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Regeneration and Establishment of Potato Plantlets Through Callus Formation with BAP and NAA

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Abstract: The experiment was conducted in the USDA Biotechnology Laboratory, Biotechnology Department, Bangladesh Agricultural University, Mymensingh to observe the effect of NAA and BAP on callus formation and regeneration from leaf and internodal segment explants. Five levels of each of NAA (0, 1.25, 2.5, 5 and 10 mg l^{-1}) and BAP (0, 0.5, 1, 2 and 4 mg l^{-1}) were applied to MS media for callus induction as well as plantlet regeneration. Twenty explants were cultured in each combination. Leaf showed better performance in callus induction and plantlet regeneration. The highest percentage of callus (95%) was induced with 2.5 mg l^{-1} NAA + 2 mg l^{-1} BAP and also minimum time (8.13 days) was required for callus induction in the same concentrations. The percentage of regeneration showed the highest value (80%) with 2.5 mg l^{-1} NAA + 2 mg l^{-1} BAP of all the combinations. It was also observed that callus derived from leaf produced plantlets in a shortest period of time (23.68 days) compared to that from stem (28.96 days).

Key words: NAA, BAP, callus, regeneration, establishment, potato

Introduction

Potato is a staple food in some countries and important vegetable in Bangladesh. In Bangladesh, potato represents about 53% of the total edible vegetables. The potential value of tissue culture in crop improvement has been widely recognized. The regeneration of plants from cell and tissue culture is an important and essential component of biotechnology that is required for the genetic manipulation of plants. High frequency regeneration of plants from *in vitro* cultured tissues and cells is a pre-requisite for successful application of tissue culture and genetic engineering technologies for crop improvement. Many attempts have been made to enhance the frequency of plant regeneration from potato callus and a lot of research has been devoted to investigate the factors affecting plant regeneration. Great progress has been made in potatoes for plant regeneration in recent years (Ehsanpour and Jones, 2000; Fiegert *et al*., 2000; Ahn *et al*., 2001). Both callus induction and plant regeneration from explants require the presence of appropriate combinations and concentrations of plant growth regulators in the culture media. Callus induction, subsequent plant regeneration and microtuberization are somehow dependent on the type of explants, media components, growth conditions and varieties. Remarkable work has been done on plant regeneration with different explant and growth regulators. Many author work to standardize the optimum concentrations of BAP and NAA for regeneration of potato (Dobranszki *et al*., 1999; Hansen *et al*., 1999; Zel *et al*., 1999; Fomenko *et al*., 2000; Asthma *et al*., 2001). It is important to standardize the protocol for regeneration of potatoes grown in Bangladesh. The present study was undertaken to investigate the *in vitro* callus induction ability and regenerability of potato with optimum concentrations of BAP and NAA.

Materials and Methods

The present investigation was carried out in the USDA Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh in 2002. Potato tubers cv. Cardinal were collected from Bangladesh Agricultural Research Institute, Gazipur. Disease-free potato tubers were sprouted in the dark condition at room temperature.

The present experiment consisted of three factors eg. explant, NAA and BAP. There were 5 levels of both of NAA (0, 1.25, 2.5, 5 and 10 mg l^{-1}) and BAP (0, 0.5, 1, 2 and 4 mg l^{-1}) and two types of explants eg. leaf and internodal segment The experiment was laid out in Completely Randomized Design (CRD) with 5 replications. For surface sterilization, etiolated sprouts of potato were first sterilized with 70% (v/v) ethanol for few seconds. The sprouts were then rinsed twice with sterile distilled water. Afterwards the sprouts were surface sterilized by immersing in 0.1% $HgCl_2$ solution for 2 min and then washed several times with sterile distilled water. The pH medium was adjusted to 5.8. MS media was prepared and

then autoclaved at 1.16 kg cm^{-2} pressure at 121°C for 20 min. The sprouts were allowed to grow on MS solidified medium with 3% sucrose. At 25±2°C under 16 h photoperiod. One month old *in vitro* grown micro plants were used as the source of explants. Four explants were directly inoculated to each glass petridish (70 mm dia.) containing 10 ml of MS medium with different hormone concentrations as per treatment. The petridishes were covered and sealed with parafilm and were kept at 25±2°C illuminated 16 h daily (light intensity of 1500 lux) with 1.83 m florescent tubes (C84 TDFL/Phillips). Callus was initiated after 7-18 days of explant inoculation. Plantlets were developed from calli in this media after 10-25 days. Plantlets of the age of 30 days with well developed roots were removed from petridishes. Plantlets were then individually transplanted in pots containing sand, soil and cowdung (1:1:1) mixtures. Immediately after transplantation, the plantlets were irrigated with a fine spray of water and the plantlets along with pots were covered with transparent polythene bags to prevent desiccation. The pots were kept in the laboratory and were irrigated regularly. The plantlets were established within 5 to 7 days and then the polythene bags were removed. Data were recorded on different parameters of callus and plantlets. The analysis of variance for different characters were performed and means were compared by the Duncan's Multiple Range Test (DMRT).

Results and Discussion

Auxin and cytokinin showed significant variation for plant regeneration via induced calli from leaf and internodal stem explants.

Callus formation: The main effect of explants and different supplements of BAP and NAA on callus induction have been presented in Table 1. Twenty explants were inoculated in each treatments. Leaf was more responsive for induction of callus and showed better callusing (11.38) than internodal segment (9.14) out of 20 cultured explants. The highest callusing was obtained in 5 mg l^{-1} NAA (13.00) followed by 2.5 mg l^{-1} NAA (12.90). On the other hand, 2 mg l^{-1} BAP showed highest callusing (12.60) followed by 1 mg l^{-1} BAP (12.50) from 20 explants.

The main effect showed that the percentage of callus induction from leaf was higher (56.58%) than internodal segment (45.76%). Also the percentage of callus induction was found significantly higher in 5 mg l^{-1} NAA (65.00%) followed by 2.5 mg l^{-1} NAA (64.50%); and in 2 mg l^{-1} BAP (63.00%) followed by 1 mg l^{-1} BAP (62.50%). Lowest callus was found when the treatment contained no NAA (12.30%) and no BAP (27.50%). Leaf explants required minimum time (12.26 days) for callus induction and internodal explants required more time (14.48 days). Minimum time was required for 2.5 mg l^{-1} NAA (12.30 days) and 1 mg l^{-1} BAP (12.50 days) than that of other supplements.

Table 1: Main effect of different combinations of NAA and BAP in MS medium on callus induction from leaf and internodal segment of potato cv. Cardinal. Data were recorded at 10 days of explantation. Twenty explants were placed in each treatment

Treatments		No. of callus inducing explants	Days to callusing	% of callus induction
Leaf		11.38A	12.26B	56.58A
Internodal segment		9.14B	14.48A	45.76B
NAA (mg l^{-1}) :	0	2.46D	12.70C	12.30D
	1.25	12.10B	13.40B	60.44B
	2.5	12.90A	12.30C	64.50A
	5	13.00A	13.14B	65.00A
	10	10.84C	15.30A	53.60C
BAP (mg l^{-1}) :	0	5.50D	12.34D	27.50C
	0.5	10.54B	14.10B	51.94B
	1	12.50A	12.50CD	62.50A
	2	12.60A	12.90C	63.00A
	4	10.16C	15.00A	50.90B

Among the different combinations, 2 mg l^{-1} BAP + 2.5 mg l^{-1} NAA and 1 mg l^{-1} BAP + 1.25 mg l^{-1} NAA showed better callus induction i.e 19.00 and 18.00, respectively in a shortest period out of 20 explants (Table 2). The explants cultured without growth regulator did not produce any callus; these results are in support of the results obtained by Fiegert *et al.* (2000) and Jayasree *et al.* (2001).

The percentage of callus induction was the highest in MS media containing 2 mg l^{-1} BAP + 2.5 mg l^{-1} NAA (95%) followed by 1 mg l^{-1} BAP + 1.25 mg l^{-1} NAA (90%) from leaf explants (Table 2). NAA was essential for callus induction and the amount of callus induction increased with increasing concentrations of NAA and BAP which was similar to the results of Martel *et al.* (1992).

Regeneration through callus: The results on the main effect of explants and different supplements of BAP and NAA on regeneration of potato cv. Cardinal have been presented in Table 3. Leaf showed better performance in regeneration (5.40) through callus from 20 cultured explant. The percentage of regeneration was recorded higher in case of leaf (25.60%) than internodal segment (11.60%). Also leaf took minimum time (23.68 days) for regeneration compared to internodal segment (28.96 days). The highest regeneration potentiality was found in 2.5 mg l^{-1} NAA (6.60) followed by 1.25 mg l^{-1} NAA (6.10) out of 20 explants. But there was no regeneration without growth regulator. The percentage of regeneration was the highest in 2.5 mg l^{-1} NAA (33.00%) followed by 1.25 mg l^{-1} NAA (30.50%) and 2 mg l^{-1} BAP (30.50%).

Table 2: Combined effect of different combinations of NAA and BAP in MS medium on callus induction from leaf and internodal stem of potato cv. Cardinal. Data were recorded at 10 days of explantation. Twenty explants were placed in each treatment

Treatment combinations					
	Hormones				
Explants	NAA (mg l^{-1})	BAP (mg l^{-1})	No. of callus inducing explants	Days to callusing	% of callus induction
Leaf	0	0	-	-	-
	0	0.5	4.00LM	14.20DE	20.00LM
	0	1	5.00L	13.30EF	25.00L
	0	2	3.00MN	15.1CD	15.00MN
	0	4	3.00MN	16.20BC	15.00MN
	1.25	0	5.00L	14.5DE	25.00L
	1.25	0.5	14.00EF	12.4FG	69.40EF
	1.25	1	18.00AB	10.28H	90.00AB
	1.25	2	16.00CD	12.16FG	80.00CD
	1.25	4	13.00FG	13.47EF	65.00FG
	2.5	0	8.00K	13.26EF	40.00K
	2.5	0.5	13.00FG	12.37FG	65.00FG
	2.5	1	16.00CD	10.61H	80.00CD
	2.5	2	19.00A	8.13I	95.00A
	2.5	4	14.00EF	13.48EF	70.00EF
	5	0	12.00GH	12.40EFG	60.00GH
	5	0.5	14.00EF	12.13FG	70.00EF
	5	1	15.00DE	11.21GH	75.00DE
	5	2	16.00CD	11.27GH	80.00CD
	5	4	14.00EF	13.25EF	70.00EF
	10	0	8.00K	17.45AB	40.00K
	10	0.5	13.40F	15.94CD	60.00GH
	10	1	14.00EF	13.48EF	70.00EF
	10	2	15.00DE	12.12FG	75.00DE
	10	4	12.00GH	15.17CD	60.00GH
Internodal Segment	0	0	-	-	-
	0	0.5	2.00N	17.31AB	10.00N
	0	1	3.00MN	16.26BC	15.00MN
	0	2	2.00N	18.41A	10.00N
	0	4	2.60N	18.17A	13.00N
	1.25	0	3.00MN	17.61AB	15.00MN
	1.25	0.5	12.00GH	14.63DE	60.00GH
	1.25	1	16.00CD	12.43FG	80.00CD
	1.25	2	13.00FG	15.51CD	65.00FG
	1.25	4	11.00HI	15.31CD	55.00HI
	2.5	0	5.00L	16.71BC	25.00L
	2.5	0.5	11.00HI	14.61DE	55.00HI
	2.5	1	14.00EF	12.10FG	70.00EF
	2.5	2	17.00BC	10.51H	85.00BC
	2.5	4	12.00GH	15.67CD	60.00GH
	5	0	9.00JK	16.37BC	45.00JK
	5	0.5	12.00GH	14.42DE	60.00GH
	5	1	13.00FG	13.81EF	65.00FG
	5	2	14.00EF	14.37DE	70.00EF
	5	4	11.00HI	15.42CD	55.00HI
	10	0	5.00L	18.19A	25.00L
	10	0.5	10.00IJ	17.27AB	50.00IJ
	10	1	11.00HI	15.31CD	55.00HI
	10	2	11.00HI	14.34DE	55.00HI
	10	4	9.00JK	17.12AB	46.00J

Table 3: Main effect of different combinations of NAA and BAP in MS medium on plant regeneration of potato cv. Cardinal. Data were recorded at 21 days of explantation. Twenty explants were placed in each treatment

Treatments		No. of regenerants through callus	Days required for regeneration	% regeneration
Leaf		5.40A	23.68B	25.60A
Internodal stem		2.32B	28.96A	11.60B
NAA (mg l^{-1}) :	0	0.00E	0.00D	0.00E
	1.25	6.10B	24.10C	30.50B
	2.5	6.60A	32.50B	33.00A
	5	3.90C	32.40B	20.50C
	10	1.80D	42.60A	9.00D
BAP (mg l^{-1}) :	0	0.00C	4.90E	0.00D
	0.5	3.40B	35.70A	17.00C
	1	5.70A	30.40C	28.50B
	2	5.90A	25.70D	30.50A
	4	3.40B	34.90B	17.00C

Table 4: Combined effect of different combinations of NAA and BAP in MS medium on plant regeneration of potato cv. Cardinal. Data were recorded at 21 days of explantation. Twenty explants were placed in each treatment

Treatment combinations					
	Hormones				
Explants	NAA (mg l^{-1})	BAP (mg l^{-1})	No. of regenerants through callus	Days required for regeneration	% regeneration
Leaf	1.25	0.5	9.00DE	31.20MN	45.00E
	1.25	1	14.00B	26.10RS	70.00B
	1.25	2	10.00D	29.50OP	50.00D
	1.25	4	8.00EF	32.80LM	40.00F
	2.5	0	0.00	0.00	0.00
	2.5	0.5	7.00FG	36.20I	35.00G
	2.5	1	12.00C	27.50QR	60.00C
	2.5	2	16.00A	25.80S	80.00A
	2.5	4	9.00DE	30.60NO	45.00E
	5	0	0.00	0.00	0.00
	5	0.5	6.00GH	34.10JK	30.00H
	5	1	8.00EF	33.50KL	40.00F
	5	2	8.00EF	28.30PQ	50.00D
	5	4	6.00GH	35.70IJ	30.00H
	10	0	0.00	0.00	0.00
	10	0.5	2.00KL	51.50D	10.00L
	10	1	4.00IJ	47.30FG	20.00J
	10	2	5.00HI	43.70H	25.00I
	10	4	2.00KL	55.50C	10.00L
Internodal Stem	1.25	0.5	4.00IJ	48.10EF	20.00J
	1.25	1	8.00EF	33.50KL	40.00F
	1.25	2	5.00HI	42.60H	25.00I
	1.25	4	3.00JK	49.80E	15.00K
	2.5	0	0.00	000	0.00
	2.5	0.5	3.00JK	48.40EF	15.00K
	2.5	1	7.00FG	31.30MN	35.00G
	2.5	2	9.00DE	32.60LM	45.00E
	2.5	4	3.00JK	47.80FG	15.00K
	5	0	0.00	0.00	0.00
	5	0.5	2.00KL	51.40D	10.00L
	5	1	3.00JK	46.20G	15.00K
	5	2	4.00IJ	48.30EF	20.00J
	5	4	2.00KL	49.50E	10.00L
	10	0	0.00	0.00	0.00
	10	0.5	1.00LM	58.20B	5.00M
	10	1	1.00LM	61.60A	5.00M
	10	2	2.00KL	52.80D	10.00L
	10	4	1.00LM	59.30B	5.00M

No regeneration was noticed in absence of either of NAA or BAP

Various combinations of supplements showed significant variation in regeneration ability. Among the combinations used, 2 mg l^{-1} BAP +2.5 mg l^{-1} NAA showed the highest regeneration of plantlets from leaf (16.00) and internodal segment (9.00), respectively out of 20 cultured explants (Table 4).

The percentage of regeneration was recorded the highest (80%) in MS media containing 2 mg l^{-1} BAP + 2.5 mg l^{-1} NAA followed by 1 mg l^{-1} BAP + 1.25 mg l^{-1} NAA (70%) from leaf (Table 4). Also it was observed that the above combinations required shorter period of time for regeneration (25.80 and 26.10 days, respectively). It was also found that leaf was always more responsive explant than internodal segment. Yadav and Sticklen (1995), Alphonse *et al.* (1998), Hamdi *et al.* (1998) observed that leaf was the best explant for regeneration. The regenerants were successfully established *ex vitro* on sand, soil and cowdung mixture (1:1:1).

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