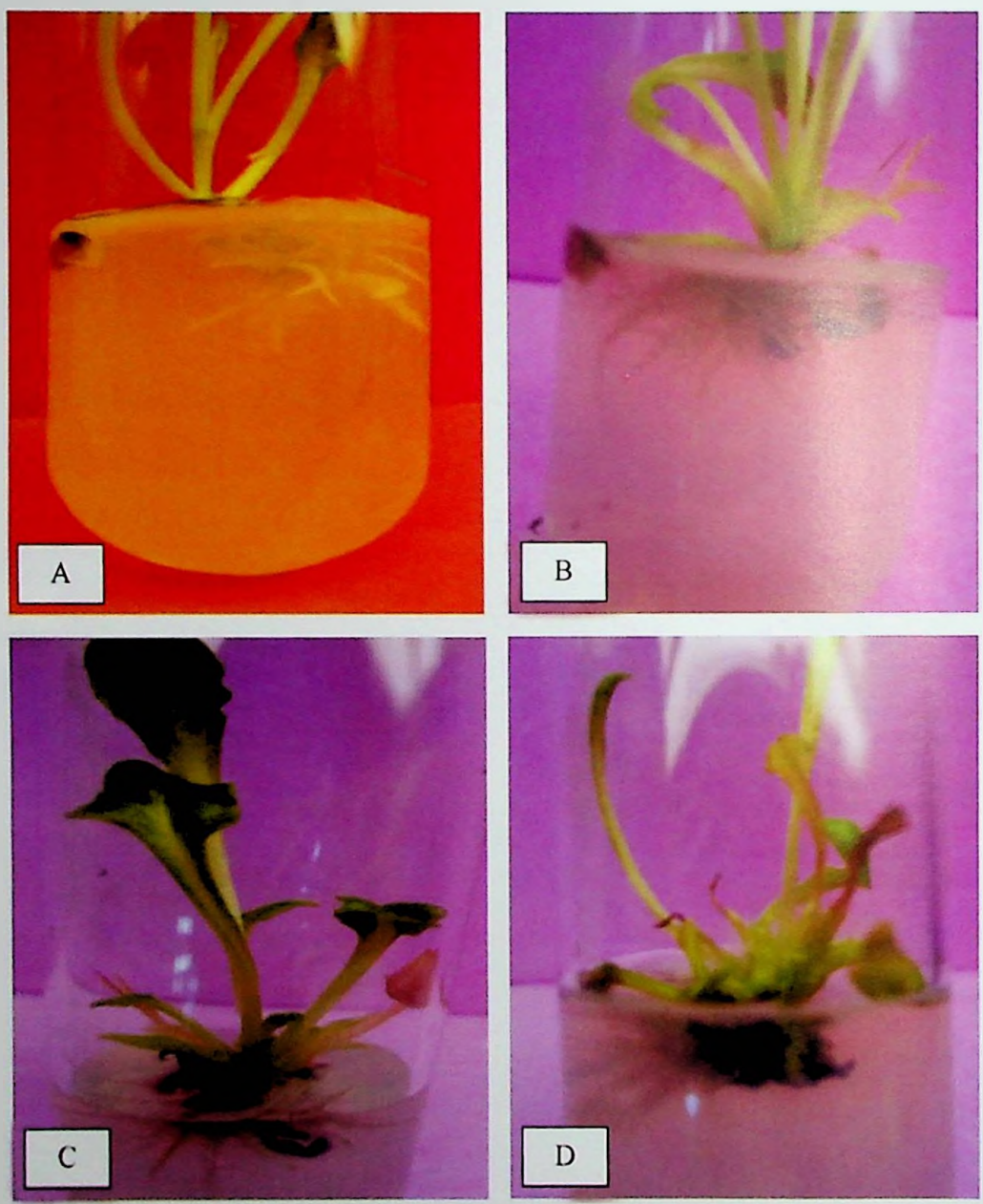

Figure 3. Effect of different concentration of NAA on rooting of *in vitro* grown shoot of sugarbeet (A) 1.0 mgl^{-1} (B) 2.0 mgl^{-1} (C) 3.0 mgl^{-1} (D) 4.0 mgl^{-1}

Table 6. Effects of different concentrations of NAA on root characters of *in vitro* grown shoot

Concentrations of NAA (mgl^{-1})	No. of root/plant	Root length (cm)
0.0	0.00 d	0.00 c
1.0	13.67 bc	2.20 b
2.0	18.00 a	3.10 a
3.0	16.00 ab	2.43 ab
4.0	11.00 c	2.00 b
LSD (0.05)	5.867	0.7015

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Combined effects of auxins concentrations on root characters

The data on root formation percentage, number of root per shoot and length of root showed significant variations due to combined effect between various auxins and their different concentrations in this study (Table 7). Among the treatment combinations, 2.0 mgl^{-1} NAA produced the highest rooted plant (64.00%) which was statistically different from other treatments. The second highest root formation (52.00%) was also observed in the same auxin (NAA) in concentration of 1.0 mgl^{-1}, whereas the lowest percentage of root formation was noted in MS medium supplemented with 4.0 mgl^{-1} IBA. Similarly 2.0 mgl^{-1} NAA also produced the highest number of root (18.00) per plant while, the second highest root per plant (16.00) was found in treatment NAA 3.0 mgl^{-1}. Significantly lowest number of root per plant (6.67) was calculated from treatment combination 4.0 mgl^{-1} IAA. Correspondingly, NAA produced the highest length (3.10 cm) of root in concentration of 2.0 mgl^{-1} which was statistically different from other treatments. Significantly lowest length of root (1.10 cm) was observed in MS medium supplemented with 1.0 mgl^{-1} IAA. Without application of auxins (IBA, IAA and NAA) on MS medium shoot did not produce any root during the study.

Table 7. Combined effects of auxins concentrations on root characters of *in vitro* grown shoot

Sl no.	Auxins x concentrations (mgl^{-1})	Root formation (%)	No. of root/plant	Root length (cm)
1	IBA x 0.0	0.00 g	0.00 h	0.00 f
2	IBA x 1.0	19.00 de	11.00 def	1.33 de
3	IBA x 2.0	25.00 d	15.00 abc	2.37 b
4	IBA x 3.0	21.00 d	13.67 bcd	2.17 b
5	IBA x 4.0	9.00 fg	7.33 fg	1.90 bcd
6	IAA x 0.0	0.00 g	0.00 h	0.00 f
7	IAA x 1.0	20.00 de	9.67 efg	1.10 e
8	IAA x 2.0	22.00 d	14.00 bcd	2.43 b
9	IAA x 3.0	27.00 d	11.67 cde	2.03 b
10	IAA x 4.0	11.00 ef	6.67 g	1.40 cde
11	NAA x 0.0	0.00 g	0.00 h	0.00 f
12	NAA x 1.0	52.00 b	13.67 bcd	2.20 b
13	NAA x 2.0	64.00 a	18.00 a	3.10 a
14	NAA x 3.0	47.00 bc	16.00 ab	2.43 b
15	NAA x 4.0	41.00 c	11.00 def	2.00 bc
LSD (0.05)		9.6466	3.9225	0.6058

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Effects of auxin concentrations on root development of different sugarbeet genotypes

The data on root development percent showed significant difference due to different concentrations in six sugarbeet genotypes (Table 8). Genotype CS 0327 produced the highest rooted plant (12.67%) in treatment 2.0 mgl^{-1} NAA which was statistically different from other concentration and second highest rooted plant (5.00%) was obtained from treatment 1.0 mgl^{-1} NAA. The lowest rooted plant (2.67) was calculated from treatment 4.0 mgl^{-1} NAA. Similarly, genotype CS 0328 also produced the maximum rooted plant (8.33%) in the same treatment (2.0 mgl^{-1} NAA), while the lowest (2.33%) was found in concentration 4.0 mgl^{-1} which was statistically identical with treatment 1.0 mgl^{-1} NAA. In case of genotype HI 0044 treatment 2.0 mgl^{-1} NAA produced the highest rooted shoot (64.00%) which was significantly different from other concentrations. The lowest root development was noted (41.00%) in 4.0 mgl^{-1}. The highest (57.00%) rooted shoot was observed in MS medium supplemented with 2.0 mgl^{-1} NAA by genotype HI 0473 and the second highest was found in concentration 1.0 mgl^{-1} NAA (46.33%). Whereas, the lowest root development (28.67%) was calculated in treatment combination 4.0 mgl^{-1} NAA. Correspondingly, genotypes Shubhra and Cauvery also produced the significantly highest rooted plant in same treatment (2.0 mgl^{-1} NAA) 49.33% and 40.00% respectively. The same treatment (4.0 mgl^{-1} NAA) produced the lowest rooted plant in case both of the genotypes 26.33% and 20.33% respectively. Without supplementation of NAA on MS medium shoot did not produce any root in any genotype during the study.

Table 8. Effects of different concentrations of NAA on root development (%) of six sugarbeet genotypes

Concentrations of NAA (mgl^{-1})	Genotypes					
	CS 0327	CS 0328	HI 0044	HI 0473	Shubhra	Cauvery
0.0	0.00 c	0.00 c	0.00 c	0.00 d	0.00 d	0.00 c
1.0	5.00 b	3.33 bc	49.67 b	46.33 b	39.67 b	34.67 a
2.0	12.67 a	8.33 a	64.00 a	57.00 a	49.33 a	40.00 a
3.0	4.67 b	4.33 b	47.00 b	34.67 c	33.67 bc	31.67 a
4.0	2.67 bc	2.33 bc	41.00 b	28.67 c	26.33 c	20.33 b
LSD (0.05)	3.8161	3.8449	9.1447	10.608	9.4998	9.6952

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Interaction effects of genotypes and NAA concentrations on root development

Interaction effect of genotypes to NAA concentration presented in Table 9 revealed that root development significantly varied due to concentration of NAA. Different genotypes responded differently in time of culture. The highest per cent root development (64.00) was obtained in genotype HI 0044 at 2.0 mgl^{-1} of NAA. The second highest root development (57.00%) was observed in genotype HI 0473 at same concentration of PGR. Similar result was obtained in genotypes CS 0327, CS 0328, Shubhra and Cauvery, where they produced 12.67, 8.33, 49.33 and 40.00% rooted plant respectively. However, no rooted plant was obtained at control where no NAA was supplemented into medium. Different concentrations of IBA, IAA, NAA and strength of media had a significant effect on root characters of grown shoot of sugarbeet. Results showed that half strength MS media is more responsive compare to full strength MS media for root development of grown sugarbeet shoot in this study. The present result agreed to earlier report of Tripathi and Kumar (2010) who stated that relatively low salt concentration in the medium is known to enhance rooting efficiency of microshoots.

Different concentrations of auxins supplemented in MS medium enhance root development of sugarbeet shoot. Auxin NAA was more effective for root production in tissue culture derived sugarbeet plantlets compared to that of IBA and IAA. These findings are in good agreement with those confirmed by Farhad . (2013). Most effective concentration for root development was observed at 2.0 mgl^{-1} NAA. However, the highest value of root length was also obtained when 2 mgl^{-1} of NAA was incorporated into the medium.

Table 9. Interaction effects of genotypes and NAA concentrations on root development of sugarbeet

Sl no.	Genotypes x NAA concentration (mgl^{-1})	Root formation (%)
1	CS 0327 x 0.0	0.00 k
2	CS 0327 x 1.0	5.00 jk
3	CS 0327 x 2.0	12.67 i
4	CS 0327 x 3.0	4.67 jk
5	CS 0327 x 4.0	2.67 jk
6	CS 0328 x 0.0	0.00 k
7	CS 0328 x 1.0	3.33 jk
8	CS 0328 x 2.0	8.33 jk
9	CS 0328 x 3.0	4.33 jk
10	CS 0328 x 4.0	2.33 jk
11	HI 0044 x 0.0	0.00 k
12	HI 0044 x 1.0	49.67 bc
13	HI 0044 x 2.0	64.00 a
14	HI 0044 x 3.0	47.00 cd
15	HI 0044 x 4.0	41.00 de
16	HI 0473 x 0.0	0.00 k
17	HI 0473 x 1.0	46.33 cd
18	HI 0473 x 2.0	57.00 ab
19	HI 0473 x 3.0	34.67 ef
20	HI 0473 x 4.0	28.67 fg
21	Shubhra x 0.0	0.00 k
22	Shubhra x 1.0	39.67 de
23	Shubhra x 2.0	49.33 c
24	Shubhra x 3.0	33.67 efg
25	Shubhra x 4.0	26.33 gh
26	Cauvery x 0.0	0.00 k
27	Cauvery x 1.0	34.67 ef
28	Cauvery x 2.0	40.00 de
29	Cauvery x 3.0	31.67 fg
30	Cauvery x 4.0	20.33 h
LSD (0.05)		7.4188

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