

Meristem Culture of Potato (*Solanum tuberosum* L.) for Production of Virus-Free Plantlets

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Abstract: The effect of three different auxins viz, naphthalene acetic acid (NAA), indole acetic acid (IAA) and indole butyric acid (IBA) each at four levels (0, 0.1, 0.5 and 1.0 mg l⁻¹) was evaluated on meristem culture of Potato (*Solanum tuberosum* L.) for production of virus-free plantlets. Maximum plantlet height (8.3 cm), largest number of nodes/plantlet (7.3) and highest number of leaves/plantlet (8.9) were recorded at 0.5 mg l⁻¹ of NAA followed by IBA at 1 mg l⁻¹, whereas extensive number of roots/plantlet (23.7) as well as the earliest microtuber formation (17 days, after transplantation) were recorded at 1 mg l⁻¹ of IBA followed by NAA at 0.1 mg l⁻¹. Largest root (4.2 cm) was observed for IAA at 1 mg l⁻¹ concentration. Enzyme Linked Immunosorbent Assay (ELISA) test proved that all plants were free of Potato Virus X (PVX), Potato Virus Y (PVY) and Potato Leaf Roll Virus (PLRV), except one which was positive for Potato Virus X (PVX) virus.

Key words: *Solanum tuberosum* L., meristem culture, ELISA

Introduction

Potato (*Solanum tuberosum* L.) is widely grown over the globe. Currently the area and production of potato in Pakistan was 109.5 thousand hectares and 1810.5 thousand, respectively Annon (1998-99). Potato plays an important role in the food chain as it ranks 4th importance after rice, wheat and maize. It is a good and cheap source of carbohydrates, vitamins, minerals and proteins and provides most of the trace elements which can meet the energy requirements of the people living in the developing countries like Pakistan (Rahman, 1986). Potato viruses cause severe losses in production of potatoes. The most prevalent viruses are Potato Leaf Roll Virus (PLRV), Potato Virus X (PVX) and Potato Virus Y (PVY) (Khalid *et al.*, 2000).

These viruses are tuber borne, which reduces plant vigor, and yield potential of potato, transmitted by aphids *Myzus persicae* that colonize potato crop. Aphid's transmission occurs from tuber to tuber during seed storage. These infected tubers also serve as virus source.

Meristem culture along with thermotherapy have become a powerful and successful tool for virus elimination from infected plants (Paet and Zamora, 1990). Meristem culture has been successfully applied in potato for development of virus-free plants. The *in vitro* technology combined with traditional practices have enhanced the commercial production of virus-free seeds an important pre-requisite to maximize yield in potato (Faccioli and Colombarini, 1996). It is also the first major food crop where biotechnology has been successfully applied for virus elimination (Bajaj and Sopory, 1986). Yousaf *et al.* (1997) developed virus-free potatoes by meristem culture on MS medium with 2 mg l⁻¹ NAA. 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.

This study was conducted to standardize culture media for regeneration of the meristem, develop virus-free plants for commercial multiplication and detect the effect of viruses viz, PVX, PVY & PLRV, by Enzyme Linked Immunosorbent assay (ELISA) technique.

Materials and Methods

The research work was envisaged at Potato Tissue Culture Laboratory, Agricultural Complex, Northern Areas Gilgit. The meristem of potato cv. Desiree was used as explant. After disinfection in 10% clorox for 10 min. and washing in distilled water, the tip and sub-tending leaf primordia were removed with in aseptic condition and placed in test tubes containing

Murashige and Skoog (MS) medium (1962). Test tubes were then incubated at 23-24°C in continuous light of 100-Lux intensity. Plantlets regenerated were multiplied through stem cuttings on MS basal medium in culture tubes, containing auxins viz, NAA, IAA and IBA at four levels (0, 0.1, 0.5 and 1 mg l⁻¹) and incubated at 22-25°C and a light intensity of 2500 Lux. After 2-3 weeks plantlets were studied for various parameters and transferred to green house. The leaf samples of 10 plants of the cultivars Desiree were tested for viruses, PLRV, PVX and PVY by ELISA technique.

Results and Discussion

Highly significant differences were observed in the mean of plant height for different hormones and their levels. The tallest plantlets (8.3 cm) were observed on 0.5 mg l⁻¹ NAA followed by 0.1 mg l⁻¹ of the same hormone (7.9 cm) (Table 1). Means of auxins showed that NAA had the tallest plantlets (7.0 cm) for all levels followed by IBA (6.6 cm), while IAA was the least in plantlet height. Shakya *et al.* (1993), Bedavi *et al.* (1995) and Yousaf *et al.* (1997) reported similar results in potato, in which vigorous axillary shoots were produced at 0.1 mg l⁻¹ NAA and IBA.

The maximum number of nodes/plantlet (7.3) were recorded on 0.5 mg l⁻¹ NAA and 1 mg l⁻¹ IBA followed by 1 mg l⁻¹ IAA (7.1), while minimum number of nodes/plantlet (4.5) were recorded at 0 mg l⁻¹ IAA (Table 2). NAA was superior to other auxins in mean number of nodes/plantlet (6.4) followed by IBA (6.3). Kong-Xiangsheng (1998) and Yousaf *et al.* (1997) also reported that MS medium supplemented with NAA would be better for maximum number of nodes/plantlet and rapid propagation of virus-free seedlings of potato.

Highest number of leaves/plantlet (8.9) were observed at 0.5 mg l⁻¹ NAA followed by 1 mg l⁻¹ NAA and IBA (8.3 each), while the lowest number of leaves/plantlet (4.6) were recorded at 0 mg l⁻¹ IAA (Table 3). Merja *et al.* (1997) found same results and regenerated potato meristem on MS medium with NAA, GA₃, IAA or IBA. Yousaf *et al.* (1997) produced highest number of leaves per plantlet in potato at 2 mg l⁻¹ NAA.

Largest roots (4.2 cm) were recorded on IAA at 1 mg l⁻¹ followed by 1 mg l⁻¹ NAA (3.8 cm), whereas the shortest roots (1.7 cm) were observed at 0 mg l⁻¹ NAA (Table 4). Similar results were found by Khattak *et al.* (1994) who reported that IAA increased root length in guava.

The maximum number of roots (23.7) were recorded at 1 mg l⁻¹ IBA, followed by 20.1 in 1 mg l⁻¹ NAA (Table 5). The auxin IBA was the most responsive (16.4) followed by NAA (13.6), while IAA was the least responsive hormone (12.5) in

Table 1: Mean plantlet height (cm) observed at various levels of auxins on stem cutting of potato cultivar Desiree

Levels (mg ⁻¹)	NAA	IAA	IBA	Mean
0	5.0g	5.1g	5.3g	5.1c
0.1	7.9ab	6.2f	6.5ef	6.8b
0.5	8.3a	6.8de	7.2cd	7.4a
1.0	7.3d	7.2cd	7.7bc	7.3a
Mean	7.0a	6.3c	6.6b	

Table 2: Mean number of nodes/plantlet observed at various levels of auxins on stem cuttings of potato cultivar Desiree

Levels (mg ⁻¹)	NAA	IAA	IBA	Mean
0	5.5fg	4.5h	5.2g	5.1c
0.1	6.6cd	5.6efg	6.7bc	6.2b
0.5	7.3a	6.0def	6.1de	6.4a
1.0	6.4cde	7.1ab	7.3ab	6.9a
Mean	6.4a	6.2a	6.3a	

Table 3: Mean number of leaves/plantlet observed at various levels of auxins on stem cuttings of potato cultivar Desiree

Levels (mg ⁻¹)	NAA	IAA	IBA	Mean
0	6.6e	4.6g	5.0g	5.4d
0.1	7.7c	5.7f	7.9bc	7.1c
0.5	8.9a	7.0e	7.1de	7.6c
1.0	8.3b	7.6cd	7.3b	8.1a
Mean	7.9a	6.2c	7.0b	

Table 4: Mean root length (cm) observed at various levels of auxins on stem cutting of potato cultivar Desiree

Levels (mg ⁻¹)	NAA	IAA	IBA	Mean
0	1.7f	2.4de	2.3e	2.1c
0.1	2.3de	3.3b	2.7cd	2.8b
0.5	3.2b	3.3b	1.8f	2.8b
1.0	3.8a	4.2a	3.0bc	3.7a
Mean	2.7b	3.5a	2.4c	

Table 5: Mean number of roots/plantlet observed at various levels of auxins on stem cuttings of potato cultivar Desiree

Levels (mg ⁻¹)	NAA	IAA	IBA	Mean
0	10.0fg	9.2g	10.0fg	9.7c
0.1	12.0ef	12.0ef	16.3c	13.4b
0.5	12.6e	15.0cd	15.8c	14.5b
1.0	20.1b	13.6de	23.7a	19.1a
Mean	13.6b	12.5c	16.4a	

Table 6: Mean number of days to microtuber formation observed at various levels of auxins on stem cuttings of potato cultivar Desiree

Levels (mg ⁻¹)	NAA	IAA	IBA	Mean
0	34.0a	30.0bcd	33.0a	32.4a
0.1	21.3g	31.0abc	27.0de	26.4b
0.5	26.0e	28.0cde	21.3g	25.1b
1.0	22.0fg	25.3ef	17.0h	21.4c
Mean	25.8b	28.6a	24.5b	

** Mean values followed by same letter (s) in respective category are not significantly different from each other at 5% level of probability

the mean number of roots per plantlet. Kayim and Koc (1992), also obtained best rooting in potato cultivars Brintje and Escort at 1 mg l⁻¹ IBA.

The earliest microtuber formation (17 days after transplantation to the green house) was recorded for auxin IBA at 1 mg l⁻¹ followed by NAA (21.3 days) at 0.1 mg l⁻¹, and also by 0.5 mg l⁻¹ of IBA whereas 0 mg l⁻¹ NAA took maximum number of days (34 days, Table 6). IBA was the earliest of all auxins (24.5 days) followed by NAA (25.8 days),

Table 7: Incidence of potato viruses in plantlets of cultivar Desiree

S. No.	Code No.	PVX	PVY	PLRV
1	GH-01	-	-	-
2	GH-02	-	-	-
3	GH-03	-	-	-
4	GH-04	-	-	-
5	GH-05	-	-	-
6	GH-06	-	-	-
7	GH-07	-	-	-
8	GH-08	-	-	-
9	GH-09	+	-	-
10	GH-10	-	-	-

- minus : virus-free + (plus): virus infected

while IAA was the most late auxin (28.6 days) in microtuber formation.

Wood and Coke (1990) investigated IBA at 1 mg l⁻¹ for earlier tuberization in potato.

All 10 plants tested for virus detection by ELISA technique were free of all three viruses, except one plant, which was positive for PVX virus (Table 7). The results of this study are in conformity with the findings of Truskinov and Rogozina (1997) developed virus-free plantlets from meristem of potato and used ELISA test to detect potato viruses.

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