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Role of Explants and NAA on Callus Induction of Potato (*Solanum tuberosum*)

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Abstract: An experiment was conducted to study the role of NAA and different explants on callus induction of potato varieties. There were three factors such as variety (Diamant, Heera and Cardinal), explants (leaves and internodes) and NAA levels (0, 2, 3, 4, 5 mg L^{-1}). The experiment was laid out in complete randomized design with three replications. Minimum 6-8 days was required for callus initiation of internode of Cardinal in 3 mg L^{-1} NAA while the maximum 22-25 days was required for internode of Cardinal in 5 mg L^{-1} NAA. Both the leaf and internode of Heera produced 100% callus while Cardinal produced minimum callus (37.7 and 20.8% for leaf and internode, respectively) in 5 mg L^{-1} NAA. Neither leaf nor internode produced callus without NAA. Internode of Diamant, Heera and Cardinal produced 100% compact calli in 4, 3 and 5 mg L^{-1} NAA respectively. Leaf of Diamant without NAA produced the highest weight (0.667 g) of callus after one month. After two months, internode of Cardinal showed the highest weight (0.205 g) of calli in 3 mg L^{-1} NAA. Therefore, the present protocol has the potential for the rapid multiplication of true-to-type clones without changing the genetic fidelity.

Keywords: Potato, Callus Induction, NAA, Explant

1. Introduction

Potato (*Solanum tuberosum L.*) belongs to the family Solanaceae is regarded as staple food in many countries of the world. Potato ranks 7th in respect of land occupancy and 5th in its production among the global food crops [1] while it ranks 1st both in area and production among the vegetable crops grown in Bangladesh and, about 9.5 million tons of potato was produced from nearly 0.475 million hectares of land with an average yield of 19.93 t ha^{-1} [2]. This yield is very low compared to many other countries. Plant tissue culture techniques have been developed as a powerful tool for crop improvement [3]. The focus of research for many crop species put into tissue culture is able to develop callus and ultimately regenerate a normal plant in appropriate culture media. For this purpose different plant parts such as leaf, internode, root, microtuber etc. can be used as explants and different hormones can be used as growth regulators. After passing several steps such as callusing, shooting, rooting etc., a complete potato plant can be developed from the tissues of different plant parts in presence of different hormones [4]. Different explants and different combinations of growth regulators had an influence on callus induction from various *Solanum tuberosum* varieties [5]. Both induction and rate of callus growth were strongly influenced by genotype [6] and in vitro callus induction was greatly influenced by the type of explants and growth regulators [7]. NAA stimulated both callus induction and root formation [8]. NAA is essential for callus formation and the amount of callus formed increased with the increase of NAA concentrations [9]. α-NAA, is essential for dynamic callus formation in potato from tuber explants [10]. Many authors worked to standardize the optimum concentrations of NAA for regeneration of potato [10] [11]. Hence the present study was initiated to find out the suitable concentration of NAA on leaf and internode explants of potato varieties in callus induction.

2. Materials and Methods

There were three factors such as varieties (Diamant, Cardinal and Heera), explants (leaves and internodes) and NAA levels (0, 2, 3, 4, 5 mg L^{-1}). The experiment was laid out in a complete randomized design with three replications. The potato tubers were sprouted in the dark at room temperature (25°C). Sometimes the tubers were sprayed with 2 ppm GA_3 for sprout initiation. After initiation, selected sprouts (app. 1 cm long) were harvested for culture. Harvested sprouts were sterilized with 70% ethanol, 5.25% NaOCl, 2-3 drops of Tween-20 and required amount of double distilled water. The sprouts were then transferred to culture MS medium [12]. Microplants derived from cultured sprouts were ready for multiplication within 20-25 days.

Required amount of MS medium was prepared and supplemented with a common level (2 mg L^{-1}) of BAP. Then the medium was divided into five equal parts. Four parts were supplemented with 2, 3, 4 and 5 mg L^{-1} of NAA separately and one part remained without NAA that was treated as control. After sterilization, 10 ml of medium was taken to each sterilized petridish. The number of petridishes was arranged as per treatments and replications. All the cultures were kept in a growth room at 25±2°C temperature. The temperature was maintained by air cooler and measured by thermometer. The growth room was lighted (2500-3000Lux) for 16 hours. Leaves and internodes of potato cultivars were cut into pieces with sterile sharp scalpel. 5 to10 pieces of leaf discs and internodes were separately arranged horizontally to each petridish and gently pressed onto the surface of the medium. Then the petridishes were placed under dark in the growth chamber/room at a controlled temperature of 25±2°C. The petridishes were checked daily to note the response and the development of contamination. To start of callus initiation in every treatment was recorded. After one month of explantation, per cent callus formed was calculated on the basis of total explants placed and category of calli according to compactness (compact, less compact) were calculated on the basis of total callus formed and then average weight of each callus was calculated by weighing 10 calli which dividing by 10. After two month of explantation, average weight of each callus was calculated by weighing 10 calli and then dividing by 10, and number of roots $callus^{-1}$ was calculated on the basis of total root forming calli as well as number of shoots $callus^{-1}$ was calculated on the basis of total shoot forming calli. The analysis of variance (ANOVA) for various parameters were done following the F-test and the mean values were adjudged by Duncan's Multiple Range Test (DMRT) (p = 0.05) [1] [13]. Data were analyzed following standard procedure using MSTATc program.

3. Results and Discussion

Table 1. *Main effect of variety, explant and NAA on compactness of calli and on weight of callus.*

Sole treatment		Days to callus initiation (range)	After one month		% callus formed	Average weight of callus (g)	Average weight of callus (g) after two months
			Category of calli according to compactness				
			% compact calli	% less compact calli			
Variety	Diamant	7-23	90.88	9.12	56.31	0.092	0.078 b
	Heera	9-21	87.89	12.11	64.01	0.034	0.100 a
	Cardinal	6-25	84.59	15.41	66.26	0.035	0.095 a
Explant	Leaf	8-25	78.73	21.27	59.14	0.074	0.085 a
	Internode	6-25	92.62	7.38	55.7	0.033	0.096 a
NAA (mg L^{-1})	0	-	-	-	-	0.111	0.000 d
	2.0	13-23	90.53	9.47	51.87	0.034	0.086 c
	3.0	7-15	88.94	11.06	83.17	0.046	0.133 a
	4.0	6-14	94.56	5.44	77.01	0.047	0.138 a
	5.0	7-25	68.67	31.33	74.03	0.030	0.097 b

Figures followed by same letter(s) are statistically similar as per DMRT

3.1. Days to Callus Initiation

Days required for callus initiation varied widely in the varieties. Among the varieties, callus initiation was taken place within 7-23 days in Diamant, 9-21 days in Heera and 6-25 days in Cardinal. Among the explants, leaf started callus initiation within 8-25 days and internode within 6-25 days. Among the different concentrations of NAA, it was observed that callus initiation started at 13 days and continued up to 23 days in 2 mg L^{-1} NAA. Concomitantly the duration is 7-15 days in 3 mg L^{-1} NAA, 6-14 days in 4 mg L^{-1} NAA and 7-25 days in 5 mg L^{-1} NAA while no callus initiation was observed without NAA (Table 1).

Considering all the three factors it was observed that internode of Cardinal in 4 mg L^{-1} NAA required minimum days (6-8) for callus initiation followed by internode of Cardinal in 3 mg L^{-1} NAA and internode of Diamant in 5 mg L^{-1} NAA (7-8). Maximum days (22-25) were required for callus initiation in case of internode of Cardinal in 5 mg L^{-1} NAA. Neither leaf nor internode of any of the varieties produced callus without NAA in the media. Both induction and rate of callus growth were significantly influenced by potato genotype, medium and their interactions which were proved earlier by [6] (Table 2).

Table 2. *Combined effect of variety, explant and NAA on days to callus initiation.*

Explant	NAA (mg L^{-1})	Variety		
		Diamant	Heera	Cardinal
Leaf	0	-	-	-
	2.0	18-22	17-20	14-16
	3.0	11-13	10-11	8-11
	4.0	9-11	10-12	8-10
	5.0	8-11	9-12	20-25
Internode	0	-	-	-
	2.0	21-23	17-21	13-14
	3.0	14-15	10-11	7-8
	4.0	11-14	11-13	6-8
	5.0	7-8	10-12	22-25

3.2. Per Cent Callus Formed

Among the varieties, the highest amount of callus was formed in Cardinal (66.26%) followed by Heera (64.01%) whereas the lowest was formed in Diamant (56.31%). Similar result was also observed by Tikk and Kollist [14], where all the varieties showed callus forming ability but there were differences in the rate of callus formation and the stability of callus. Comparing the two explants, leaf (59.14%) performed better than the internode (55.70%). This result corroborated with the findings of [15]. Among the different NAA levels, 3 mg L^{-1} NAA produced the highest (83.17%) callus followed by 4 mg L^{-1} NAA (77.01%) and the lowest (51.87%) was in 2 mg L^{-1} NAA. No callus was formed in the control. The present results supported the findings of [15] where MS medium supplemented with 3.0 mg L^{-1} NAA and 0.5 mg L^{-1} BAP was found the best for callusing of both leaf and internode explants (Table 1).

Considering the individual treatment it was found that both leaf and internode of Heera produced 100% callus in 5 mg L^{-1} NAA, whereas, both leaf (37.67%) and internode (20.83%) of Cardinal produced the minimum callus in 5 mg L^{-1} NAA. Neither leaf nor internode of any of the varieties produced callus without NAA (Table 3).

Table 3. *Combined effect of variety, explant and NAA on percent callus formed after one month.*

Explant	NAA (mg L^{-1})	Diamant	Heera	Cardinal
Leaf	0	-	-	-
	2.0	44.20	47.27	62.27
	3.0	85.23	78.33	95.00
	4.0	78.00	67.50	98.00
	5.0	93.67	100	37.67
Internode	0	-	-	-
	2.0	38.63	46.67	72.17
	3.0	84.17	65.10	91.17
	4.0	64.70	59.53	94.33
	5.0	98.20	100	20.83

3.3. Category of Calli According to Compactness

Among the different varieties, Diamant produced the highest compact calli (90.88%) followed by Heera (87.89%) and the lowest was in Cardinal (84.59%). Accordingly, the percentage of less compact calli was reverse where Diamant produced the lowest (9.13%) followed by Heera (12.11%) and the highest in Cardinal (15.41%). The result is in agreement with the findings of Tikk and Kollist [14], where the 11 potato varieties showed differences both the rate of callus formation and the stability of callus. Comparing the two explants, internode produced more compact calli (92.62%) than the leaf (78.73%). The production of less compact calli was reverse where leaf produced more (21.27%) than the internode (7.38%). The result was directly supported by [6] where friable calli were obtained from leaf explants. Among the different concentrations of NAA, it was observed that the maximum calli were compact (94.56%) in 4 mg L^{-1} NAA followed by 2 mg L^{-1} NAA (90.53%), while the lowest (68.67%) was in 5 mg L^{-1} NAA. Accordingly, the percentage of less compact calli was the lowest (5.44%) in 4 mg L^{-1} NAA and the highest (31.33%) in 5 mg L^{-1} NAA whereas, no calli either compact or less compact were produced without NAA. Compactness of calli may vary with NAA concentration which is supported by [16] where calli in medium supplemented with 3.0 mg L^{-1} NAA and 1 mg L^{-1} BA became hardy and green (Table 1).

Considering the combined effects, internode of Diamant, Heera and Cardinal produced 100% compact calli in 4, 3 and 5 mg L^{-1} NAA respectively. Production of less compact calli was the highest (78.33%) in leaf of Cardinal with 5 mg L^{-1} NAA. Similar result was observed by Kollist and Tikk [17], where callus formation and regeneration ability depended on variety and composition of medium (Table 4).

Table 4. *Combined effect of variety, explant and NAA on Category of calli according to compactness after one month.*

Explant	NAA (mg L^{-1})	% compact calli			% less compact calli		
		Diamant	Heera	Cardinal	Diamant	Heera	Cardinal
Leaf	0	-	-	-	-	-	-
	2.0	81.67	91.10	84.87	18.33	8.90	15.13
	3.0	83.43	92.77	78.77	16.57	7.23	21.23
	4.0	93.60	90.73	100	6.40	9.27	0
	5.0	81.67	44.43	21.67	18.33	55.57	78.33
Internode	0	-	-	-	-	-	-
	2.0	95.00	96.67	93.90	5.00	3.33	6.10
	3.0	98.00	100	80.67	2.00	0	19.33
	4.0	100	85.23	97.77	0	14.77	2.23
	5.0	91.67	72.57	100	8.33	27.43	0

3.4. Average Weight of Callus After One Month

After one month of explantation, Diamant, Heera and Cardinal produced 0.092, 0.034 and 0.035 g of callus, respectively. In case of explants, leaf showed 0.074 g of callus while internode produced only 0.033 g; however, the variation was statistically insignificant. The highest weight (0.111 g) of callus was found in 0 mg L^{-1} NAA and the lowest weight (0.030 g) was in 5.0 mg L^{-1} NAA but the differences were not significant (Table 1).

The interaction among varieties, explants and different concentrations of NAA was found statistically significant where leaf of Diamant without NAA produced the highest weight (0.667 g) of callus (Table 5).

Table 5. *Combined effect of variety, explant and NAA on average weight of callus (g).*

Explant	NAA (mg L^{-1})	Diamant	Heera	Cardinal
Leaf	0	0.667 a	0.000 b	0.000 b
	2.0	0.023 b	0.033 b	0.042 b
	3.0	0.024 b	0.060 b	0.042 b
	4.0	0.045 b	0.032 b	0.056 b
	5.0	0.038 b	0.031 b	0.014 b
Internode	0	0.000 b	0.000 b	0.000 b
	2.0	0.030 b	0.030 b	0.053 b
	3.0	0.025 b	0.076 b	0.050 b
	4.0	0.041 b	0.033 b	0.074 b
	5.0	0.029 b	0.046 b	0.021 b

Figures followed by same letter(s) are statistically similar as per DMRT

3.5. Average Weight of Callus After Two Months

After two months, average weight of callus was significantly variable among different varieties. The highest weight (0.100 g) was found in Heera and the lowest (0.078 g) was in Diamant. The weight of callus obtained from internode explant was comparatively higher (0.096 g) than the leaf (0.085 g) but the difference was not significant. NAA showed significant variation in callus formation where the highest weight (0.138 g) of calli was found in 4 mg L^{-1} NAA while the control did not produce any callus (Table 1).

The interaction effect of these three factors was significantly difference in case of average weight of callus. Internode explant of Cardinal showed the highest weight (0.205 g) of calli in 3 mg L^{-1} NAA while none of the explants of the varieties showed no callus without NAA after two months (Table 6).

Table 6. *Combined effect of variety, explant and NAA on average weight of callus after two month.*

Explant	NAA (mg L^{-1})	Diamant	Heera	Cardinal
Leaf	0	0.000 l	0.000 n	0.000 n
	2.0	0.059 jkl	0.072 ijk	0.112 fg
	3.0	0.072 ijk	0.198 ab	0.119 ij
	4.0	0.135 e	0.088 hi	0.161 d
	5.0	0.123 ef	0.095 gh	0.047 lm
Internode	0	0.000 n	0.000 n	0.000 n
	2.0	0.053 klm	0.064 jkl	0.155 d
	3.0	0.075 ij	0.205 a	0.132 e
	4.0	0.154 d	0.107 fg	0.184 bc
	5.0	0.113 fg	0.167 cd	0.037 m

Figures followed by same letter(s) are statistically similar as per DMRT

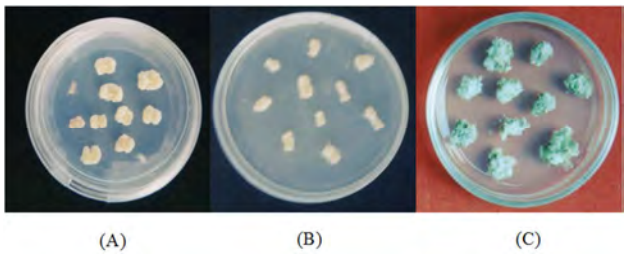

Figure 1. *Callus formed under different treatments at two months of explantation.*

A. Leaf of Heera with 5.0 mg L^{-1} NAA
B. Internode of Diamant with 2.0 mg L^{-1} NAA
C. Internode of Cardinal with 5.0 mg L^{-1} NAA

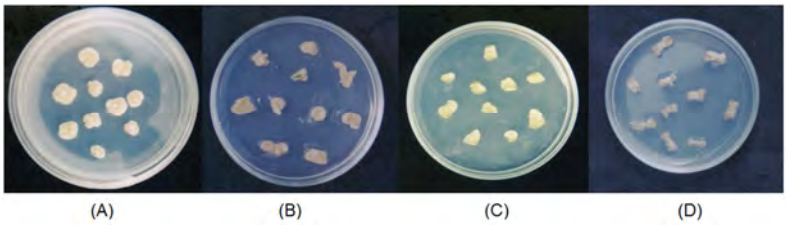

Figure 2. *Comparison of callus weight at one and two months of explantation.*

A. Internode of Heera with 3.0 mg L^{-1} NAA at one month of explantation
B. Leaf of Diamant with 2.0 mg L^{-1} NAA at one month of explantation
C. Internode of Heera with 3.0 mg L^{-1} NAA at two months of explantation
D. Leaf of Cardinal with 5.0 mg L^{-1} NAA at two months of explantation

4. Conclusion

Internode of Cardinal with 4 mg L^{-1} NAA required minimum days (6-8). For callus initiation, 100% explants of both leaf and internode of Heera in 5 mg L^{-1} NAA formed callus, whereas, internode of Diamant, Heera and Cardinal with 4, 3 and 5 mg L^{-1} NAA respectively produced 100% compact calli. Hence, the present protocol has the potential for the rapid multiplication of true-to-type clones without changing the genetic fidelity.

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