LIFE SCIENCE

Khulna University Studies, 6(1&2): 43-47, 2005

EVALUATION OF SOMACLONES OF POTATO (*SOLANUM TUBEROSUM* L.)

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KUS- 03/29-200703
Manuscript received: July 20, 2003; Accepted: June 06, 2005

Abstract: Evaluation of somaclones in the field of three potato varieties (Diamant, Multa and Cardinal) were examined through callus culture using node, internode and leaf segments. Multiple shoot regeneration from callus at various frequencies was observed using different concentrations and combinations of growth regulators. The highest percentage of callus induction from internode was observed in MS medium containing 3.0 mg l^{-1} of 2,4-D + 150 mg l^{-1} of L-asparagine. In this combination, the percentage of callusing was 90% for Diamant, 80% for Multa and 70% for Cardinal. The best response for multiple shoot formation was observed in MS medium supplemented with 4.0 mg l^{-1} of KIN + 0.5 mg l^{-1} of NAA. Diamant responded better than Multa and Cardinal for shoot regeneration from callus. Best rooting was observed from node or internode derived plantlets of primary calliclone in MS medium supplemented with IBA. Rooted calliclones were transplanted in the field and evaluated somaclones variation using parameters viz. plant height, number of leaves/plant, number of tuber/plant and tuber weight/plant. The result of statistical analysis on individual character supports existence of significant variation among the different somaclones.

Key wards: *Solanum tuberosum*, potato, callus, somaclonal variation

Introduction

Potato (*Solanum tuberosum* L.) is considered to be the fourth major food crop of the world. In Bangladesh, it ranks third after rice and wheat in respect of land under cultivation (Sharkar and Mostafa, 2002). It covers 56 thousand hectares (about 10 percent) of cultivated area of Bangladesh (Islam *et al.*, 2000). The average per hectare yield (10.95 t) of potato in Bangladesh is very low compared to the average per hectare yield (15.18 t) of the world (FAO, 1995). Larkin and Scowcroft (1981) have promoted the term somaclonal variation for any kind of genetic variation detected in plants derived from any form of cell or tissue culture. Initial studies on potato generated much interest in somaclonal variation since some of the somaclones of these crops had useful traits of agronomic interest. So, in this time somclonal variation is a tissue cultural method to improve for crop races. For the improvement of potato crops Shepard *et al.* (1980) suggested that it would be more profitable to improve a popular variety selectively rather than to create new one. Somaclonal variation with useful properties have been produced in potato and selected (Chaudhury, 1994). Such variants are resistant to early blight (c.o. *Alternaria solani*) and to multiple races of *Phytopthora infestans* (De, 1992). The diverse variable characteristic of somaclones, highlights the fact that somaclonal variation may be an additional tool for crop improvement rather than an interesting scientific phenomenon (Bajaj, 1990). Genetic variability in potato arising from regeneration through tissue culture can be transmitted to the progeny through vegetative propagation (Ramulu, 1986). In the present paper, we have studied callus induction, regeneration and evaluation of somaclones in the field on three varieties of potato through callus culture using node, internode and leaf explants.

Materials and Methods

The three varieties of potato (Diamant, Multa, Cardinal) were grown in the field of Department of Botany, Rajshahi University, Rajshahi, Bangladesh. Actively growing shoot tips of three varieties of potato were collected as primary explants .for the meristem culture and they were rinsed with tap water for 5-10 min. The explants were washed again with distilled water containing few drops of Tween 20 and few drops of Savlon (1% v/v) for 7-10 min. The materials were then taken into laminar flow cabinet and finally surface sterilization was done with 0.1% (w/v) $HgCl_2$ for 2-6 min followed by five times rinse with sterile distilled water. Then excised meristems were placed on Paper bridge in liquid MS (Murashige

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DOI: https://doi.org/10.53808/KUS.2005.6.1and2.0329-L

and Sookg, 1962) medium. After that node, internode and leaf explants were collected from meristem derived *in vitro* grown shoots for callus induction.

In vitro grown explants (node, internode and leaf disc) were collected and cultured on MS medium supplemented with different concentrations of 2,4-D alone or in combinations with KIN and L-asparagine for callus induction. For plantlet regeneration, callus was transferred to MS medium supplemented with BAP and KIN either alone or in combination with NAA. The pH of the medium was adjusted to 5.7±0.1 and solidified with a 0.6% agar. The cultures were maintained with 16h photoperiod in the growth chamber at 26±2°C under fluorescent light of 18-32 PPF. Subculturing was done every three - four weeks. *In vitro* regenerated rooted shoots were established *ex vitro* in specially made plastic tray containing coco-peat and kept under misting condition. For field evaluation, acclimated 25-30 days old ten plants selected from independent callus for each variety were transferred to field. Data were recorded for estimating somaclonal variation in different agronomic traits such as plant height, leaf/plant, number of tuber/plant and tuber weight/plant and significant test was done by F-test.

Results

Induction of callus: Callus induction was observed within four weeks after inoculation of the node, internode and leaf explants on MS medium containing different concentrations of 2,4-D (0.5-4.0 mg l^{-1}) alone and in combination with L-asparagine (150 mg l^{-1}) and KIN (0.5-1.0 mg l^{-1}). The best callus induction was observed in MS medium with 3.0 mg l^{-1} of 2,4-D + 150 mg l^{-1} of L-asparagine for the three varieties from internode explants. On this medium, the calli produced by internode explants were light green for Diamant, greenish brown for Multa and Cardinal. The percentage of callus induction from internode was 90, 80 and 60 for Diamant, Multa and Cardinal, respectively (Table 1). In this combination, the percentage of callus induction from node explant was 80, 70 and 60, and from leaf explant was 60, 40 and 40 for Diamant, Multa and Cardinal respectively.

Table 1. Effects of different concentrations of 2,4-D alone or with L-asparagine or KIN on callus induction of three cultivars of potato in MS medium. Data were recorded after 6 weeks of inoculation.

Growth regulators (mg l^{-1})	CULTIVERS								
	Diamant Explants induce callus (%)			Multa Explants induce callus (%)			Cardinal Explants induce callus (%)		
	Node	Internode	Leaf	Node	Internode	Leaf	Node	Internode	Leaf
2,4-D									
0.5	10	20	10	10	15	10	10	15	10
1.0	30	40	20	25	35	15	20	30	20
1.5	50	60	30	35	40	25	30	40	20
2.0	60	70	30	40	50	35	40	50	30
2.5	65	70	40	45	60	35	40	50	35
3.0	75	80	50	50	70	40	45	60	40
3.5	40	50	20	30	45	20	25	40	20
4.0	30	40	20	20	30	20	15	25	15
2,4-D +150 L-asparagine									
1.0+	45	50	30	25	30	20	20	25	15
1.5	65	70	40	35	40	30	30	35	25
2.0	70	75	45	40	45	30	40	60	30
2.5	70	80	50	60	70	40	50	65	35
3.0	80	90	60	70	80	50	60	70	40
3.5	40	50	35	30	40	20	30	40	20
4.0	30	40	25	20	30	15	15	30	10
2,4-D+KIN									
2.0+0.5	45	55	35	35	50	25	30	40	25
2.0+1.0	40	50	30	30	45	20	30	35	20
3.0+0.5	60	70	50	55	60	40	45	55	35
3.0+1.0	50	65	40	40	50	35	40	45	30
3.5+0.5	35	50	30	30	45	25	25	40	20
3.5+1.0	30	40	20	20	30	15	15	20	10

Shoot regeneration from callus: Various concentrations of cytokinin (BA, KIN) singly or in combination with auxin (NAA) were used for shoot regeneration. Shoot formation was highly influenced by explant type and type of the growth regulators used in the experiment. Among the different concentrations and combinations for shoot multiplication, the best performance was observed in MS medium supplemented 4.0 mg l^{-1} KIN + 0.5 mg l^{-1} NAA from internode derived calli (Table 2). The percentages of internode derived calli produced shoots were 75, 50 and 45 for Diamant, Multa and Cardinal, respectively. The number of shoots per culture was 6.1, 5.1 and 4.2 for Diamant, Multa and Cardinal, respectively. The same concentration and combination of growth regulator was effective for plant regeneration callus induced from other source of explants. In case of node it were 60, 45 and 35 for Diamant, Multa and Cardinal, respectively. Similar trends were observed for number of shoot formation per culture.

Root induction on regenerated plantlets: The regenerated shoots were transferred to MS semi-solid medium containing IBA singly and in combination with BA. Among the different combination and concentration of IBA and BA+IBA, the highest percentage of shoot (100%) induced root along with highest root number/explant was obtained in 0.5 mg l^{-1} IBA and 0.5 mg l^{-1} BA+0.5 mg l^{-1} IBA containing MS semi-solid medium (Table 3).

Evaluation of somaclones in the field: For plant evaluation, regenerated plantlets with well developed root systems from selected ten individual calli for each of the cultivar were excised and cultured separately to detect somaclones. The somaclonal variation was measured through plant height (cm), number of leaves/plant, number of tuber/plant and tuber weight/plant (g). The data were analyzed using range, mean, standard error of mean and F-test. The results are presented in Table 4. Distinct morphological variation was noticed among the different calliclone lines for each of the studied genotype.

Table 2. Effects of different concentrations of BAP and KIN alone or in combination with NAA in MS medium on shoot regeneration from callus. Data were recorded after 6 weeks of inoculation for regeneration.

Variety	Growth regulators (mg l^{-1})	Source of explants induced callus					
		Node		Internode		Leaf	
		Regenerated shoots (%)	No. of shoots/ culture	Regenerated shoots (%)	No. of shoots/ culture	Regenerated shoots (%)	No. of shoots/ culture
	BAP						
	3.0	30	3.2	40	3.4	25	2.9
	4.0	25	2.8	30	3.0	15	2.4
	KIN						
	3.0	35	2.9	45	3.6	30	3.0
Diamant	4.0	30	3.0	35	3.2	20	2.6
	BAP+NAA						
	4.0+0.5	55	3.5	65	5.6	40	3.9
	4.0+1.0	40	3.7	60	4.7	30	4.1
	KIN+NAA						
	4.0+0.5	60	5.6	75	6.1	50	3.4
	4.0+1.0	50	5.1	70	5.8	45	5.1
	BAP						
	3.0	20	2.6	30	2.8	15	2.6
	4.0	15	2.2	25	2.4	10	2.0
	KIN						
	3.0	25	3.0	35	3.1	20	2.4
Multa	4.0	20	2.4	30	2.6	15	2.1
	BAP+NAA						
	4.0+0.5	40	3.2	45	5.2	30	2.5
	4.0+1.0	30	2.9	40	4.2	20	2.7
	KIN+NAA						
	4.0+0.5	45	5.0	50	5.1	35	3.1
	4.0+1.0	40	4.6	45	4.1	30	3.4
	BAP						
	3.0	15	2.4	20	2.4	10	2.2
	4.0	10	2.0	15	2.1	10	2.0
	KIN						
	3.0	20	2.7	25	2.8	20	2.4
Cardinal	4.0	15	2.2	15	2.2	15	2.0
	BAP+NAA						
	4.0+0.5	30	3.2	30	3.6	25	3.0
	4.0+1.0	20	3.0	25	3.0	20	2.4
	KIN+NAA						
	4.0+0.5	35	3.8	45	4.2	30	3.2
	4.0+1.0	30	3.2	30	3.4	20	2.6

Table 3. Effect of different concentrations and combinations of cytokinin and auxin in MS medium on root development in regenerated shoots. Data were recorded 28 days after inoculation.

Variety→ Treatment↓			Diamant	Multa	Cardinal
IBA	0.5 mg l^{-1}	Number of root/shoot	18.20±2.44	14.70±2.52	12.45±0.24
		Shoot induced root (%)	100	100	100
	2.0 mg l^{-1}	Number of root/shoot	16.30±2.31	12.10±1.66	9.24±0.21
		Shoot induced root (%)	100	100	100
BA+IBA	0.5+0.5 mg l^{-1}	Number of root/shoot	14.00±2.38	11.43±1.86	7.20±0.13
		Shoot induced root (%)	80	60	45
	1.0+0.5 mg l^{-1}	Number of root/shoot	9.30±1.59	7.83±1.20	5.28±0.14
		Shoot induced root (%)	60	50	40

This is supported by the significant value of F-test for each of the genotype. Among the studied four characters maximum variation was observed in plant height in all genotypes. It range from 102 to 144.8 cm for Diamant, 82.2 to 126.0 cm for Multa and 78.2 to 124.6 cm for Cardinal. Similar trend of variation was also noticed for the other characters. For the trait, number of leaves/plant the range of variation was 16.3–37.4, 14.2-27.9 and 11.2-53.3 for Diamant, Multa and Cardinal respectively. In case of number of tubers/plant the range of variation was 22.2-62.2, 10.3-22.2 and 10.8-22.2 for Diamant, Multa and Cardinal respectively. On the other hand, for tuber weight/plant the range variation was 285.6-802.6, 251.6-531.5, and 216.9-532.2 for Diamant, Multa and Cardinal respectively. All these data supported that *in vitro* callus culture can induce genetic variation among the regenerated plant of any genotype.

Discussion

The present investigation shows that 2,4-D with L-asparagine was effective for callus induction in potato. Many workers

observed 2,4-D as the best auxin for callus induction as in monocot and even in dicot (Evans *et al*., 1981). Among the three varieties, Diamant showed the better performance to induce callus either form node, internode or leaf segments. This is due to different genotypic effect (Bhattacharya *et al.,* 1990). Among the three types of explants, internode was found to be the best explant for callus induction.

Table 4. Statistical analysis of morphological characters of three *in vitro* regenerated potato varieties, under field condition.
Data were recorded after 60 days for plant height (cm) and no. of leaves/plant and after 90 days for no. of tuber and tubers weight/plant (g) of transplantation.

Plant accession of 3 varieties	Plant height		No. of leaves /plant		No. of tubers/plant		Tubers wt. /plant	
	Range	$\bar{x}$ ±SE	Range	$\bar{x}$ ±SE	Range	$\bar{x}$ ±SE	Range	$\bar{x}$ ±SE
Diamant								
1	126-130.6	128.5±1.23	21.2-28.5	25.7±0.54	44.8-49.6	47.7±0.26	561.2-567.6	564.4±2.54
2	114.2-118.4	116.4±0.15	22.1-26.3	24.6±0.26	25.5-32.5	30.7±0.24	320.1-326.4	323.9±1.85
3	140.5-144.8	142.8±0.95	31.3-37.4	35.5±0.12	62.2-72.4	68.4±0.35	802.6-809.2	806.3±1.65
4	102-111	108.4±0.26	21.5-27.3	24.9±0.23	32.2-39.2	36.5±0.16	475.2-483.5	480.6±1.76
5	110-118.2	115.7±0.85	20.8-25.6	23.9±0.15	22.2-32.9	29.3±0.35	412.1-420.6	418.4±2.10
6	118-130.2	125.5±2.31	24.4-30.5	28.4±0.18	31.2-36.5	34.6±0.16	458.6-464.3	462.8±2.54
7	115.3-125.4	123.4±1.54	16.3-22.2	20.7±0.17	32.6-38.9	36.6±0.26	395.2-400	398.5±2.26
8	110-121.2	118.6±1.65	18.8-22.4	20.4±0.16	42.3-49.1	48.3±0.24	671.3-678.2	674.2±2.24
9	111.4-128	123.5±2.30	24.2-28.2	26.2±0.14	32.2-36.5	34.2±0.21	458.6-466.8	462.8±3.30
10	102.3-108.2	106.3±2.40	27.2-32.5	29.3±0.13	25.5-30.2	29.4±0.13	285.6-291.7	289.7±1.23
F value		182.2***		51.4***		202.5***		8264.5***
Multa								
1	90.2-95.3	92.5±0.23	16.3-20.6	18.3±0.15	11.2-17.0	14.7±0.26	275.9-280.6	278.8±0.34
2	82.2-85.5	83.6±0.15	15.6-22.6	18.5±0.32	10.5-14.3	12.6±0.13	251.6-258.9	256.3±0.62
3	82.2-87.9	86.5±0.26	15.3-20.6	19.6±0.15	22.2-26.3	24.3±0.42	448.3-454.6	451.5±0.54
4	111.3-116.0	114.3±0.25	22.5-27.9	26.9±0.26	10.3-15.6	12.8±0.16	260.3-268.5	263.4±1.23
5	82.3-88.6	85.84±0.23	13.5-1705	15.3±0.24	14.5-18.6	16.7±0.18	305.9-310.5	308.7±2.35
6	104.3-110.0	108.4±0.24	17.2-21.6	19.5±0.36	12.3-18.4	16.5±0.19	478.6-485.6	482.8±0.45
7	122.2-126.0	124.2±0.25	22.6-27.9	25.8±0.28	11.3-18.0	14.2±0.13	272.5-278.3	275.6±0.98
8	120.2-124.3	122.4±0.29	22.6-26.5	24.8±0.24	8.9-12.8	11.7±0.16	531.6-538.2	536.3±0.85
9	98.1-104.3	102.7±0.21	15.5-20.6	18.6±0.26	12.5-19.3	16.4±0.14	312.6-320.5	318.5±1.65
10	102.5-107.3	105.5±0.26	14.2-18.6	17.2±0.16	11.6-16.3	14.3±0.41	288.6-292.6	290.8±1.10
F value		172.6***		40.4***		132.5***		2216.4***
Cardinal								
1	88.3-92.5	90.3±0.10	11.2-16.3	15.3±0.17	11.7-15.7	13.7±0.16	216.9-123.3	221.8±0.56
2	78.2-85.5	81.6±0.11	19.2-14.3	22.4±0.16	12.3-18.5	15.6±0.16	248.6-252.2	250.3±0.43
3	78.3-85.1	83.4±0.06	16.0-19.2	18.6±0.13	9.8-15.3	11.3±0.14	261.5-265.3	263.5±0.63
4	109.1-115.0	111.2±0.15	21.3-26.5	24.3±0.05	22.2-26.8	24.8±0.13	451.2-458.3	456.4±0.42
5	78.3-84.3	81.7±0.19	14.5-19.3	18.2±0.11	14.4-19.6	17.7±0.11	299.1-304.0	302.6±0.36
6	92.3-98.2	96.4±0.10	11.3-16.9	15.5±0.24	8.8-12.5	10.4±0.11	252.2-257.0	255.0±0.43
7	108.5-112.6	110.2±0.14	19.1-25.5	23.9±0.32	10.8-14.5	12.2±0.09	461.2-467.2	465.8±0.40
8	115.6-124.6	122.4±0.31	48.5-53.3	51.8±0.15	8.1-11.9	10.7±0.15	532.2-538.1	535.8±0.51
9	94.5-99.3	98.7±0.25	15.2-20.0	18.2±0.16	12.6-18.3	16.4±0.08	312.5-317.1	315.4±0.16
10	94.2-97.5	96.4±0.12	14.3-18.5	16.2±0.08	12.2-15.3	13.3±0.18	268.1-272.2	270.4±0.37
F value		153.4***		34.5***		107.6***		1628.6***

Among the three types of explant, leaf derived calli showed poor performance for both plant regeneration and number of shoot formation ability. Suh and Park (1986) reported that KIN with NAA in MS medium composition also stimulated proliferation and elongation of shoots. A high level of cytokinin (4.0 mg l^{-1}) and low level of auxin (0.5 mg l^{-1}) combination seem to play an important role for differentiation of adventitious shoot, as suggested by Islam *et al.* (1982) and Karim *et al.* (2002). For proper root development from plantlets, use of IBA (1.0 mg l^{-1}) was also reported by others (Marani and Pisi, 1977; Zaman *et al.,* 2001). Our results are supported by the work of others (Ramulu 1986; Thomson *et al*., 1986; Chandra *et al*., 1985). They also observed distinct somaclonal variation in potato regenerated through callus culture. Besides potato, variations among the somaclone are also reported in other crops (Marty, 1988; Galiba and Sutka, 1988; Bajaj, 1990). These results further indicate that use of somatic cell culture can be used as an alternative way to induce variation in any genotype that can eventually be used as a method of crop improvement technique. As we know commercial varieties of potato are tetraploid and vegetatively propagated, very often breeders are faced many difficulties for the improvement of potato through conventional breeding method. Therefore, this *in vitro* technique for creating variations would be used for further improvement of potato genotype against biotic and abiotic stresses.

Conclusion

The present study indicates that, MS medium containing 3.0 mg l^{-1}, 2,4-D + 150 mg l^{-1} L-asparagine was very suitable for callus induction in potato. It was also found that MS medium containing 4.0 mg l^{-1} KIN + 0.5 mg l^{-1} NAA was effective for shoot formation from potato callus. MS medium containing 0.5 mg l^{-1} IBA was found adequate for rooting from stem cuttings of primary calliclones. Significant somaclonal variation of different yield components was observed among different regenerants using three commercial varieties of potato. This investigation could be used as an alternative and innovative method of ameliorating qualitative and quantitative traits of potato.

Acknowledgement
The authors acknowledge the Ministry of Science and Information and Communication Technology of the Peoples Republic of Bangladesh for financial supports of this study.

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