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RESPONSE OF POTATO TO IN VITRO PROPAGATION

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Potato shoots taken from sprouted tubers were cut into piecies and propagated *in vitro* on modified MS medium. The multiplication rate of propagation was X 9 per month. Morphology of the plantlets incubated in longer day length (16, 24 h) was desirable for *in vitro* propagation. Cultures incubated over three months in shorter day length (8 h) showed loss of apical dominance and produced erect scaly leaves but supported the growth to continue by branching. However cultures incubated at longer day length indicated reduced growth and produced mini aerial tubers on stolons developed in leaf axis.

INTRODUCTION

Potato being susceptible to a number of viral, bacterial and fungal pathogens suffers heavy losses from these organisms. To reap the benefits from the potential of a variety continuous production ofdisease free seed potato is, therefore, essential. Starting from a single tuber nearly 800-900 plants are produced over Ist three years by conventional vegetative propagation methods using virus tested stem cuttings (Hussey and Stacey, 1981). Tissue Culture being a technique of great potential provides quick means of vegetative propagation and when applied to potato it produces not only many thosand plants in single year but also is oriented with the mechanism of built-in disease protection. The health status of *in vitro* propagated material will not deteriorate as long as it remains in culture vessels. Under Punjab conditions the period for seed production is very short and virus vectors are very active (Mirza, *et al.*, 1982) during crop season. With *in vitro* propagation, the cost of retesting to ensure disease freedom during conventional propagation period is also saved. Rapid propagation also offers the bulking up of a newely selected genotype under disease free conditions for commercial use.

A number of asexual reproductive phenomena could be exploited by *in vitro* technique for rapid propagation of heterozygous crops (Hussy, 1978).

These fall under two main categories i. e. (i) The enhanced production of axillary meristems and (ii) The regeneration of adventitious meristoms in the form of shoot apices or embroyo either directly on plant tissue or via callus, cell suspention or protoplast culture.

Potato, under appropriate conditions, shows considerable activity of producing adventitious shoots both directly from organ tissue as well as from callus (Wang and Huang, 1975; Roest and Bokelmann, 1976). Coventional methods of propagating potato via stem cutting and tubers depend on the production of axillary shoots which can also be multiplied *in vitro*. To ensure maximum genetic uniformity of the material produced by Tissue Culture, formation of adventitious shoots is avoided as far as possible for the various reasons (Broertjes and Van Harten, 1978; Shepard, Bedney and Shahin, 1980).

Many workers, therefore, focussed their efforts to develop a system of *in vitro* propagation based entirely on proliferation by axillary shoots avoiding, as far as possible, the conditions and procedures that favour the formation of adventitious meristematic structures. The response of potato to *in vitro* propagation and its usefulness in seed potato production created interest to take up the studies. This, effort, therefore, describes the general vegetative behaviour of potato shoots under *in vitro* culture conditions.

MATERIALS AND METHODS

Tuber samples from a certified crop of a popular cultivar 'Home Guard' were obtained from a commercial source to carry out the experiment. The tubers washed clean with water were incubated at 25°C in dark to obtain the sprouts. The sprout sexcised from the tubers were washed in a flask containing 1% sodium hypochlorite solution, carrying 0.05% Tween-20 as wetting agent, to eliminate surface contamination. Washing was done by shaking the flask on a mechanical flask shaker for 10 minutes; followed by two simular washings with sterile water to remove traces of bleach. Later on sprouts were further rinsed 3-4 times with sterile water. The explants consisted of single nodes including 5 mm of stem excised from sproutes. The explants were placed into culture vessel (test tubes) on sterilized solidified media under aseptic conditions in an air-flow-cabinet.

Nutrient Media

Murashige and Skoog (1962) salt mixture with an addition of 0.25mgl^{-1}

$NiCl_2$ was used. Sucrose was added at the rate of 3% and medium was gelled with 0.4% purified washed agar. The nutrient medium was adapted by myo-inocitol (100 mg l^{-1}), Pyridoxin Hcl, Biotin, Choline Chloride, L-ascorbic acid, adenine, Nicotinic acid, Ca. Pentothenate (5mg l^{-1}), Kinetin (10^{-6}M) and GA_3 (2.5 x 10^{-6}M). Activated charcoal was also added at the rate of 0.25%. The pH was adjusted with 0.1 M NaoH and 0.1 M Hcl at 5.7 prior to sterilization at 15 lbs PSI (121°C) for 20 minutes in autoclave.

Culture conditions

Cultures were grown in rimless 100 x 25 mm glass test tubes containing 10 ml of agar medium covered with polyproplene sheet gripped tightly with a rubber band. These cultures were incubated at 25°C ± 2°C in an environment controlled installation with a day length of 12 h and light intensity of 6000 lx. Source of light was white fluorescent tubes. After 4 weeks plantlets were cut into saprate nodes and cultured in fresh medium. Cultures were divided into three sets each containing twenty five sub-cultures. Each set was subjected to 8, 16, 24, h, day lengths with light intensity of 6000 lx in growth cabinets run at 25°C. Data was recorded on 4 week old shoots for plant characters like stem thickness, Number of nodes and inter nodal length.

RESULTS AND DISCUSSION

In vitro shoot production and subculturing

Sprout cuttings placed on solidified agar nutrient medium produced 40-60mm high upright shoots from axillary buds after 4 weeks; each shoot containing 8-12 nodes. Similar uprignt shoots developed from each subcultured mode again after 4 weeks. After 8th subculturing the plantlets were as vigorous and healthy as were in the begining of the experiment, indicating that the successive shoot production could apparently be continued.

Effect of Daylength

The effect of daylength in the general morphology of the shoots was studied and observation on characters like numbr of nodes, thickess of the stem and rate of shoot elongation were record. Analysis of variance (Table-I) showed significant effects of daylength (P 0.05) on all the characters.

Table 1. *Effect of day length on plant morphology*

Day lengths	Data recorded after 4 weeks		
	No. of nodes	Stem thickness (mm)	Mean inter nodal length. (mm).
8	6.8	0.65	5.14
16	8.9	0.82	3.11
24	10.2	1.01	2.09

At longer daylengths (16, 24 hours), thicker stems and shorter internodes were formed as compared to shorter (8 hours) daylength were a marked tendency in shoots to develop thin stems and narrow scale like leaves was noticed. Thicker and shorter stems were found to be desirable for subculturing because these were easy in handling and manipulation as compared to thin stems.

Effect of Longer Incubation on Cultures

The results tabulated above refer to the cultures grown over one month. When the cultures were incubated further over three months the plantlets produced miniature aerial tubers at the end of stolons formed in the leaf axis in 16 and 24 h day length. Mostly the stolons grew from middle or lower nodes which culminated into tubers. Generally one or occasionaly two tubers were formed. The morphology of the arial tubers appeared to be normal except that the greyish white colour and tender skin. These mini tubers behaved like the tubers produced in the soil and produced normal plants when planted.

The cultures grown under 8 h day length continued growth by branching and producing leaves. The shoot tips showed loss of terminal dominance by ending up on needle like tip with scaly leaves. However on subculturing, these shoots produced normal plantlets under 24 h daylength conditions.

The potato plant produces a succession of nodes each containing a leaf and its associated axillary bud during its vegetative growth. According to the age of the plant and the environmental conditions each axillary bud can grow either into an upright leafy shoot or a more or less horizontal stolon, some of which may culminate in tubers. Both stolons and tubers carry axillary buds; all of which have the potential to grow into leafy shoots, stolons or tubers (Hussy and Stacey, 1981). The *in vitro* method used in this study exploits this pheno-

menon for the rapid and continuous production of nodes with small rooted shoots or miniature tubers as an end product.

Cytokinins, commanly known to accelerate the rate of adventitious meristems are undesirable in maintaining the genetic purity of potato cultivars during its multiplication *in vitro*. In this study a small amount of hormone (10^{-6}M) was used to enhance the growth rate of the axillary meristem. However, when this objective was achieved, careful observations failed to detect any shoot arising from adventitious meristem. Probably the quantity of the horme was too small to promote the formation of adventitious meristem. It may also be possible that axillary meristem exerts some special dominance effect on the adventitious meristem as suggested by Hussey and Stacey, 1981).

The aim of the study was to produce *in vitro* plantlets which should grow as normally as possible and produce the crucial organs i. e. axillary meristems at some increased rate. Nozeran, Bancilhon-Rossignol and Grenan (1977) reported average multiplication rates of x5 to x7 per month *in vitro* culture of potato shoot tips using node cuttings on agar medium. They incubated the cultures at 19°C in 12 h day length and a light intensity of 12000-13000 lx. Goodwin, Kim and Adisarwanti (1980) using liquid cultures in flasks, agitated for 2-4 h per day, obtained multiplication rate of around x5 per month at 18-20°C in continuous light of approximately 1000 lx intensity.

The work reported here shows rapid production of x8 to x11 with an average of 9 nodes per month when temperature was maintained at 25°C and daylength of 12 hours. This rate of node production is slightly better than that of Hussy and Stacey (1981) who obtained x8 to x10 nodes per month. They suggested that by subculturing serial shoot production could apparently be continued indefinitely. Probably the media used in these studies was responsible for the slightly better rate of node production. It may also be possible that it was the behaviour of the genotype as cultivar Home Guard was used in this study against cultivars Arran Pilot, Majestic and King Edward used by them.

With this average rate of x9 per month very large number of plantlets can be produced. Nodes in the range of 50-100 can be established from the sprouts of a medium size tuber by sprouting and resprouting in 6 weeks. On a conservative estimate the number of *in vitro* cultures would exceed 6000 plantlets after 12 weeks.

Tuber formation in conventionally grown potato generally occurus in short days and low temperature. Contrary to this, formation of, *in vitro* aerial tubers in this experiment was favoured by long days and at a temperature of 25°C. Similar are the observations of Hussy and Stacey (1981) and Sunderland (1974).

The establishment of optimal conditions for *in vitro* tuber formation would be of considerable value. The small tubers would seem to be the ideal end product of micropropagation process for the production of disease free potato seed. These mini potatoes are highly convenient for stotage and transporation to long distances as elite materials.

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