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Induction and Evaluation of Somaclonal Variation in Potato (*Solanum tuberosum* L.)

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Abstract: Callus was induced from nodal internodal explants of potato Cvs. Multa and Diamant through the proper adjustment of auxin and cytokinin combinations. Callusing response was the best in MS supplemented with 1.0 mg l^{-1} NAA + 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA+ 0.5 mg l^{-1} BA. The callus developed from both nodal and internodal segments induced to develop shoot when subculture on to MS containing same growth regulator formulations. MS containing 3 mg l^{-1} KIN with 1.5 mg l^{-1} NAA was the most responsive medium for shoot regeneration. Plants regenerated through callus culture after transplanting in field displayed somaclonal variation for plant height, number of laves/plant, number of tubers/plant and tuber weight/plant. This variability may incorporate for the potato breeding program.

Key words: Potato, variety, callus

Introduction

The potato (*Solanum tuberosum* L.) is a vegetable crop of major economic importance world wide. It is the fourth most cultivated food crop after wheat, rice and maize and therefore, the most important dicotyledonous and tuber crop (Jones, 1994). As such, potato growers produce about 325 million tons of potato annually (World Book, 2000). Potato is the only crop, which substantially supplements the food requirement of many countries. It is the cheapest source of carbohydrates that is used as a supplementary diet to rice. The potato is a short duration crop that produces a large amount of calories in a short period of times Vrolijk (1994). It is also enriched with protein, iron, magnesium, potassium and vitamin B & C.

Plants derived from tissue have been variously referred to as somaclones or calliclones or proto clones and the variations displayed by such plants are simply called somaclonal variations. Shepard ***et al.*** (1980), Larkin and Scowcroft, (1981), Barwale and Wodholm, (1987), D. Amato (1978) reported a detailed cytological study of plant tissue culture. Potato is amenable to protoplast and tissue culture techniques. This leads to the possibility of producing new plants by somaclonal variation, protoplast fusion or genetic transformation Thomas ***et al.*** (1992), Nelson,

(1983). Various factors have been attributed to the origin, of somaclonal variation eg. cytoplasmic or nuclear mutation Brettel ***et al.*** (1986), polyploidy or other chromosomal abnormalities ***in vivo*** or ***in vitro*** Ahloowahlia (1982), Orton, (1983) or possibly transposable elements Larkin ***et al.*** (1984). It has been suggested that somaclonal variation may be a way of generating useful genetic variation and selection for desired traits can be performed ***in vitro***. Somaclonal variation may be a powerful tool for the production of regenerants tolerant to environmental stresses Nabors and Dykes (1985).

Attempt has made in this study to induce callus subsequent, plant regeneration from the callus and the field evaluation of calliclones of potato with the ultimate aim of the selection of noval somaclones for the improvement of the two potato cultivars, diamant and multa.

Materials and Methods

The research work has done in the Laboratory of Plant Breeding and Biotechnology in the University of Rajshahi from June 1999 to September 2000. Shoot tip meristem of potato (***Solanum tuberosum***) were used for the initiation of primary culture. The shoot tip potato cultivars multa and diamant collected from 25-30 days old field grown seedling (Biotek. Narsary, Namu Bhadra, Rajshahi, Bangladesh) were used as primary explants for meristem culture. Nodal and internodal segments were collected from meristem derived ***in vitro*** grown shoots for callus induction.

The shoot tips of potato were excised with the help of sharp blade and collected in a reagent both containing distilled water with few drops of dettol and few drops of tween twenty and then the explants were washed with gradual change of sterile distilled water. Surface sterilization was carried out by deeping the material in 0.1% $HgCl_2$ solution with gentle shaking for 2-8 minutes followed by 3-5 times washing with sterile distilled water in the front of running laminar air flow cabinet.

Initiation of meristem culture, plantlet production and field transplantation were carried out according to the protocols established by Ahmed (1999) and Rahman (1999).

In vitro grown explants (node, internode) derived meristem were cultures onto MS (Murashige and Skoog, 1962) solidified nutrient medium supplemented with different concentrations and combinations of Napthalene acetic acid (NAA) with Benzyl amino purine(BA) for callus induction. Plants were regenerated by transferring the selected calli in MS medium supplemented with Benzyl amino purine (BA) and Kinetin (KIN) either alone or in combination with Napthalene acetic acid (NAA). All media were supplemented with 3% sucrose. The pH of the media was adjusted to 5.8 before autoclaving and the media were gelled 0.8% difco bacto agar.

The calli, which were induced to develop shoot were subculture onto agar, solidified MSO (Murashige and Skoog medium without hormone) for further shoot elongation and further multiplication. The plantlets were initially planted in phytotrary containing decomposed coir dust and acclimated in misting house condition. The acclimated 25-30 days old plants were

Fig. (A): Induction of callus from nodal segment of ***in vitro*** grown plant of multa in MS+1.0 mg l^{-1} NAA+1.0 mg l^{-1} BA 6 weeks of culture

(B): Induction of callus from nodal segment of ***in vitro*** grown plant of diamant in MS mg l^{-1} +1.0 mg l^{-1} NAA+1.0 mg l^{-1} BA, 6 weeks of culture

(C): Shoot regeneration from internodal segment derived callus in multa in MS+3.0 mg l^{-1} Kn+1.5 mg l^{-1} NAA after 42 days of subculture

(D): Plants in net house, developed from callus of multa (left) and diamant (right), 60- day after plantation

(E): Tubers developed callus derived plants of multa, 90 days after plantation

(F): Tubers developed callus derived plants of diamant, 90- day after plantation.

transplanted in to the field (Fig.D). Data were collected on plant heightber of leaf/plant, number of tuber/plant and tuber wt/plant (Fig. E and F) for assessing somaclonal variation. Test of significance was done by F-test and LSD test.

Results and Discussion

The present investigation was carried out with a view to establish a protocol for the induction of callus, plant regeneration from the callus and field evaluation of different somaclones. The effect of different concentrations of auxin and cytokinin in MS medium on callus induction and shoot regeneration of potato cvs. diamant and multa were studied. Among the many combinations of different growth regulator supplements studied as callus inducing media and only the responsive combinations are presented in Table 1. Callus proliferation was initiated from the cut surface of the explants and finally covered the whole surface. This investigation was carried out for callus induction from nodal and internodal explants and maintenance for further growth.

Among all the treatments of 2, 4-D and NAA with BA in the multa and the diamant, the maximum (90 + 80%) induced callus in media having 1.0 mg l^{-1} NAA+ 0.5 mg l^{-1} BA from internodal segments (Fig. 1 A, B).

In this medium the callus were brown and degree of callus formation was massive. In this investigation different concentrations of 2, 4-D alone and in combinations of NAA with BA were found to be the most suitable culture media composition to induce the explants develop maximum callus in two potato cultivars (multa and diamant) (Table 1). Among the different concentrations and combinations of NAA with BA were found to be the best for callus induction from the both types of explants of both multa and diamant cultivars.

Similar results were reported by Kothari ***et al.*** (1984) in African marigold. In present study reveals that, nodal and internodal explants of the multa showed the better performance to induce callus than diamant. This difference in callus induction may be due to different genotypes Bhattacharya ***et al.*** (1990).

Callus derived from different types of explants obtained different media composition were used for shoot regeneration. In the present study, MS supplemented with different concentrations of cytokinins alone or in combinations of cytokinins with auxin were used to see the response of multiple shoot induction from nodal and internodal explants derived callus from the two cultivars (multa and diamant) of potato.

The combinations of BA with and Kn with NAA showed better performance for shoot regeneration of two potato cultivars from nodal and internodal segments derived callus (Table 2, Fig. 1C). Two successive culture phases are required for inducing multiple shoots also reported by, to many earlier researchers (Conover and Litz, 1978; Litz and Conover, 1981). Similarly Rejeevan and Pandey (1986) observed that shoot tip explant induced to form shoot when successive cultured auxin with cytokinins.

Table 1: Effect of different concentrations of 2, 4-D and NAA with BA in MS medium on callus induction from nodal and internodal explant of two potato cultivars. Data were recorded after 6 weeks of culture

		Cultivars					
		Multa			Diamant		
Explants	Growth Regulation mg l^{-1}	% of explants induced callus	Callus colour	Degree of callus formation	% of explants induced callus	Callus colour	Degree of Callus formation
Nodal	MS+2,4-D						
explants	2.5	40	LG	+	30	GB	+
	3.0	50	LG	+	40	GB	+
		40	LG	+	30	GB	+
	NAA+BA						
	0.5+0.5	50	LG	+	50	GB	+
	0.5+1.0	70	LG	++	60	GB	++
	1.0+0.5	70	LG	++	70	GB	++
	1.0+1.0	80	B	+++	70	GB	++
Internodal	MS+2, 4-D						
explants	3.0	50	LG	+	40	GB	+
	3.5	60	LG	++	50	GB	+
	4.0	40	LG	+	30	GB	+
	NAA+BA						
	0.5+0.5	50	LG	+	40	GB	+
	0.5+1.0	70	LG	++	60	GB	++
	1.0+0.5	90	B	+++	80	B	+++
	1.0+1.0	80	B	+++	70	GB	++

LG = Light green, B= Brown, += Slight callus, ++= Moderate callus, +++= Massive callus

Results on morphological characters of the calliclones of multa and diamant are presented in Table 3 respectively. Remarkable variation was noticed among the plants of the ten callus line of multa and diamant for all of the characters (Table 3) whereas, variation among the plants of same callicloses were less pronounced.

Statistical analysis of the individual character support the existence of significant variation observed among the different cell lines. Like multa modification in other morphological characters were also among the somaclones regenerated from the different callus of diamant. However, variation among the calliclones were less pronounced than those of multa. Somaclonal variation in among plants regenerated through callus culture were early reported by Chandra ***et al.*** (1985). They observed that plantlets regenerated through callus culture were not genetically stable. In this study, statistically significant variations regarding some morphological characters

Table 2: Effect of different concentrations of BA. Kn and combinations of BA with NAA. Kn with NAA in MS medium on shoot regeneration from nodal and internodal explant derived callus of potato cvs. Multa and Diamant. Data were recorded after 42- day of subculture

		Cultivars					
		Multa			Diamant		
Explants	Growth Regulation mg l^{-1}	% calli regenerated shoots	No of shoots/ callus	Shoot lengths (cm)	% calli regenerated shoot	No of shoots/ callus	Shoot lengths (cm)
Nodal	BA						
explants	1.5	40	3.5	3.41	40	4.0	4.42
	2.0	40	3.8	4.3	40	4.0	4.60
	KIN						
	2.5	40	2.8	3.2	40	4.0	4.25
	3.0	40	4.4	4.5	40	4.5	5.0
	BA+NAA						
	2.0+1.0	50	6.3	2.81	40	3.4	4.64
	2.0+0.5	60	6.4	7.2	50	5.0	6.22
	KIN+NAA						
	3.0+1.0	50	4.3	5.7	40	3.7	5.08
	3.0+1.5	60	5.8	6.8	50	4.8	6.43
Internodal	BA						
explants	1.5	40	4.3	5.112	40	3.7	4.55
	2.0	40	3.8	4.95	40	4.7	5.42
	KIN						
	2.5	40	3.2	4.587	40	4.5	4.34
	3.0	40	4.2	1.957	40	4.8	5.28
	BA+NAA						
	2.0+0.1	50	4.8	5.83	40	3.8	4.50
	2.0+0.5	60	6.3	7.2	50	5.2	6.23
	KIN+NAA						
	3.0+1.0	50	5.8	5.53	40	4.0	5.1
	3.0+1.5	60	6.3	7.7	50	4.9	6.42

Table 3: Means±SE of morphological characters of the calliclones of Multa and Diamant

Varieties	Multa			Diamant		
Characters	Mean±SE	F	LSD	Mean±SE	F	LSDs
Plant height/cm	102±.08	177.08	1.25	102 ±0.88	177.08	1.25
No. of leaves/Plant	20±0.51	42.21	0.58	27±0.62	72.25	0.70
No. of tubers/Plant	19±0.45	109.75	0.56	46±0.82	185.70	1.01
Tubers wt/Plant (gm)	368±1.83	1695.22	2.77	598±1.62	7187.33	1.85

Data on plant height and no. leaves/plant were recorded 60 days after plantation and on No. of tubers/plant and tubers wt/plant were recorded 90 days after

were also observed among the different calliclones of two potato cultivars which support the tending of Chandra ***et al.*** (1985)[1] Somaclonal variation was also observed and reported in many other crops by Secor and Shepard (1981), Shepard ***et al.*** (1980), Larkin and Scowcroft (1981) and Scowcroft (1977).

Plant regenerated through callus culture displayed somaclonal variation. This variability may be incorporated for the potato breeding program. However, further study is necessary to find out the nature and stability of the seasonal variants developed for multa and diamant cultivars in the present study.

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