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In vitro Response of Potato (*Solanum tuberosum* L.) to Various Growth Regulators

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Abstract: The effect of three different growth regulators viz: Naphthalene Acetic Acid (NAA), Indole Acetic Acid (IAA) and Indole Butyric Acid (IBA) each at five concentration levels (0.0, 0.05, 0.15, 0.25 and 0.35) was evaluated on meristem culture of Potato (***Solanum tuberosum*** L.) for production of virus-free plantlets. The parameters included were plantlet height, number of nodes/plantlet, number of leaves/plantlet, root length and number of roots/plantlet. Maximum plantlet height (9 cm) with NAA at 0.15 mg l^{-1}, higher number of nodes/plantlet (9.714) with IBA at 0.35 mg l^{-1} and maximum number of leaves/plantlet (6.143) with IAA at 0.25 mg l^{-1} were recorded.

Key words: *In vitro*, potato, growth regulation, naphthalene acetic acid, indole acetic acid, indole butyric acid

Introduction

Potato (***Solanun tuberosum*** L.) an important food crop belongs to the family Solanaceae. Potato is also grown successfully in Pakistan. Currently, the area of potato in Pakistan is 109.5 thousand ha with annual production of 1810.5 thousand tones (Anonymous, 1998-99). However, the present yield is still very low, due to non availability of virus-free and certified seed potatoes varieties.

The successful production of potatoes for nutritional and seed purposes demands the control of these viruses, which cannot be sufficiently controlled by any physical or chemical agent. Tissue culture is an important technique of biotechnology and has a potential to improve the quality and quantity of vegetatively propagated potato plants. It is also the first major food crop where biotechnology has been successfully applied for virus elimination (Bajaj and Sopory, 1986). Hussain and Rashid (1991) obtained an average of about 60.5% of virus-free plants in Cardinal variety with the highest number 80% after heat treatment. Fiki ***et al.*** (1992) reported that elimination of potato X potexvirus increased total number, weight and diameter of microtubers. Shakya ***et al.*** (1993) observed that when the meristems were provided the media supplemented with 0.1 mg l^{-1} NAA and 0.1-1 mg l^{-1} BA developed healthy potato plantlets. A solid medium was superior to liquid medium. The plantlets developed from meristems could then be further propagated by single node cuttings. Saldana ***et al.*** (1996) obtained the highest therapy efficiency values at 15 mill amperes for 15 min. Under these conditions, 40-80% of the buds regenerated, and 60-100% of the regenerated plantlets tested virus negative. Yousaf ***et al.*** (1997) found that

NAA at 2 mg l^{-1} + BAP at 0.5 mg l^{-1} gave the longest main shoot (22 cm), highest node number (23) and leave numbers (25). In combination 2 mg l^{-1} BAP and 0.1 mg l^{-1} NAA gave the largest number of axillary shoots per main shoot. Sucrose at 40 g L^{-1} in combination with 0.1 mg l^{-1} BAP was optimal for obtaining the maximum number of microtubers. Largest microtuber weight and size was given in media containing 80 l^{-1} sucrose and 0.1 mg l^{-1} BAP. Merja ***et al.*** (1997) concluded that explants of 0.2 and 0.4 mm were less susceptible to virus infection than larger plants. Truskinov and Rogozina (1997) concluded that the efficiency of virus elimination depends on both antiviral and regeneration factors of tissue culture and can be improved by thermotherapy and chemotherapy. Cecilio - Fitho ***et al.*** (1998) derived potato plants from meristem culture gave total and commercial yields respectively 52.5% and 89% higher than plants obtained from conventional methods of propagation. Brazalandia Rosada had significantly higher commercial yield while Pecanha Rosea had a significantly lower total yield than the other cultivars. Zaman ***et al.*** (2001) obtained maximum plant height (8.3 cm), largest number of nodes per plantlet (7.3) and highest number of leaves per plantlet (8.9) at 0.5 mg l^{-1} of NAA. Shah ***et al.*** (2002) reported that maximum number of leaves per plantlet (6.143), root length (4.429) and number of roots per plantlet (17.43) were obtained at 0.25 mg l^{-1} of IAA. Thus keeping the importance of tissue culture techniques in producing virus-free potato plants, the present study was under taken to standardize the culture media for regeneration of meristem and to obtain virus-free plants for commercial multiplication.

Materials and Methods

The research work was conducted at Potato Tissue Culture Laboratory, Agricultural Complex, Northern Areas Gilgit. A completely randomized design (CRD) was used with three growth regulators with their five levels of concentrations repeated seven times. Buds of cultivar Desiree were cultured in test tubes to obtained ***in vitro*** bud plants. After disinfection in 0.1% NaOCl with 1-3 drops of Tween-20 for 10 min and washing with distilled water thrice, the tip and sub-tending leaf primordial were removed with in aseptic condition and placed in test tubes containing Murrashige and Skoog (MS) medium (1962). The size of the meristem was about 0.1-0.3 mm at the time of culture. Test tubes were incubated at 23-24°C temperature in 16 h light of 1000 Lux intensity. The meristems normally took 3-4 months to develop into a plantlet ready for sub culture. Meristematic plantlets were multiplied through stem cuttings containing single nodes. Stem cuttings cloned separately on basal MS medium in culture tubes, containing auxins viz, NAA, IAA and IBA at various levels (0.0, 0.5, 0.15, 0.25, 0.35). The cultures were incubated at 22-25°C at a light intensity of 2500 Lux. After 2-3 weeks plantlets were studied for various parameters and transferred to green house. During the research work data were recorded on number of sterile tubes, plant height (cm), number of nodes/plantlet, number of leaves /plantlet, number of roots/plantlet, root length (cm). The data recorded for each trait was subjected to the analysis of variance technique and the significant means were reported by Least Significant Differences Test (Steel and Torrie, 1980).

Results

Number of sterile tubes

The number of sterile tubes recorded is given in Table 1. Best performance was observed for IBA in which 98 contamination free tubes were observed out of 105 tubes showing 93% non-contaminated tubes. In NAA 84 tubes were free of contamination out of 105 indicating 80% infestation free. IAA was the least responsive auxin showing only 72 infestation free tubes out of 105 tubes, only 38% were recorded free of infestation. The entire experiment consisted of 315 tubes out of which 254 regenerated successfully representing 80% non-contaminated culture.

Plantlet height (cm)

The data regarding plantlet height (cm) showed that both the growth regulators and concentration levels and their interaction were differed significantly at 5% level of probability. The tallest plantlets (9.000 cm) were produced by NAA at concentration level of 0.15 mg l^{-1} followed by IAA (8.429 cm) at 0.35 mg l^{-1} and NAA with 8.00 cm at 0.05 mg l^{-1}. The shorter plant height (4.286 cm) was observed in IAA at concentration level of 0.0 mg l^{-1} (Table 2).

Regarding three growth regulators, the higher plant height (7.343 cm) was produced by NAA followed by IAA with 7.086 cm. Whereas, higher the concentration of the growth regulator, maximum would be the plant height, therefore, 0.35 mg l^{-1} statistically proved the better by giving higher plant height (7.952 cm) while shorter plant height (4.381 cm) was observed in concentration level of 0.0 mg l^{-1}. Zaman ***et al.*** (2001) also reported that NAA has produced the tallest potato plants.

Number of nodes per plantlet

The results indicated that both the growth regulators and concentration levels and their interaction were statistically significant at 5% level of probability. Maximum number of nodes per plant (9.714) was produced by IBA at concentration level of 0.35 mg l^{-1} followed by IAA (8.571) at 0.35 mg l^{-1}. The shorter number of nodes per plant (5.000) was observed in NAA at concentration level of 0.0 mg l^{-1} (Table 2).

As far as growth regulators are concerned, the maximum nodes per plant (8.229) and (8.057) was observed in growth regulators IBA and IAA, respectively. Both are statistically at par to each other. The minimum number of nodes per plant (6.114) was produced by NAA. Our results are in agreement with Kong-Xiangsheng (1998) and Yousaf ***et al.*** (1997) they reported that MS medium supplemented with IBA would be better for maximum number of nodes/plantlet and rapid propagation of virus free seedlings of potato. Concentration level of 0.35 mg l^{-1} gave better number of nodes per plant (8.429) while the shorter number of nodes per plant (6.429) was observed in concentration level of 0.0 mg l^{-1}. Zaman ***et al.*** (2001) also reported that higher the concentration of auxins, higher would be the number of nodes per plant produced.

Number of leaves per plantlet

The highest number of leaves per plant (6.143) was produced by IAA at concentration level of 0.25 mg l^{-1} followed by IBA (6.000) at 0.35 mg l^{-1} level. The minimum number of leaves per plant

Table 1: Percentage of Sterile tubes observed at various growth regulators on potato cultivar desiree

Auxins	Total tubes cultured	Infestation free tubes	%age
NAA	105	84	80
IAA	105	72	68
IBA	105	98	93
	315	254	80

Table 2: Mean plantlet height (cm), Number of nodes per plantlet and number of leaves per plant observed at various levels of growth regulators on potato cultivar desiree

Levels (mg l^{-1})	NAA	IAA	IBA	Mean
Plantlet height (cm)				
0.0	4.429d	4.286d	4.429d	4.381c
0.05	8.000abc	7.286c	7.429bc	7.571b
0.15	9.000a	7.429bc	7.143c	7.857ab
0.25	7.429bc	8.000abc	7.286c	7.571b
0.35	7.857bc	8.429ab	7.571bc	7.952a
Mean	7.343a	7.086ab	6.771B	
Number of nodes per plantlet				
0.0	5.000e	7.286bc	7.000bcd	6.429c
0.05	5.571de	7.571bc	8.429ab	7.190bc
0.15	6.571cde	8.429ab	8.429ab	7.810ab
0.25	6.429cde	8.429ab	7.571bc	7.476ab
0.35	7.000bcd	8.571ab	9.714a	8.429a
Mean	6.114b	8.057a	8.229a	
Number of leaves per plant				
0.0	2.429g	2.857fg	2.714fg	2.667c
0.05	3.286efg	4.286cde	3.714def	3.762b
0.15	4.143cde	4.143cde	2.714fg	3.667b
0.25	5.714ab	6.143a	5.000abc	5.619a
0.35	4.857bcd	5.714ab	6.000ab	5.524a
Mean	4.086b	4.629a	4.029b	

Mean values followed by the same letter(s) in respective category are not significantly different from each other at 0.05 probability level.

(2.429) was observed in NAA at concentration level of 0.00 mg l^{-1} (Table 2).

Among three growth regulators data showed the non-significant difference as IAA proved to be the best auxin amongst the all, as it produced the highest number of leaves per plant (4.629) as compared to other growth regulators.

As far as the concentration levels are concerned, highest number of leaves (5.524 and 5.619) were developed at 0.25 mg l^{-1} and 0.35 mg l^{-1}, respectively and both were statistically at par to each other. While the minimum of leaves per plant (2.667) were observed in concentration level of 0.0 mg l^{-1}. Zaman ***et al.*** (2001) who concluded that higher the concentration of auxin level will be helpful for the production of highest number of leaves per plant.

Root length (cm)

Highest root length (4.429 cm) was produced by IAA at concentration level of 0.25 mg l^{-1} followed by NAA and IAA with similar value (4.286 cm) at the same concentration level of 0.35

Table 3: Mean root length and mean number of roots/plantlet at various levels of growth regulators on potato cultivar desire

Levels (mg l^{-1})	NAA	IAA	IBA	Mean
Mean root length (cm)				
0.0	2.857e	3.571abcde	3.143de	3.190c
0.05	3.143de	3.857abcd	3.429bcde	3.476bc
0.15	3.429bcde	3.429bcde	3.286cde	3.381c
0.25	4.143abc	4.429a	3.429bcde	4.000ab
0.35	4.286ab	4.286ab	3.857abcd	4.143a
Mean	3.571AB	3.914A	3.429B	
Mean number of roots per plantlet				
0.0	10.14f	11.14def	10.57ef	10.62c
0.05	12.00def	13.14cde	15.14abc	13.43ab
0.15	13.00cde	16.00ab	13.14cde	14.05ab
0.25	11.43def	17.43a	10.86ef	13.24b
0.35	16.00ab	13.86bcd	12.86cdef	14.24a
Mean	12.51b	14.31a	12.51b	

Mean values followed by the same letter(s) in respective category are not significantly different from each other at 0.05 probability level.

Table 4: Incidence of potato viruses in plantlets of cultivar

Code NO.	PVX	PVY	PLRV
GH-01	-**	-	-
GH-02	-	-	-
GH-03	-	-	-
GH-04	-	-	-
GH-05	-	-	-
GH-06	+	-	-
GH-07	-	-	-

- (Minus): Virus free + (plus): virus infected

mg l^{-1}. The shorter root length (2.857 cm) was observed in NAA at concentration level of 0.0 mg l^{-1} (Table 3).

Means of three growth regulators showed that the higher root length (3.914 cm) was recorded in growth regulator of IAA followed by NAA with 3.571 cm, while the lowest root length (3.429 cm) was recorded in IBA. Zaman ***et al.*** (2001) also reported that IAA has produced the maximum (3.5 cm) root in Potato.

In case of concentration levels, 0.35 mg l^{-1} statistically produced the higher root length (4.143 cm), followed by 0.25 mg l^{-1} producing the root length of 4.000 cm, while the shorter root length (3.190 cm) was observed in concentration level of 0.0 mg l^{-1}. Zaman et al. (2001) also reported that higher the concentration of growth regulator, more lengthy will be the roots produced.

Number of roots per plantlet

Data regarding to interaction between growth regulators and level of concentration showed statistically significant results at 5% level of probability. The higher number of roots per plant

(17.43) was recorded in IAA at 0.25 mg l^{-1} level followed by NAA and IAA with similar number of roots per plant (16.000) while minimum number of roots/plant (10.14) was observed in NAA at concentration level of 0.0 mg l^{-1}.

The number of roots per plantlet was significantly different for growth regulators and their concentration levels. The growth regulator IAA was the higher in production of maximum number of roots/plantlet (14.314) followed by NAA and IBA with similar value (12.514).

In case of concentration levels, 0.35 mg l^{-1} statistically produced higher number of roots per plant (14.24) followed by concentration level of 0.15 mg l^{-1} with 14.05. Shah ***et al.*** (2002) also reported similar results, reporting that IAA and higher concentration of growth regulators are best for the production of maximum roots per plant.

Virus detection

Sensitive ELISA technique was used for detection of potato viruses viz: PX, PVY and PLRV in the regenerated plantlets. Seven (7) Meristematic plantlets were subjected to ELISA. It is clearly evident from results (Table 4) that among 7 tested plantlets only one plantlet (GH-06) was positive to PVX. No infection of PVY and PLRV was detected. This test also reveals that meristem culture is a successful technique to eliminate viruses. The results of this study are in conformity with the findings of Truskinov and Rogozina (1997) and Rancovic ***et al.*** (1997) who developed virus-free plantlets from meristem of potato and used ELISA test to detect potato viruses.

It can be concluded from the present research that MS medium with NAA at 0.15 mg l^{-1} is highly responsive for vegetative growth and recommended for maximum plant height. The better hormone recommended for number of nodes IBA 0.35 mg l^{-1}. The better hormone recommended for number of leaves per plantlet, root length (cm) and number of roots per plantlet is IAA at 0.25 mg l^{-1}.

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