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Effect of Growth Regulator on Regeneration of Two Sweet Potatoes (*Ipomoea batatas* L.)

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Abstract: The experiment was conducted to investigate the effect of various concentrations of different growth regulators on the aspect of callus induction along with shoot regeneration and root formation of two sweet potato varieties *viz.* BARI 6 and BARI 7. For callus induction, MS medium was used supplemented with different concentrations of 2,4–D (0, 0.1, 0.5 and 1.0 mg L^{-1}) and Kinetin (0, 0.1, 0.5 and 1.0 mg L^{-1})). Different concentrations of BAP (0, 1.5, 2.5, 3.5 and 4.5 mg L^{-1}) were used for shoot regeneration while different concentrations of NAA (0, 0.1, 0.5, 1.0 and 1.5) with a constant level of BAP @ 2.0 were also used for root development. All the studied parameters were significantly influenced by the effect of sweet potato varieties and various concentrations of growth regulators. The highest percentage (91.67%) of callus induction was observed in 0.5 mg L^{-1} 2,4–D + 0.5 mg L^{-1} kinetin with the variety BARI 6 within a minimum number of days (5.50 days) while similar effect also showed the higher weight of callus at 15 (4.65 g) and 35 (6.12 g) days of culture. Performance study of BAP concentration regarding two potato varieties were significant among the shoot characters where 2.5 mg L^{-1} BAP treated callus of BARI–6 obtained the maximum regeneration of shoot (85.00%) with an average requiring minimum time (6.83 days) while maximum leaves $shoot^{-1}$ (12.17) and longest shoot (5.50 cm) were also obtained. Percentage of root formation (73.33%) had also maximum in BARI–6 with an average requiring least time (6.83 days) while it was treated by 1.0 mg L^{-1} NAA which treatment also showed the longest root (10.83 cm).So, it is clear that the sweet potato variety BARI–6 showed superiority than BARI–7 while 0.5 mg L^{-1} 2,4–D + 0.5 mg L^{-1} kinetin was the best combination for callus induction, 2.5 mg L^{-1} BAP for shoot formation and 1.0 mg L^{-1} NAA for root regeneration in the present study.

Key Words: BAP, BARI, Callus, Growth regulators viz. 2, 4–D, Kinetin, NAA, Shoot, Sweet potato, Root

Introduction

Sweet potato (*Ipomoea batatas* L. Lam.) is a dicotyledonous plant that belongs to the family Convolvulaceae, order Polemoniales and found by Columbus and his shipmates. It is the fourth largest crop in Bangladesh after rice, wheat and potato. The country produced 307 thousand MT of sweet potatoes in an area of 78 thousands acres of land and the average yield was 3.94 MT $acre^{-1}$ during the year 2007–08 which was lower than before year (BBS, 2008). It is evident from the above statistics that the production of the crop is gradually decreasing in this country. Besides, simple starches, sweet potatoes are rich in complex, carbohydrates, dietary fiber, beta carotene, vitamin C and vitamin B_6. Sweet potato provides nutritionally significant quantities of riboflavin, iron, calcium, copper, potassium and protein. The sweet potato is conventionally propagated by vine cuttings in the field and thus susceptible to cumulative infection by fungi, bacteria, viruses and viroids. These infections cause degeneration of the crop resulting yield reduction. Tissue culture is an important field of biotechnology and has a potential to improve the quality and quantity of vegetatively propagated sweet potato. Establishment of an efficient plant regeneration system for the sweet potato grown in Bangladesh will be highly useful. Sweet potato plant can be regenerated in vitro from explants of many parts (Hossain *et al*., 1999; Cheng and Yeh, 2003). The techniques have not only the potential of producing a large number of propagules within a single year but also have added advantage of built in disease protection. Rapid multiplication by tissue culture would enable newly selected varieties to be bulked up in a disease free condition for commercial use. It is well documented that *in vitro* techniques are used to be an effective tool for improvement of sweet potato. So, far, *in vitro* techniques for regeneration of sweet potato are not established in Bangladesh. Therefore, there is a need for successful regeneration technique for improvement of sweet potato. The present experiment was undertaken to study the effect of various concentration of growth regulator on the aspect of callus induction, shoot regeneration and root formation of BARI released two sweet potato varieties.

Materials and Methods

The research program reported here was carried out during the period from May to September 2010 in the USDA Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh. Two sweet potato varieties *viz.* BARI–6 and BARI–7 were used as experimental materials which were collected from the Bangladesh Agricultural Research Institute, Gazipur-1701. Leaf explant of sweet potato were used for callus induction on MS medium supplemented with different concentrations of 2,4–D (0.0. 0.1, 0.5 and 1.0 mg L^{-1}) in combinations with different concentrations of Kinetin (0.0,0.1, 0.5 and 1.0 mg L^{-1}). After callus induction, calli were used for shoot regeneration in different concentrations of BAP (0.0. 1.5, 2.5, 3.5 and

4.5 mg L^{-1}). The regenerated shoot were rooted on MS medium supplemented with different concentrations of NAA (0.0. 0.1, 0.5 and 1.0 mg L^{-1}) in combination with a constant level of BAP (2.0 mg L^{-1}). To investigate the effect of different treatments of growth regulators on callus induction, shoot regeneration and root formation, the data were recorded on days to callus induction, callus induction (%), weight of callus at 15 and 35 days of culture, shoot regeneration (%), days to shoot initiation, number of leaves $shoot^{-1}$, shoot length, percent shoot sowing root, days to root and length of root. The experiment was laid out in a completely randomized design (CRD) with three replications. The collected data were analyzed statistically and means were adjudged by DMRT at 5% level of probability (Gomez and Gomez, 1984).

Results and Discussion

Percentage of callus induction and days to callus initiation varied significantly due to the effect of sweet potato varieties in combination with different hormone combinations of 2,4–D and kinetin (Table 1). The highest percentage (91.67%) of callus induction was observed in 0.5 mg L^{-1} 2,4–D + 0.5 mg L^{-1} kinetin with the variety BARI 6 within a minimum number of days (5.50 days) followed by (88.33%) was found in the same treatments with the variety BARI 7 at requiring days of 5.83. The lowest percentage (20.00%) of callus induction was recorded in 0 mg L^{-1} 2,4–D + 0.1 mg L^{-1} kinetin at both varieties BARI 6 and BARI 7.

Table 1. Effects of sweet potato varieties on regeneration of callus, days to callus and weight of callus at 15 and 35 days after callus initiation

Varieties	2,4–D + Kn (mg L^{-1})	Callus induction (%)	Days to callus initiation	Weight of callus at 15 days	Weight of callus at 35 days
BARI 6	0+0	0.0 n	0.0	0.0 o	0.00 q
	0+0.1	20.00 m	11.17	0.79 n	1.79 p
	0+0.5	25.00 lm	10.83	0.94 m	1.99 o
	0+1.0	31.67 k-m	9.33	0.98 lm	2.04 o
	0.1+0	23.33 lm	9.67	1.05 k	2.01 o
	0.1+0.1	41.67 i-k	9.67	1.71 j	2.75 n
	0.1+0.5	56.67 d-h	8.33	2.26 h	3.29 l
	0.1+1.0	68.33 cd	7.67	3.43 e	4.15 j
	0.5+0	31.67 k-m	9.17	3.04 g	4.00 k
	0.5+0.1	61.67 c-g	7.33	4.43 b	5.85 b
	0.5+0.5	91.67 a	5.50	4.65 a	6.12 a
	0.5+1.0	85.00 a	6.83	4.24 c	5.35 d
	1.0+0	60.00 c-g	7.17	3.78 d	4.99 f
	1.0+0.1	55.00 e-g	9.0	3.35 f	4.56 h
	1.0+0.5	71.67 b-c	7.5	3.34 f	5.69 c
	1.0+1.0	58.33 d–g	9.5	2.09 i	3.98 k
BARI 7	0+0	0.0	0	0.00 o	0.00 q
	0+0.1	20.00 n	11.83	0.77 n	1.78 p
	0+0.5	28.33 lm	11.00	0.92 m	1.92 o
	0+1.0	35.00 j-l	9.67	0.97 lm	1.96 o
	0.1+0	25.00 lm	10.5	1.0 kl	2.03 o
	0.1+0.1	35.00 j-l	9.3	1.69 j	2.69 n
	0.1+0.5	53.33 f-i	8.67	2.24 h	3.27 l
	0.1+1.0	66.67 c-e	8.17	3.43 e	4.42 i
	0.5+0	31.67 k-m	9.83	3.03 g	4.21 j
	0.5+0.1	44.50 h-j	8.17	4.41 b	5.42 d
	0.5+0.5	88.33 a	5.83	4.63 a	5.65 c
	0.5+1.0	81.67 ab	7.50	4.23 c	5.23 e
	1.0+0	55.00 e-h	7.83	3.76 d	4.77 g
	1.0+0.1	50.00 g-i	9.17	3.33 f	4.33 i
	1.0+0.5	65.00 c-f	8.50	3.33 f	4.34 i
	1.0+1.0	51.67 g-i	10.17	2.07 i	3.07 m
Sig. level		*	**	**	**
CV (%)		**19.34**	**13.34**	**2.21**	**2.73**

At 15 days of culture, the highest weight of callus (4.65 g) was recorded from the variety BARI 6 in 0.5 mg L^{-1} 2,4–D + 0.5 mg L^{-1} kinetin which was followed by the variety BARI 7 (4.63 g) in similar hormone treatment. Similarly, the variety BARI 6 also showed the highest weight of callus (6.12 g) in 0.5 mg L^{-1} 2,4–D + 0.5 mg L^{-1} at 35 DAS. On the other hand, the lowest weight of callus (0.77 g) was found from the both variety BARI 6 and BARI 7 in 0 mg L^{-1} 2,4–D + 0.1 mg L^{-1} kinetin at 15 days after culture statistically similar results were also found at 35 days of culture with BARI 6 and BARI 7 (1.78 and 1.79 g, respectively) in the similar hormone treatments (Table 1). These results indicated that the 0.5 mg L^{-1} 2,4–D + 0.5 mg L^{-1} kinetin showed the highest callus proliferation ability with the variety BARI 6 than that of BARI 7 and other hormone concentrations. The present results was also similar to the findings of Wang et al. (1999) where they reported that MS medium supplemented with 0.02 or 0.05 mg L^{-1} 2,4–D and 0.5 mg L^{-1} kinetin would be used for callus induction of sweet potato.

The combined effect between sweet potato varieties and various concentrations of BAP was significantly influenced on regeneration of shoot (%), days to shoot initiation, number of leaves $shoot^{-1}$ and length of shoot (Table 2). From the Table 2, it was evident that the concentration of BAP at 2.5 mg L^{-1} was more effective to produce greater results on shoot regeneration than that of other concentrations of BAP regarding to both BARI varieties. As a result, regeneration of shoot of BARI 6 had maximum (85.00%) in 2.50 mg L^{-1} BAP which was closely followed by the similar concentration of BAP in BARI 7 (78.33%). Similarly, maximum leaves $shoot^{-1}$ (12.17) was also found from the variety BARI 6 in 2.5 mg L^{-1} BAP whereas statistically similar leaves $shoot^{-1}$ (11.67) was produced from the variety BARI 7 in 2.5 mg L^{-1}. On the other hand, longest shoot (5.50 cm) was found from the variety BARI 6 in 2.5 mg L^{-1} BAP which was closely followed by the variety BARI 7 in 2.5 mg L^{-1} (5.00 cm), BAP at 3.5 mg L^{-1} in BARI 6 (4.83 cm) and BAP at 1.5 mg L^{-1} in both BARI 6 and BARI 7 (both similar 4.50 cm) whereas they were statistically identical. Similarly, BAP at 2.5 mg L^{-1} requiring the minimum time (6.83 days) for days to shoot initiation of BARI 6. Among other concentrations of BAP, BAP at 4.5 mg L^{-1} showed the minimum regeneration of shoot (41.67%) with an average requiring maximum time (13.50 days) for shoot initiation, minimum leaves $shoot^{-1}$ (3.33) and shortest shoot (2.33 cm) while statistically identical leaves $shoot^{-1}$ (3.83) and shoot length (2.50 cm) were produced from the variety BARI 6 in 4.5 mg L^{-1} BAP. From the observation of the Table 2, it was also found that without BAP (control) did not regenerate any shoot during the study. The result of the present study was similar to the finding of Wang *et al.* (1999) who reported that shoot regeneration was promoted by BAP in petiole callus of sweet potato *viz.* Genki.

Table 2. Effects of different concentrations of BAP on regeneration of shoot, days to shoot, number of leaves $shoot^{-1}$ and length of shoot

Varieties	BAP (mg L^{-1})	Shoot regeneration (%)	Days to shoot initiation	Number of leaves $shoot^{-1}$	Length of shoot (cm)
BARI 6	0	0 g	0	0.00 f	0 d
	1.5	71.67 bc	8.83	9.0 b	4.50 ab
	2.5	85.00 a	6.83	12.17 a	5.50 a
	3.5	58.33 de	11.50	7.00 cd	4.83 ab
	4.5	48.33 ef	13.17	3.83 e	2.50 c
BARI 7	0	0.0 g	0.0	0.0 f	0.0 d
	1.5	61.67 cd	10.17	8.50 bc	4.50 ab
	2.5	78.33 ab	7.83	11.67 a	5.00 ab
	3.5	50.00 d-f	12.67	6.50 d	4.33 d
	4.5	41.67 f	13.83	3.33 e	2.33 c
Sig. level		*	**	**	**
CV (%)		**16.08**	**13.78**	**15.30**	**19.27**

A significant variation was also found due to the combined effect between sweet potato varieties and various concentrations of NAA supplemented with BAP at 2.0 mg L^{-1} on root formation, days to root initiation and length of root (Table 3). Among the various concentrations of NAA, it was found that the NAA at 1.0 mg L^{-1} observed the maximum shoot sowing root (73.33%) and longest root (10.58 cm) of BARI 6 which was statistically identical with the similar concentration of BAP with the variety BARI 7 (70.00% and 10.33 cm, respectively), the variety BARI 6 in 0.5 mg L^{-1} NAA (66.67% and 10.00 cm, respectively) and the variety BARI7 in 0.5 mg L^{-1} NAA (63.33% and 9.67 cm, respectively). Among

other concentration of NAA, NAA at 0.1 mg L^{-1} observed the minimum shoot showing root (28.33%) of BARI 7 while statistically similar minimum shoot showing root (30.00%) was found from the variety BARI 6 in similar concentration of NAA. Similarly, 1.5 mg L^{-1} NAA supplemented with 2.0 mg L^{-1} BAP showed the statistically identical shortest root (5.33 and 5.83 cm) from the variety BARI 7 and 6, respectively. These results indicated that 1.0 mg L^{-1} NAA supplemented with 2.0 mg L^{-1} BAP would be optimum concentration and more effective to produce more and earlier root formation, longest root it was in this study. The result of the present study was agreed to the finding of Gao *et al*. (1999) who found that the optimum medium contained 1.0 mg NAA L^{-1} for root differentiation and 0.1 mg L^{-1} for root growth of sweet potato. Besides, they also found that the concentration of NAA seemed to be the most important factor for root differentiation and growth of sweet potatoes.

Table 3. Effects of different concentrations of NAA with BAP at 2.0 mg L^{-1} on regeneration of root, days to root and length of root

Varieties	NAA (mg L^{-1})	Shoots showing root (%)	Days to root initiation	Length of root (cm)
BARI 6	0	0.0 d	0	0.0 d
	0.1	30.00 c	11.50	7.67 b
	0.5	66.67 a	7.17	10.00 a
	1.0	73.33 a	6.83	10.83 a
	1.5	46.67 b	10.17	5.83 c
BARI 7	0	0.0 d	0.0	0.00
	0.1	28.33 c	11.50	7.0 b
	0.5	63.33 a	8.67	9.67 ab
	1.0	70.00 a	8.17	10.33 a
	1.5	45.00 b	10.50	5.33 c
Sig. level		**	**	**
CV (%)		**18.04**	**12.25**	**14.10**

From the above result it could be concluded that the 0.5 mg L^{-1} 2,4–D + 0.5 mg L^{-1} kinetin for callus induction, 2.5 mg L^{-1} BAP for shoot regeneration and 1.0 mg L^{-1} NAA in combination with 2.0 mg L^{-1} BAP for root formation were optimum concentration in the present study for both the sweet potato varieties. However, sweet potato variety BARI 6 showed the better performance than BARI 7 with the assigned growth regulators in respects of the studied all characteristics.

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