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Full Length Research Paper

Effect of growth regulators on micropropagation of potato cultivars

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The effect of growth regulators on *in vitro* micropropagation of three potato cultivars (*Solanum tuberosum* L.) were evaluated. In the present study nodal explants of potato cultivars (Diamont, 1533 and Kufri Badshah) were cultured on MS basal medium supplemented with different hormonal combinations (BAP and GA_3). Out of four different media combinations, MS + BAP (1.0 mg/l) gave maximum shoot regeneration on all the three cultivars of potato. Successful rooting was achieved by placing the shoots on half strength MS basal medium. The combination of sand: soil (1:1) was the best for plant acclimatization as 90% of the plants survived and became established.

Key words: Potato, nodal explants, micropropagation, cultivars.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an economic tuberous crop cultivated worldwide in the temperate, tropical and subtropical zones. It is the world's number one non-grain food commodity and fourth largest food crop following rice, wheat and maize (Moeinil *et al*., 2011). In India during the year 2012 its production was 436.45 lakh tons (2011-12 reports, India Infoline News Service). Potato gives an exceptionally high yield and produces more edible energy and protein per unit area and time than many other crops. While the developed countries make diversified use of potatoes as food, feed and raw material for processed products, starch and alcohol; the developing countries potatoes are increasingly adopting potato cultivation as an important source of food, employment and income. Potato is being seen as a vital food-security crop and as a substitute for costly cereal imports. It is vulnerable to a number of biotic and abiotic stresses which limit its production, particularly among small and marginal farmers with limited resources. Conventional potato seed production system is characterized by low multiplication rate (1:4-1:15), Wastage of a large quantity of food material, absence of uniformity, risk of catching diseases/pests and progressive accumulation of degenerative viruses during clonal propagations (Naik *et al*., 2000). Also, shortage of good quality planting material has been recognized as one of the major bottleneck in potato cultivation in developing countries. The problem is further aggravated by high seed rate (3-4 t/ha) and high cost of potato seed due to which the cost of seed potatoes alone accounts for about 40 to 60% of the total production costs in many parts of the world. Development of tissue culture technology has been the foundation of high quality, disease free planting material production at a mass scale, particularly in vegetatively propagated crop. Micro propagation is the alternative to conventional propagation of potatoes (Chandra *et al*., 1994). *In vitro* propagation methods using sprouts and nodal cutting are more reliable for maintaining genetic integrity of the multiplied clones (Liljana *et al*., 2012). *In vitro* approach facilitates production, mass multiplication and season independent production of disease free planting material and conservation of potato in controlled and disease free conditions using space very effectively and efficiently. Micropropagation coupled with conventional propagation has now become an integral part of seed production in

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many countries. The objective of present study was to find out a suitable growth regulators and its optimum concentration for micropropagation of three different potato cultivars.

MATERIALS AND METHODS

The present investigation for *in vitro* micropropagation was conducted during in the Tissue
Culture Laboratory, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab. Popular high yielding potato cultivars Diamont, 1533 and Kufri Badshah tubers were used as a source for explants throughout the experiment. Healthy sprouted tubers were planted in pots in the green house. When the plants were 25 to 35 cm tall with 4 to 6 nodes, they were cut into single node and large leaves were removed.

Surface sterilization of explants

Single node cuttings about (1-3 cm) long were washed under running tap water for 20 min followed by surface sterilization with Teepol^{TM} (0.5% v/v) for 15 min under tap water and used as explant throughout the experiment. Under the laminar airflow hood the single nodal segments were surface sterilized by treating with 1% (w/v) carbendazim (Bavistin^{TM} from BASF India Ltd, Mumbai) for 15 minutes and finally with 0.1% mercuric chloride for 1min. These were thoroughly rinsed with sterile water 3-4 times after each step. Disinfested nodes were put on sterilized paper tissue in sterilized petridishes and both the ends of the nodal segment exposed to sterilant were trimmed and used as explant ready for inoculation.

Establishment of explants and shoot regeneration

Maintaining the correct polarity of the single nodal segments, these were individually inoculated in 25×150 mm test tubes (Borosil, India) with Murashige and Skoog (MS) basal medium containing 3% sucrose and supplemented with different concentrations of BAP (0.5, 1.0 mg/l) alone and in combination with GA_3 (0.2 mg/l). The medium was solidified with 0.8% agar (Bacteriological grade, Hi Media, India) and pH 5.8 was adjusted with 1N NaOH and 1N HCl prior to autoclaving. All the chemicals used were of analytical grade (Hi Media, SRL, Sdfine Chem Ltd, Merck Ltd India and Sigma Chem Co. USA). Cultures were incubated at 25±2°C under a 16 hours photoperiod (illuminated with 40W white fluorescent tubes, Philips, India) followed by 8 hours dark period. Nodal explants of three cultivars of potato were cultured on MS basal medium containing different concentrations of BAP and with combinations of GA_3 (Table 1).

Multiplication of shoots and root regeneration

All the regenerated shoots were inoculated on shoot multiplication medium supplemented with lower concentration BAP. After multiplication of shoots, the regenerated shoot clumps were excised and transferred to hormone free half strength MS basal medium for root induction.

Hardening of regenerated plantlets

After *in vitro* root regeneration the rooted plantlets were removed from rooting medium and washed to remove adhering gel and kept on moist cotton for 3 days in the incubation room for initial hardening. These plantlets were then transplanted to plastic bags containing garden soil and sand at 3:1 ratio in the green house.

RESULTS AND DISCUSSION

Initial response in cultured explants was observed after 3-4 days of culturing followed by distinct response in the form of direct shoot regeneration. Each node cultured in MS medium developed into a plantlet and at 4 weeks of each culture passage the plantlets had well developed aerial parts and good root system and occupied the full length of the culture vessel (Figure 1).
Shoot regeneration from the nodal explants of three different potato cultivars was observed to be ranging from 72% to 98.70%. Maximum regeneration percentage (98.70%) was found to be on MS basal medium + BAP (1.0 mg/l) in Kufri badshah (Table1). Direct shoot regeneration was observed on MS basal medium supplemented with different concentrations of BAP, whereas, culture media supplemented with GA_3 exhibited high callus induction. Statistical analysis using factorial CRD revealed that significant differences among the media combinations, whereas, cultivar differences observed were non-significant. Interactions between media and cultivar were also non-significant.

After shoot regeneration, multiplication of shoots was obtained on MS basal medium supplemented with BAP (0.5 mg/l). It was observed that BAP played important role in shoot regeneration. Earlier reports are available on role of BAP in promoting the number of lateral shoot (Uddin, 2002; Hussain *et al*., 2005; Azar *et al*., 2011). Similar results were also reported by Sarker and Mustafa 2002 that the BAP showed better response in terms of shoot per explants, shoot length, number of nodes and leaves in potato varieties Lal Pari and Jam Alu. Similar behavior was also observed in varieties Diamont, Altamash and Cardinal. The results also coincide with the reports of Hoque *et al*. (1996a, 1996b) and Mila (1991) for other potato varieties. Hussain *et al*., 2005 obtained maximum regeneration percentage from nodal explants of potato on MS basal medium with 2.0 mg/l BAP and 0.5 mg/l IAA. Molla *et al*., 2011 also studied the effect of growth regulators on direct regeneration of potato. Seven different concentration of BAP, six different concentrations of TDZ and eight different concentrations of zeatin riboside (ZR) were tested separately for *in vitro* direct regeneration of potato along with GA_3 (0.2 mg/l) and IAA (0.01 mg/l). MS medium supplemented with 3 mg/l BAP, 0.3 mg/l TDZ and 5 mg/l ZR showed very good shoot induction.

Root initiation was observed spontaneously from the *in*

Table1: Effect of different concentrations and combinations of BAP and GA_3 on shoot regeneration of potato cultivars.

Media composition			Per cent shoot regeneration			Mean
	BAP (mg/l)	GA_3 (mg/l)	Diamont	1533	Kufri badshah	
MS +	0.5	0.0	91.00	89.00	92.33	90.78
	1.0	0.0	96.00	92.20	98.70	95.63
	0.5	0.2	72.00	75.00	77.33	74.78
	1.0	0.2	79.86	80.83	84.66	81.79
Mean			84.72	84.26	88.26	
CD (5%)	A (Variety) : NS		B (Media): 4.51		AB: NS	

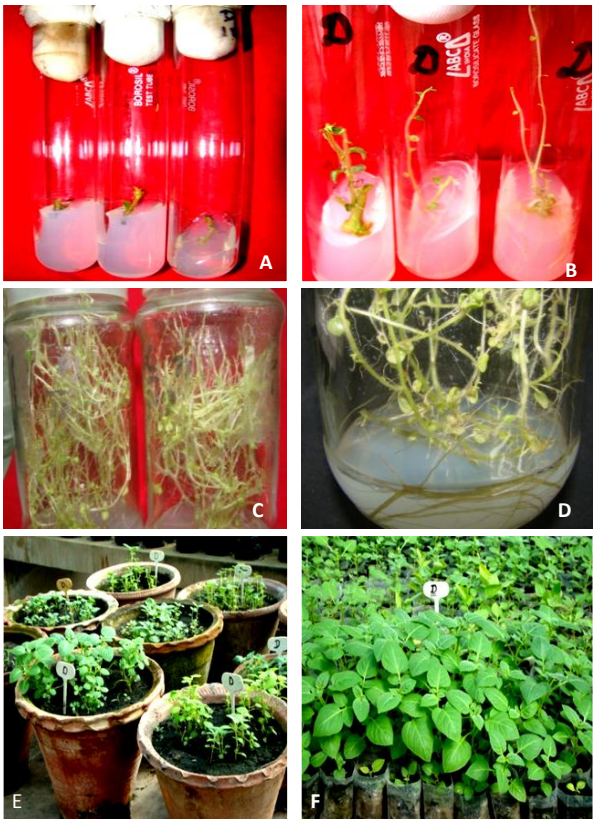

Figure 1. *In vitro* micropropagation of potato cv. Kufri Badshah using nodal explants.

A) Culturing of nodal explants of potato on MS basal medium + BAP (1.0 mg/l)
B) Initiation of shoots from nodal explants on MS (basal medium) + BAP (1.0 mg/l)
C) Elongation and Multiplication of shoots on MS (basal medium) + BAP (0.5 mg/l)
D) Regeneration of roots on half strength MS basal medium
E) *In vitro* regenerated plantlets of tomato kept for hardening in pots containing mixture of sand : soil (1:1) planting substrate .
F) *In vitro* regenerated plantlets successfully acclimatized to sand and soil (1:1) planting substrate.

vitro grown shoots. Complete rooting was achieved on growth regulator free ½ strength MS basal medium. Nearly 100% rooting was observed in all the 3 cultivars on this medium. These results are in agreement with Vinterhalter *et al*. (1997) who reported that potato is an easy to root species and nodal explants do not require exogenous hormone for rooting. Rooted plantlets were transformed to plastic pots containing sand: soil (1:1) and 90% of the plantlets survived in the greenhouse and acclimatized.

CONCLUSION

A genotype independent protocol for the micropropagation of three potato cultivars was optimized. This protocol will provide the base for the mass production of studied cultivars through *in vitro* technique.

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