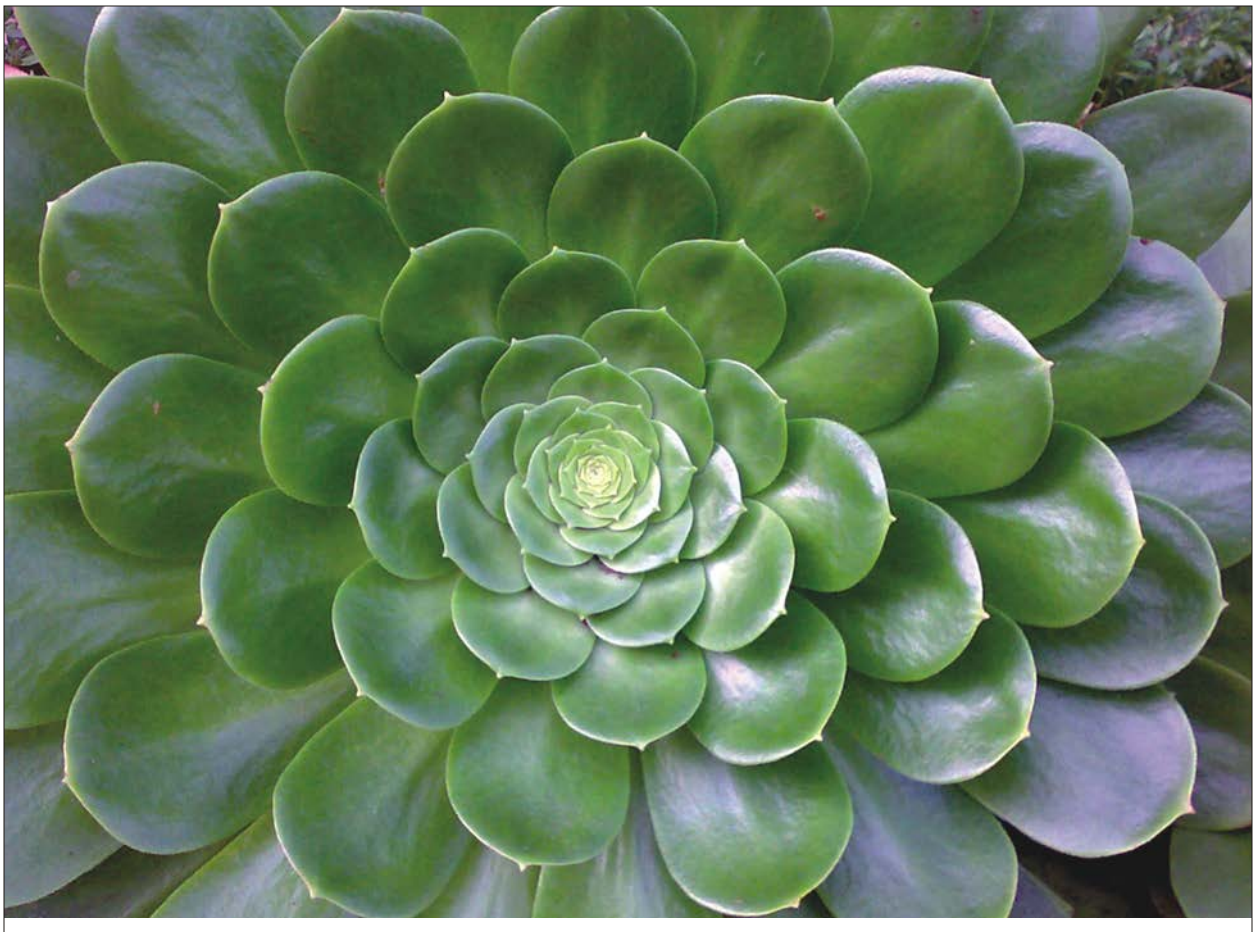

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In vitro Propagation of Elite Indigenous Potato (*Solanum tuberosum* L. Var. Indurkani) of Bangladesh

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Abstract: *In vitro* technique has been developed using single sprout bud explants of elite indigenous potato (*Solanum tuberosum* L.) cultivar (cv. Indurkani) of Bangladesh. MS basal medium supplemented with different concentrations and combinations of BA and Kinetin were used to induce shoots and roots. Shoot and root formations were found to be better in combine treatment of BA and Kn than single treatment of BA or Kn alone. The highest number of shoot were formed on MS basal medium supplemented with a combination of 3.0 mg^{-1} BA and 2.0 mg L^{-1} Kn whereas 2.0 mg L^{-1} BA alone produced only 80% shoots and 2.0 mg L^{-1} Kn alone produced 100% shoots. The induction of multiple shoots from each bud was highest (3.0) in a combination of 2.0 mg L^{-1} Kn and 1.0 mg L^{-1} BA while 2.0 mg L^{-1} BA alone produced only 1.37 shoots. The maximum number of rooting was obtained with 0.5 mg L^{-1} Kn+2.0 mg L^{-1} BA. The highest shoot growth rate (10.5 mm/week) was observed on MS basal medium supplemented with 0.2 mg L^{-1} BA and 2.0 mg L^{-1} Kn. The rooted plantlets were hardened and successfully established in the soil.

Key words: Potato, propagation, growth regulators, indurkani

Introduction

Potato (*Solanum tuberosum* L.) is a popular and major vegetable crop of Bangladesh. *S. tuberosum* L. belongs to the family Solanaceae and is native to South America. It ranks third after rice and wheat with respect to area under cultivation. Potato is the staple food in at least 40 countries, the majority of which are in the developed world. In nutritive value, boiled potato is almost as good as boiled rice. Potato is richer in vitamins while rice has more calories (Rashid, 1991). It is distributed to tropical and subtropical regions of the world. The Portugese introduced this crop in our country in the early ninteen century. The average per hectare yield of potato in Bangladesh is very low (10.95t) compared to average per hectare yield of the world (15.18 t) (FAO, 1995). A good number of High Yielding Varieties (HYV) are now cultivated in Bangladesh but the indegenous varieties still occupy about 35% of the total area under potato cultivation (Ilangantileke *et al*., 2001). Farmers of Bangladesh generally use own grown potato tuber as seed in the next season year after year rather than using certified seed tuber each year. BADC can supply only 6000 tones of certified seedsof high yielding varieties which constituted only 5.5% of the requirements of these HYV (Rashid, 1991). As a result, each year potato plants are infected by a number of viruses e.g., PVX, PVY, PLRV etc. which are the main cause of gradual reduction in vigor and yield of potato known as degeneration. Other than virus attack, the potato crop production is seriously hampered by the attack of fungal and bacterial diseases. The total loss caused by the above diseases is 30-100% during cultivation (Anonymous, 1992). Bangladesh Govt. has to import 15,000 tonnes of seed potato from the Netherland each year to supply good quality seed tubers to farmers. To overcome these problems, a system of disease free seed potato production using micropropagation technique has been proved successful in South China, Vietnam, Tiwan, India and Bangladesh having similar climatic conditions (Zaag, 1990). In Bangladesh, studies

on rapid *in vitro* multiplication, production of disease free plantlets, microtuber production of modern varieties have been done extensively. But no researches report on indigenous cultivar Indurkani yet available. The present investigation was conducted to develp a reproducible protocol of rapid *in vitro* multiplication of potato cultivar Indurkani.

Materials and Methods

The research was conducted at Plant Biotechnology Laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh during the end of 2005. An indigenous elite potato (*Solanum tuberosum* L.) variety Indurkani was collected from potato growers of Sadar thana of Bogra district, Bangladesh. The potato tubers were preserved in dark condition at 12°C at Plant Biotechnology Laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University. After sprouting the tubers, 4-5 cm long sprouts were surface sterilized in a sequential manner with 70% ethanol for 10-15 sec and 0.15% mercuric chloride along with few drops (1.0 mL in 200 mL water) of Decon for 10 min followed by thoroughly washing with sterile distilled water 3-4 times in a laminar air flow cabinate. The sprouts were cut in to pieces of 0.2 to 0.4 cm containing one bud in each explant and were cultured on MS (Murashige and Skoog, 1962) medium containing standard salts, vitamins, 3% sucrose (w/v) and 0.7% TC agar (Carolina, USA) supplemented with different combinations and concentrations of benzyladenine (BA) and kinetine (Kn) for shoot and root formation. The pH of the medium was adjusted to 5.8 before adding agar. After melting agar media, 20-22 mL media dispensed in to each culture tubes and autoclaved at 1.1 mg m^{-2} for 20 min at 121°C. The cultures were incubated at 25±2°C under 16 h light photoperiod. New shoots were initiated after 3-4 days of inoculation. Eight replications were tested for each treatment and after three weeks data on number of shoots/explant, No. of roots/explant, shoot length, root length, No. of leaves/shoot and growth rate of shoots were recorded.

Results and Discussion

The present study was undertaken to establish a reproducible protocol for mass scale micropropagation of local elite cultivar (cv. Indurkani) of potato (*Solanum tuberosum* L.). For this purpose, different concentrations of BA and Kn were used singly or in combinations in MS basal medium. Nodal segments of potato sprout were used as explant in this experiment. Six concentrations (0.1 -3.0 mg L^{-1}) of each BA and Kn were tested in MS medium for the initiation and multiplication of shoot.

Hundred percent shoots were observed for all concentrations of single use of BA and Kn. Among various concentrations of BA used in MS media, maximum number of shoots were found when the explants were cultured on MS medium containing 2.0 mg L^{-1} BA while highest number of shoots/explant were observed when the explants were cultured on MS medium containing 2.0 mg L^{-1} Kn. Shoot length was also found highest in the same treatment of Kn. Growth of shoot was retarded/deminished after 6-7 days of initiation of shoot. It was observed that lower concentrations of Kn (0.1-2.0 mg L^{-1}) found suitable for potato micropropagation.

Effect of BA and Kn on Shoot Multiplication

Cent percent shoot initiation was observed in all MS media used supplemented with (0.1- 3.0 mg L^{-1}) BA and Kn (Table 1) except 0.1, 0.5 and 3.0 mg L^{-1} BA. The highest number of shoots/explant (1.37) was found in MS medium containing 2.0 mg L^{-1} BA while 2.0 mg L^{-1} Kn produced longest shoot (4.18 cm) with second highest number of shoots/explant (1.28). The highest number of branches/explant (1.42) was produced in MS medium containing 0.1mg L^{-1} Kn. It was

Table 1: Effect of different concentrations of cytokinins (BA and Kinetin) in MS medium on shoot and root proliferation/formation from nodal buds of potato (*Solanum tuberosum* L. cv. Indurkani) sprout. (Data were recorded after three weeks of inoculation)

Growth regulators (mg L^{-1})	(%) of explant formed shoot directly	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of roots/ explant	Root length (cm)	No. of branches/ explant
BA							
0.1	62.5	1.00	1.48±0.64	2.20±0.83	-	-	1.00
0.2	100	1.12±0.35	1.73±0.83	3.25±1.83	-	-	1.20±0.25
0.5	75	1.00	1.28±0.57	1.60±0.81	0.75±0.70	1.34±0.35	-
1.0	100	1.12±0.35	1.47±0.77	1.62±1.30	-	-	1.15±0.37
2.0	100	1.37±0.74	1.61±1.50	4.5±5.70	-	-	-
3.0	75	1.00	1.60±1.70	3.0±2.70	-	-	-
Control		1.00	1.63±0.56	3.16±2.10	-	-	-
Kn							
0.1	100	1.13±0.33	2.65±0.90	9.41±2.32	0.28±0.69	0.44±1.00	1.42±1.29
0.2	100	1.00	1.28±0.51	6.28±2.51	-	-	-
0.5	100	1.00	3.01±0.88	9.42±2.82	2.42±0.90	1.60±0.37	1.28±1.60
1.0	100	1.14±0.34	2.72±0.11	8.84±2.70	1.85±1.20	1.02±0.68	1.0±0.92
2.0	100	1.28±0.48	4.18±0.98	7.85±3.10	1.57±1.51	2.79±0.30	1.00±1.15
3.0	100	1.00	1.12±10.04	3.14±1.24	-	-	-
Control		1.00	1.63±0.56	3.16±2.10	-	-	-

Table 2: Effect of different combinations and concentrations of BA and Kinetin in MS medium on shoot and root proliferation/formation from nodal buds of potato (*Solanum tuberosum* L. cv. Indurkani) sprout. Data were recorded after three weeks of inoculation

Growth regulators (mg L^{-1})	(%) of explant formed shoot directly	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of roots/ explant	Root length (cm)	No. of branches/ explant
Kn+BA							
2.0+00	100	1.28±0.48	4.18±0.98	7.85±3.10	1.57±1.51	2.97±3.00	1.00±1.15
2.0+0.1	100	1.28±0.48	4.27±1.60	10.57±3.40	2.80±1.60	2.67±1.60	2.28±1.11
2.0+0.2	100	1.12±0.35	4.72±1.81	10.0±3.46	2.26±1.06	3.48±1.80	2.00±1.50
2.0+0.5	100	1.37±0.74	4.28±1.70	8.75±3.45	3.12±1.90	2.13±1.60	2.37±1.40
2.0+1.0	100	1.38±0.09	4.83±0.91	13.12±4.40	3.50±2.13	1.65±1.06	3.63±2.26
2.0+2.0	100	1.62±0.91	4.63±0.74	14.75±5.11	2.00±1.30	0.63±0.53	2.75±1.03
2.0+3.0	100	2.0±0.91	4.00±1.70	10.1±4.01	1.50±1.80	0.40±0.60	2.00±1.50

Table 3: Effect of different auxins in MS medium on root formation of the *in vitro* grown microshoots of potato (*Solanum tuberosum* L. cv. Indurkani). Data were recorded after three weeks of inoculation

Type of auxin	Conc. of auxin (mg L^{-1})	(%) of microshoots rooted	No. of roots/shoot		Average length of roots X±SE	Intensity of callus formation
			Range	Mean		
IBA						
	0.1	50	2-3	2.5	5.5±0.35	-
	0.2	20	2-3	2.5	4.5±0.0	-
	0.5	100	2-8	4.4	4.3±0.18	-
	1.0	20	2-4	3.0		-
	2.0	50	2-4	3.0	4.5±0.0	-
	3.0	80	2-6	-	4.5±0.0	+
					2.6±0.50	
NAA	0.1	80	12-16	12	2.4±0.25	++
	0.2	100	5-8	6.6	2.0±0.55	+++
	0.5	20	0-2	1.5	0.35±0.0	+++
	1.0	-	-	-	-	+++
	2.0	-	-	-	-	+++
	3.0	-	-	-	-	+++
					-	
IAA	0.1	20	1-2	1.5	3.5±0.0	-
	0.2	33	1-2	1.5	3.5±0.0	-
	0.5	80	2-10	4.5	3.5±0.27	-
	1.0	60	1-4	2.0	3.8±0.59	-
	2.0	60	3-6	3.33	6.2±0.83	-
	3.0	20	6-7	3.0	7.5±0.0	-

(a) (b) (c)

Fig. 1: (a) Effect of BA and Kin (b) Effect of Auxin and (c) Effect of combination on shoot and root formation

observed that the shoot multiplication rate was increased with an increase of cytokinin but after certain level, this become constant or decreased indicating that beyond certain level of BA or Kn has an adverse effect (Table 1). Single application of Kn was found to be better than BA in producing number of shoot/explant, No. of branches/explant, longest shoot and No. of roots/explant (Table1) (Fig.1a).

Combined Effect of BA and Kn on Shoot Multiplication

It was revealed from Table 2 that combined effect of Kn and BA is better than single application of Kn (Fig. 1c).

Rooting of the Shoots

Microshoots containing single node were collected from *in vitro* grown potato shoots and were inoculated on MS medium containing different concentrations (0.1-3.0 mg L^{-1}) of IBA, IAA and NAA for rooting. Cent percent microshoots produced root at 0.5 mg L^{-1} IBA (Table 3). The root induction gradually decreased with increasing concentration of IBA. The highest number of roots (4.4) was found at 0.5 mg L^{-1} IBA. No callus was formed at the cut portion of the shoot proving ideal concentration for root induction. While all concentrations (0.1-3.0 mg L^{-1}) of NAA produced calli at the cut portion of shoot. IAA also induces roots with out callus formation but average number of roots/explant were lower and average length of roots was smaller than IBA (Table 3) (Fig. 1b). No similar result was found in earlier works because this is an indigenous variety in Bangladesh and this is the first work with variety Indurkani.

Establishment of Plantlets

Rooted plantlets of *Solanum tuberosum* L. cv. Indurkani were taken out from culture tubes and washed thoroughly with tap water to remove the culture medium from the roots. Washed plantlets were grown on earthen pots containing one part sterile garden soil, one part sand and one part compost. The potted plants were irrigated adequately and were kept covered by transparent polythene bag to maintain high humidity and kept inside the culture room for one week. More than 80% plantlets were survived. Then the hardened plantlets were transferred in the field.

Conclusions

The combination of BA and Kin was found better than alon of BA and Kin for shoot formation. On the other hand IBA was found to be better than NAA or IAA for root induction of potato. The Potato Variety Indurkani, thst is mainly found in Bangladesh and most probably this is the first work. So there was no scope to compare the findings of present study with previous one. More research should be done with this variety to develop a suitable protocol for rapid propagation and to increase its unit production. Then it will be an excellent profitable variety for large-scale production. The finding of this study will help the researchers for further research on variety Indurlani.

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