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THERMOTHERAPY AND MERISTEM TIP CULTURE OF *SOLANUM TUBEROSUM* FOR ELIMINATION OF POTATO VIRUSES X, S AND Y

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Abstract

Meristem tips of 0.4 mm excised from *Solanum tuberosum* cultivars cardinal and desiree having undergone thermotherapy at 34°C for 11-60 days regenerated into rooted plantlets. The effect of duration of thermotherapy on regeneration potential of meristems was insignificant ($P > 0.95$). Thermotherapy for 60 days in cv. cardinal resulted in 8, 35 and 46% elimination of potato viruses S, X and Y respectively, and 9, 48 and 59% from cv. desiree.

Introduction

Potato cultivars grown in Pakistan are seriously affected by tuber borne viruses but no physical or chemical agent has yet been found to effectively eradicate these viruses. Yield losses upto 80%, have been attributed to viruses (Beemster & Rozendal, 1972). In Pakistan, 20-70.5% reduction in yeild of potato tubers due to virus diseases, have been reported (Hussain, *et al,* 1978). The seed tubers were found to degenerate by 37-56% due to build up of viruses during miltiplication in field (Jagirdar, *et al,* 1982).

Meristem tip culture has been introduced to recover virus free plants from the infected material since healthy plantlets can be raised from a small, virus free zone at the extreme tips of the shoot apices (Crowley & Hanson, 1960; Pennazio & Redolfi, 1974; Takeuchi *et al,* 1972). Complete eradication of viruses by meristem tip culture alone has so far been considered difficult or unsuccessful (Sajid *et al.*, 1985; Pennazio & Redolfi, 1974, Quazi & Martin, 1978).

The virus may be partially or completely inactivated, when virus infected plants or plant parts are kept at higher than normal temperature. Alfalfa mosaic virus (AMV), potato leaf roll virus (PLRV) and tomato black ring virus (TBRV) have been eradicated by hot air treatment at 37°C for 3-6 weeks (Kaiser, 1980) although virus detection method used was not very sensitive. This paper reports the behavior of potato viruses X, S and Y, when meristem tips excised from potato plants subjected to thermotherapy for 11-60 days were cultured to regenerate into plantlets *in vitro*.

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Materials and Methods

(i) *Plant growth:* Potato tubers cvs. cardinal and desiree, were treated with 50 mg/l gibberellic acid (GA_3) for one hour to break dormancy and incubated at 20-22°C for 20 days. Single eye-pieces for the tubers were sown in pots containing 1:2:1 mixture of sand, farm yard manure (FYM) and soil, at 22 ± 2°C under 2500 lux with 16h photo-period. The plants were periodically irrigated with 25% Hoagland nutrient solution, through a pore in the bottom of pots. Fifty potato plants of each variety, 18-25 cm high, were subjected to high temperature treatment for 11-60 days before meristems of 0.4 mm size were excised from them. The temperature was gradually increased to this point during the first week.

(ii) *Preparation of culture material:* The axillary buds were aseptically removed from the plants before commencement of thermotherapy and on the 11, 26, 34, 45 and 60th day of thermotherapic treatment. The buds washed in 5% domestic detergent "Zip" and sterile distilled water were disinfected by a quick dip in 70% ethanol (Wang *et al.*, 1980) and finally washed in sterile distilled water three times for 10 minutes each. Meristem tips, 0.4 mm, were then excised from the axillary buds using hypodermic needles under a stereoscopic zoom microscope (40X) in laminar flow cabinet with an air velocity of 30 meters per min. The meristem tips were cultured on filter paper bridges suspended in liquid Murashige – Skoog's (MS – 1962) medium containing 0.5 mg/l indol-3-acetic acid (IAA), 0.1 mg/l gibberellic acid (GA_3) and 0.04 mg/l kinetin, at pH 5.7, and autoclaved at 12 p.s.i for 10 min.

(iii) *Virus testing:* Latex test based on mixing plant sap with latex (antibody coated polystyrene balls, 810 nm in diam.) was used. Lower leaves of experimental plantlets were aseptically removed. The leaves of healthy potato plantlets obtained from Neoplants, Ltd; England and also from a virus infected plant, were taken for negative and positive controls, respectively. Each leaf was placed in a plastic bag with 0.3 ml tris HCl buffer (0.05 M, pH 8, containing 1% 2-marcaptoethanol and 0.05% Tween-20). They were rolled over by test tubes to squeeze the juice. The juice of each leaf was then diluted 1:10 and 1:100 with tris HCl buffer. Equal volumes (25 μl) of the sensitized latex for each of potato viruses X, S and Y, and of diluted leaf extract of each plantlet were mixed on Petri plates which were then shaken at 130 rpm at 25°C for one h. The aggregation of latex particles indicated the presence of virus tested.

Results and Discussion

Thirty eight to 44% meristem tips of potato cv. cardinal and 39 to 46% meristems of cv. desiree depending upon the duration of thermotherapy were regenerated into rooted plantlets. Thermotherapic duration, however, did not significantly affect the regeneration potential of meristem tips ($P > 95$). The number of virus free plantlets

Table 1. Effect of thermotherapy on meristem regeneration and virus elimination from Cardinal variety of potato cvs. cardinal and desiree.

Duration of thermotherapy and meristem excision in day	Number of meristems cultured	Mortality (%)	Regeneration (%)	Percent plantlets free from potato virus S	X	Y
cv. cardinal						
0	135	57	43	0	1.7 (1)	3.5 (2)
11	98	56.1	43.9	0	4.7 (2)	7.0 (3)
26	118	57.6	42.4	0	10.0 (5)	24.0 (12)
34	113	57.5	42.5	0	14.6 (7)	35.4 (17)
45	109	59.6	40.4	2.3 (1)	27.3 (12)	43.2 (19)
60	97	61.6	38.1	8.1 (3)	35.1 (13)	46.0 (17)
cv. desiree						
0	105	54.3	45.7	0	2.1 (1)	4.2 (2)
11	82	54.9	45.1	0	5.4 (2)	13.5 (5)
26	91	56.0	44.0	0	17.5 (7)	42.5 (17)
34	98	57.1	42.9	2.4 (1)	21.4 (9)	45.2 (19)
45	116	58.6	41.4	6.3 (3)	39.6 (19)	56.3 (27)
60	112	60.7	39.3	9.1 (4)	47.7 (21)	59.1 (26)

Actual numbers of the virus free plantlets are given in the parenthesis.

among the regenerants was dependent on the duration of thermotherapy given to the plants prior to meristem excision. Potato virus S was only slightly affected by thermotherapy. There was no effect on PVS for 34 days of treatment to cardinal and 26 days treatment to desiree variety (Table 1). After 60 days treatment, only 8% regenerants of cardinal and 9% regenerants of desiree variety were PVS free. Thirty five percent regenerants of cardianl and 48% regenerants of desiree variety were free of PVX after full duration of heat treatment. PVX was more easily eliminated from desiree as compared to cardinal variety and so was PVY. Fifty nine percent regenerants of desiree and 46% regenerants of cardinal variety were free of PVY after 60 days of thermothearapy.

The meristems excised from desiree plants subjected to thermotherapy for a given duration, regenerated into PVX and PVY free plantlets more frequently, than those excised from cardinal plants. Our results are similar to Stace – Smith & Mellor (1968) who obtained 50% PVX free plantlets from meristems of potato plants of white rose variety subjected to thermotherapy for 8 weeks.

Two months of thermotherapy resulted in 38 and 39% regeneration of meristems of cardinal and desiree, respectively, as compared to 43 and 47% regeneration of meristems without prior thermotherapy. This is an insignificant effect ($P > 0.95$) of thermotherapy on regeneration potential of the meristems. Using of higher temperature much lower percentage of regeneration has been reported by Zakulukiewiez (1971).

PVX eradication reportedly leads to many adverse effects like increased susceptibility to *Phytophthora infestans* (Muller & Munro, 1951). This may be attributed to change in nutritional and physiological status of the virus free plants. The possible increase in susceptibility to fungal infection or appearance of suppressed viruses in PVX – free plants, is unlikely to detract greatly from the importance of virus free potato production program because the benefits more than compensates for any adverse effects.

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