The Agriculturists 12(1): 15-25 (2014) ISSN 2304-7321 (Online), ISSN 1729-5211 (Print)
A Scientific Journal of Krishi Foundation ***Indexed Journal***

Somaclonal Variation in Potato (*Solanum tuberosum* L.) Using Chemical Mutagens

M. E. Hoque[1]* and M. N. Morshad[2]

[1]*Dept. of Biotechnology,* [2]*Dept. of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh*

**Corresponding author and Email: ekramul.sau@gmail.com*

Received: 17 February 2014 Accepted: 14 June 2014

Abstract

An experiment was conducted with three popular potato varieties *viz*. Cardinal, Diamant and Asterix to create somaclonal variation in potato. The chemical mutagens *viz*. Ethyl methane sulphonate (EMS), Methyl methane sulphonate (MMS), 5-Bromo Uracil (BU) and 2,4-D were used in three different concentration (1.0, 2.0 and 4.0 mg/L). Among them only 2,4-D regenerated callus in potato. Higher concentration (4.0 mg/L) of 2,4-D showed variant type of callus, which regenerated abnormal plantlet and some of the plantlets died within 45 days after inoculation. The higher concentration (4.0 mg/L) of EMS, MMS and BU showed huge abnormality on *in vitro* regeneration in all three varieties of potato. Thin stem, deformed shoot development and very less leaf formation were observed in 2.0 mg/L and 4.0 mg/L of EMS, MMS and BU. Due to toxic effect some of the plantlets died. The mutagen treated variants were acclimatized in plastic tray and subsequently in the field condition. It was noticed that, only 37.16% plants survived in natural field condition. Morphological characterization and yield potentiality of all somaclones were studied. It revealed that only one variants *viz*. SVP-53 showed higher yield as compared with two check varieties. The first generation mini tubers were kept for further research.

Keywords: Somaclonal variation, chemical mutagen, Potato

1. Introduction

Potato (*Solanum tuberosum* L.) is an important vegetable crop in Bangladesh which has versatile use in our daily consumption as well as industrial purpose. The total area under potato cultivation is near about 0.6 million hectares and the average yield was 19.69 t/ha, (BBS, 2012). The development of high yielding, more starch enriched, disease resistant varieties is needed for sustainable potato production. In any crop improvement program, genetic diversity has been considered as an important factor for developing cultivar with increased yield and wider adaptability in adverse environmental condition. Till now, only few potato varieties have been cultivated throughout the country which has less genetic variability.

Genetic recombination and variability creation by conventional hybridization method is very difficult in potato due to its shy flowering habit and vegetative mode of propagation. Hence, advanced tissue culture technology can be applied in potato for creation of novel variation. Larkin and Scowcroft (1981) adopted the term

"somaclonal variation" to describe the genetic variations occurring *in vitro* cultured cells, tissues and plants. It has the potentiality to assist plant breeders to create new variation for crop improvement. Many varieties have been developed in sugarcane, mustard, rice, *Apium* etc. (Karp, 1995) by using this technique.

Different factors are identified which influence whether or not variation will be produced and how much variation will be created. It depends on the genetic constitution of source materials, the nature of mutagenic agents, type of explants, etc. Potato being a vegetatively propagated crop, its traditional sexual true breeding approaches are limited. On the other hand, its polyploid genomic structure gives an opportunity to work on somaclonal variation methods for its improvement. Hence, the experiment was designed to create somaclone using different chemical mutagens to create economically important traits in potato.

2. Materials and Methods

2.1. Callus induction and plantlet regeneration and plantlet acclimatization

The experimental materials were three popular varieties *viz.*, Cardinal, Diamant and Asterix which were collected from the Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur. Fresh sprouts were used as explants. Those explants were washed with running tap water followed by dipping in 70% ethanol for 1 minute, then rinsed with double distilled water and dipped in 0.1% $HgCl_2$ for 2-3 minutes for surface sterilization. The explants were cultured in Murashige and Skoog (MS) medium (1962) supplemented with three concentrations *viz.*, 1.0, 2.0 and 4.0 mg/L of each chemical mutagen, EMS (Ethyl Methane Sulphonate), MMS (Mthyl Methane Sulphonate), BU (5-Bromo-Uracil) and 2, 4-D. All these works were done under aseptic condition under Laminar Airflow Hood to avoid contamination. The culture vials were incubated on 3000 lux for 16:8 hour photoperiod at a temperature of 23±1 ^{o}C.

The experiment was laid out in a completely Randomized Design (CRD) having two factors (variety and treatment) with three replications. Data were analyzed using MSTAT-C statistical program. The differences among the means were compared by the least significant different test at 5% level. Data were recorded on days to callus induction, callus size, callus weight, days to shoot initiation, days to root induction, regeneration of normal and abnormal plantlets. Autocleaved garden soil, sand and cowdung were mixed in the ratio of 1:2:1 and were placed in a plastic tray having 4×4 cm small chamber. The plantlets of 40-50 days having well developed roots were removed from culture vial and the roots were washed gently on running tap water. It was immediately transplanted into plastic tray and irrigated with fine spray of water and were kept in a shaded place. After 10-15 days the plantlets were transferred to the main field. Chemical mutagenic treated plantlets were named as SVP (somaclonal variant of potato) and numbering was done chronologically like SVP-01, SVP-02 etc.

2.2. Morphological characterization of somaclonal variants

The mutagen-treated somaclonal variants were nourished under field condition. The whole experimental materials were covered by mosquito net to protect from viral infection through insect. All inter cultural operations were done whenever needed. Good crop management practices were followed to produce minitubers (1st generation tuber) from the different SVP genotypes. Data were recorded on plant height, stem colour, leaf colour, leaf shape, number of tubers per plant, tuber size, tuber weight and yield per plant.

3. Results and Discussion

The chemical mutagens *viz*. EMS, MMS, 5-BU and 2, 4-D were used to create somaclone in three potato varieties - Cardinal, Diamant and Asterix. It was observed that, out of them only 2, 4-D had the ability to induce callus in different potato cultivars.

3.1. Callus induction and somaclonal variant creation in potato

Callus induction and its morphological parameters in different the varieties are presented in Tables 1 and 2. It was observed that simple MS medium had no ability to induce callus in all the three varieties. MS media supplemented with 4.0 mg/L of 2, 4-D required less time to induce calli in all the genotypes. The maximum time (10.0 days) was noticed in Asterix with the treatment MS +1.0 mg/L of 2, 4-D. Vigorous and robust shoot initiation was noticed in all three varieties in MS+2.0 mg/L of 2, 4-D. (Fig. 1).

The biggest size of callus (2.2cm) was observed in the varieties Cardinal and Asterx at 45 days after initiation. Although, the size of calli was the same in both the genotypes but the final weight of calli at 45 days showed little difference between Cardinal and Asterix. Shoot iniciation, root iniciation, normal and abnormal variant regeneration data are presented in Table 2. Shoot per plantlet increased gradually in 30 days and 45 days but healthy, abnormal and deformed shoot formation were observed (Fig. 2) in the higher concentration (4.0 mg/L) of 2, 4-D. The maximum no. of 13.2 shoots were found in the treatment MS+2.0 mg/L 2, 4-D in the variety Asterix at 45 days. Roots per variant were the highest (17.9) in Cardinal with the same treatments. Root per plantlet showed positive correlation with shoot per plantlet. Ehsanpour *et al*. (2007) obtained calli from *in vitro* grown potato leaf segment on MS medium containing 2, 4-D, NAA, Kinetin and yeast extract. They reported the changed DNA pattern as the source of genetic variation.

However, they mentioned that, somaclonal variation could be used for selection of potato calli toward desirable traits, such as salt or drought stress tolerance. Somaclonal variation in potato meristem culture was also reported by Rosenberg *et al*. (2010). They showed that meristem clone differed in yield, number and weight of tubers and late blight resistance. In addition, they reported deviation from true to type in morphological characteristics of meristem clones. This finding was in conformity with our present observations. Patricia *et al*. (2004) reported that 1.65 mM of piclorm and 11.5 mM of 2, 4-D created somaclonal variation in potato.

3.2. Effect of mutagen on in vitro regeneration

The effects of chemical mutagen on *in vitro* regeneration in potato are presented in Table 3. Direct shoots were developed from the explants treated with the mutagens. Toxic effect was observed in higher concentration of mutagens and took more time to shoot initiation in all three varieties. In some cases, regenerated variants died within 15 days of culture. Among the mutagens, BU had more corrosive effect on the plantlets. However, the individual effect of each treatment is given below. Days to shoot initiation were the maximum (13.0) in the treatment MS+ 4.0 mg/L of BU in Cardinal. It was the minimum (4.5) in the treatment MS+1.0 mg/L of EMS in the variety Diamant. Number of shoot per plantlet was the highest (19.6) at 45 days in treatment MS+ 4.0mg/L of MMS in Asterix. Huge number of branches and very thin stems were noticed in higher doses of treatments, which indicate that abnormal variants were created at high doses of mutagen [Fig. 3(a)(b)(c)]. Length of shoot per plantlet was the highest (12.47 cm) in the treatment MS+2.0 mg/L of EMS in Asterix. The lowest length (5.32 cm) was recorded in the treatment MS+2.0 mg/L BU with Cardinal. Due to application of chemical mutagen, there was a great variation in shoot formation and shoots per plantlet. In some treatments abnormal shoot development occurred but eventually those shoots died. The treatment MS+4.0 mg/L EMS, MS+2.0 mg/L MMS, MS+4.0 mg/L MMS, MS+2.0 mg/L BU and MS+4.0 mg/L BU had negative effect on shoot development. Some of the experimental materials died at 30 or 45 days of culture in the above treatments which proved toxic effect of chemical mutagens (EMS, MMS and BU) on potato genotypes.

Table 1. Effect of 2, 4-D on callus induction in different potato varieties

Variety	Treatments (mg/L)	Days to Callus Initiation	Size of callus (cm)			Fresh weight (gm)	
			15 days	30 days	45 days	Initial weight 15 days	Final weight 45 days
Diamant	T_1= Normal MS	-	-	-	-	-	-
	T_2=MS+1.0	9.3	0.45	1.10	1.40	0.44	1.90
	T_3=MS+2.0	7.2	0.70	1.50	1.70	0.34	1.90
	T_4=MS+4.0	6.3	0.50	0.98	1.30	1.04	1.39
Cardinal	T_1= Normal MS	-	-	-	-	-	-
	T_2=MS+1.0	8.5	0.72	1.39	1.50	0.99	1.75
	T_3=MS+2.0	7.0	0.72	1.54	1.70	0.64	1.39
	T_4=MS+4.0	5.9	1.02	1.90	2.20	1.04	3.67
Asterix	T_1= Normal MS	-	-	-	-	-	-
	T_2=MS+1.0	10.0	0.61	1.99	2.20	0.98	1.92
	T_3=MS+2.0	8.0	0.70	1.56	2.00	0.77	1.67
	T_4=MS+4.0	7.1	0.79	1.55	1.96	1.04	2.99
	SE±	0.19	0.05	0.30	0.19	0.19	0.09
	LSD	0.58	0.09	0.41	0.21	0.31	0.08
	Level of significance	**	**	**	**	**	**

Table 2. Effect of 2,4-D on plantlet regeneration and variant creation in potato

Variety	Treatments (mg/L)	Shoot per plantlet			Root per plantlet		
		15 days	30 days	45 days	15 days	30 days	45 days
Diamant	T_1= Normal MS	1.0	1.50	2.61	2.0	5.3	7.6
	T_2=MS+1.0	1.3	2.50	3.0	2.6	4.8	6.9
	T_3=MS+2.0	1.9	3.0	7.0 (AB)	3.0	6.7	9.3
	T_4=MS+4.0	2.0	5.0	9.3 (AB)	6.7	10.8	15.6
Cardinal	T_1= Normal MS	1.2	2.3	2.3	1.0	3.0	5.0
	T_2=MS+1.0	1.7	2.9	3.2	2.5	3.1	4.7
	T_3=MS+2.0	3.0	6.8	9.5 (AB)	4.0	4.5	17.90
	T_4=MS+4.0	3.2	9.8 (AB)	12.5 (AB)	3.0	4.0	5.0
Asterix	T_1= Normal MS	1.5	2.8	2.8	2.1	3.5	4.2
	T_2=MS+1.0	2.6	3.5	4.1	3.7	4.9	4.9
	T_3=MS+2.0	3.9	8.7	13.2 (AB)	5.0	7.8	15.3
	T_4=MS+4.0	1.0	- (AB)	- (AB)	-	-	-
	SE±	0.53	0.91	0.87	0.71	0.01	0.05
	LSD	0.18	0.10	0.43	0.26	0.06	0.05
	Level of significance	*	**	**	**	*	**

AB= Abnormal shoot development due to application of chemical mutagen

Table 3. Effect of mutagenic agents on normal and abnormal shoot initiation in potato

Variety	Treatments (mg/L)	Days to shoot initiation	No. of shoots per plantlet			Length of shoot per plantlet (cm)		
			15	30	45	15	30	45
Diamant	T_1= Simple MS	5.1	1.1	2.6	4.2	1.90	5.63	9.3
	T_2=MS+1.0 EMS	4.5	2.3	3.5	4.9	1.70	5.42	10.65
	T_3=MS+2.0 EMS	9.3	1.4	3.1	5.9	1.90	6.41	11.25
	T_4=MS+4.0 EMS	8.8	2.9	-	-	1.80	-	-
	T_5=MS+1.0 MMS	10.7	2.1	4.8	5.6	2.10	7.00	11.46
	T_6=MS+2.0 MMS	8.0	1.0	-	-	2.05	-	-
	T_7=MS+4.0 MMS	9.9	2.1	6.5	13.20	2.00	7.01	12.21
	T_8=MS+1.0 BU	9.6	1.9	4.0	5.10	1.80	6.64	11.12
	T_9=MS+2.0 BU	10.4	2.1	-	-	1.90	-	-
	T_{10}=MS+4.0 BU	7.3	-	-	-	2.10	-	-
Cardinal	T_1= Simple MS	6.1	2.1	3.5	4.9	2.10	5.44	8.6
	T_2=MS+1.0 EMS	7.5	2.7	3.9	4.60	1.70	5.45	10.87
	T_3=MS+2.0 EMS	7.5	2.3	3.2	10.9	1.80	5.50	10.10
	T_4=MS+4.0 EMS	10.1	1.6	9.5	15.8	2.00	4.90	9.37
	T_5=MS+1.0 MMS	8.2	2.6	4.8	5.1	1.60	7.10	12.27
	T_6=MS+2.0 MMS	9.3	1.7	3.5	15.0	2.10	7.05	12.00
	T_7=MS+4.0 MMS	10.4	1.8	13.6	24.8	3.10	6.37	11.27
	T_8=MS+1.0 BU	11.1	1.7	3.9	4.9	1.70	5.22	10.72
	T_9=MS+2.0 BU	12.0	1.0	12.80	14.40	1.80	5.21	5.32
	T_{10}=MS+4.0 BU	13.0	-	-	-	-	-	-
Asterix	T_1= Simple MS	7.2	1.7	3.8	4.40	2.08	7.00	8.2
	T_2=MS+1.0 EMS	7.9	2.9	3.1	4.50	1.90	6.00	11.45
	T_3=MS+2.0 EMS	9.2	2.8	13.0	-	2.10	6.10	12.47
	T_4=MS+4.0 EMS	10.3	2.9	2.6	3.5	2.70	6.20	-
	T_5=MS+1.0 MMS	8.7	1.1	2.2	4.45	1.70	5.35	10.75
	T_6=MS+2.0 MMS	9.5	1.9	5.2	8.0	2.05	5.90	11.52
	T_7=MS+4.0 MMS	9.2	3.1	13.2	19.6	2.91	6.30	10.99
	T_8=MS+1.0 BU	7.9	2.9	5.6	7.3	1.30	4.70	9.00
	T_9=MS+2.0 BU	8.0	3.7	6.8	10.0	1.90	4.80	10.20
	T_{10}=MS+4.0 BU	9.5	2.0	-	-	2.00	-	-
	SE±	0.16	0.76	0.87	0.29	0.71	0.09	0.03
	LSD	1.35	0.99	0.67	0.12	0.28	0.90	0.03
	Level of significance	**	**	**	**	*	*	**

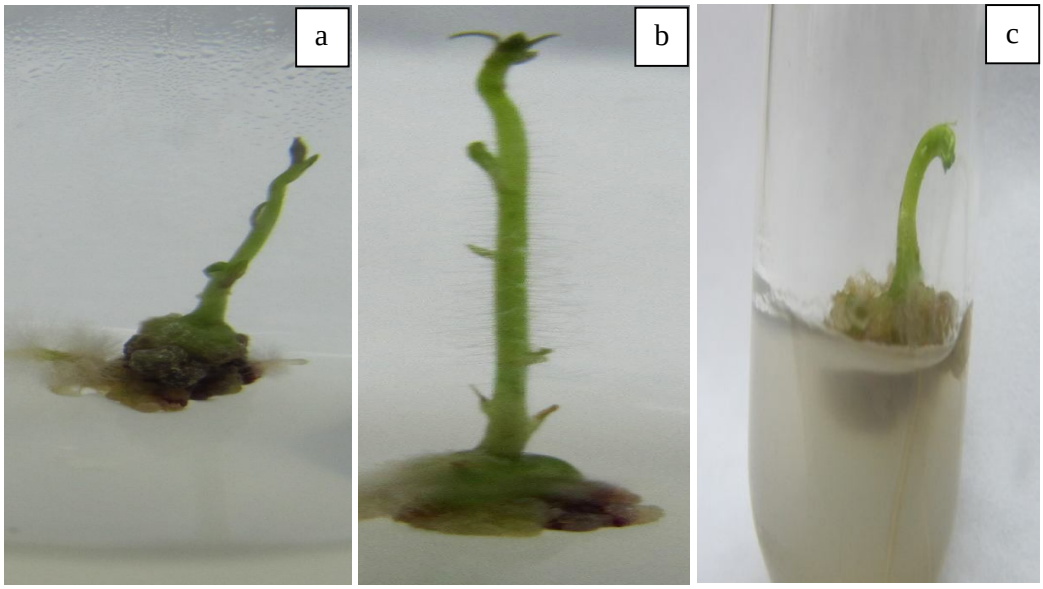

Fig. 1. Healthy plantlet development from callus in the varieties: (a) Cardinal (b) Aterix and (C) Diamant

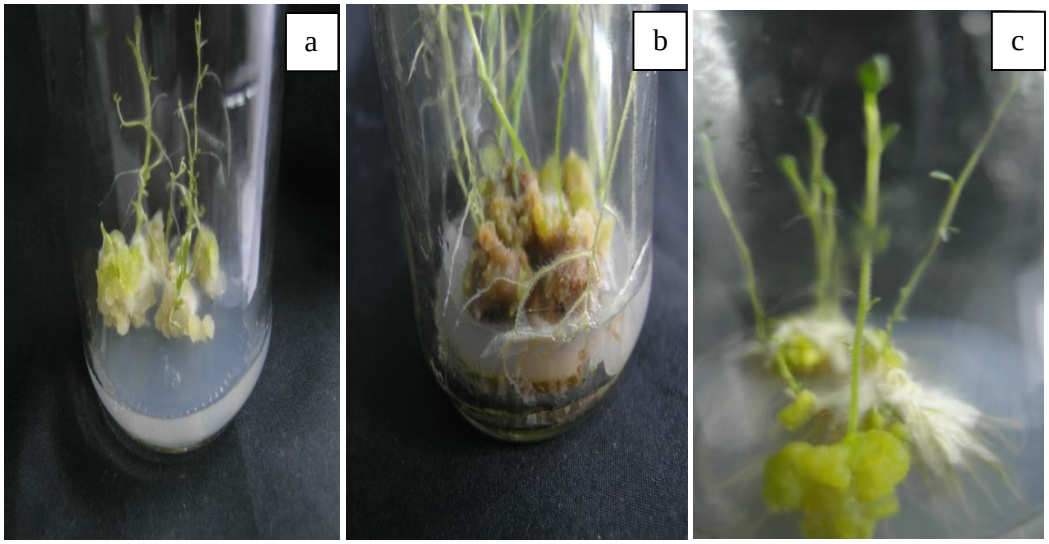

Fig. 2. Abnormal shoot development from callus in (a) Cardinal (b) Asteix and (c) Diamant

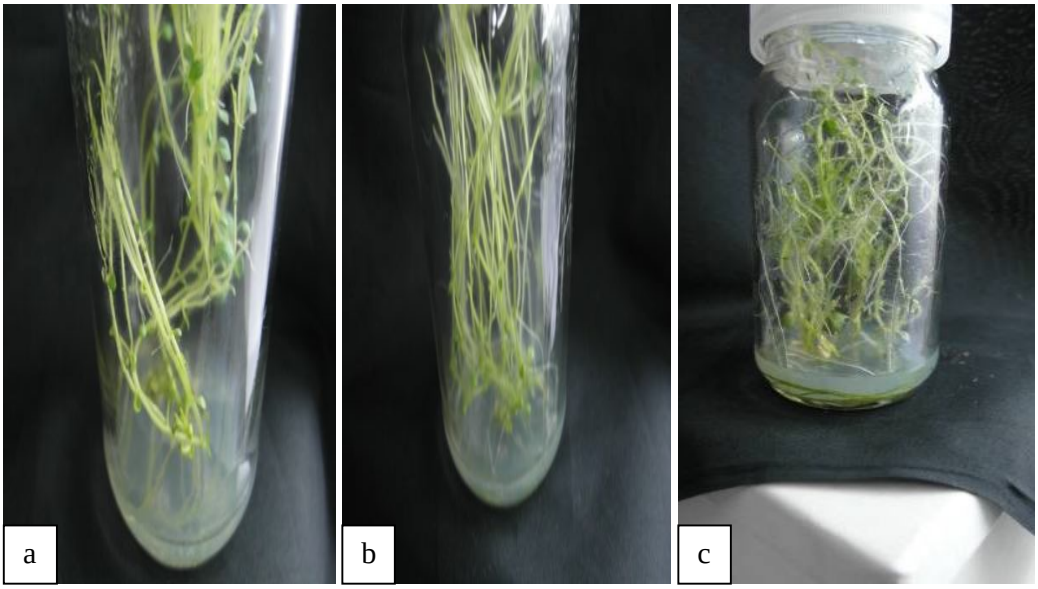

Fig. 3. (a) Abnormal shoot formation due to EMS treatment in Variety-Cardinal
(b) Abnormal shoot formation due to MMS treatment in Variety – Diamant
(c) Abnormal shoot formation due to BU treatment in Variety -Asterix

Fig. 4. Somaclonal variants sown in plastic tray

Table 4. Effect of mutagenic agents on root initiation in potato

Variety	Treatments (mg/L)	Days to root initiation	No. of roots per plantlet		
			15 days	30 days	45 days
Diamant	T_1= Normal MS	9.0	0	5.1	7.2
	T_2=MS+1.0 EMS	10.5	2.1	7.5	18.9
	T_3=MS+2.0 EMS	11.0	3.4	7.6	10.9
	T_4=MS+4.0 EMS	12.3	4.8	-	-
	T_5=MS+1.0 MMS	11.0	0	6.5	8.3
	T_6=MS+2.0 MMS	7.0	1.7	-	-
	T_7=MS+4.0 MMS	8.9	3.2	7.2	11.2
	T_8=MS+1.0 BU	12.5	0	5.5	7.6
	T_9=MS+2.0 BU	10.10	2.5	-	-
	T_{10}=MS+4.0 BU	9.9	-	-	-
Cardinal	T_1= Normal MS	11.1	1.5	4.8	6.9
	T_2=MS+1.0 EMS	9.6	1.1	5.1	9.3
	T_3=MS+2.0 EMS	8.7	3.5	7.7	10.5
	T_4=MS+4.0 EMS	10.5	2.5	6.5	11.6
	T_5=MS+1.0 MMS	14.6	2.1	5.2	7.1
	T_6=MS+2.0 MMS	17.2	2.9	6.5	9.3
	T_7=MS+4.0 MMS	19.2	4.1	6.5	11.7
	T_8=MS+1.0 BU	18.6	1.5	4.2	7.2
	T_9=MS+2.0 BU	10.1	2.1	4.5	9.2
	T_{10}=MS+4.0 BU	11.9	-	-	-
Asterix	T_1= Normal MS	13.0	1.0	3.5	5.5
	T_2=MS+1.0 EMS	9.1	1.8	3.3	7.8
	T_3=MS+2.0 EMS	12.1	3	5.1	8.2
	T_4=MS+4.0 EMS	10.5	2	6.6	-
	T_5=MS+1.0 MMS	6.5	1	4.7	6.1
	T_6=MS+2.0 MMS	8.7	3	5.7	7.1
	T_7=MS+4.0 MMS	9.1	4	5.0	8.0
	T_8=MS+1.0 BU	13.2	2	5.3	7.0
	T_9=MS+2.0 BU	14.8	2	4.4	4.2
	T_{10}=MS+4.0 BU	17.2	3	-	-
	SE±	0.83	0.22	0.63	0.81
	LSD	0.93	0.13	0.32	0.10
	Level of significance	**	**	**	*

Table 5. Survival rate of different regenerated plantlets after transplantation

Acclimatization	Variety	No. of transplanted Plantlets	No. of Plants Survived	Percentage of Survival (%)
In small plastic tray	Asterix	369	66.50	18
	Cardinal	725	152.25	21
	Dimant	847	127.05	15
Sub total		1941	345.80	17.81
In natural field condition under netting	Asterix	66.0	19.8	30
	Cardinal	150.0	49.5	33
	Dimant	125	53.75	43
Sub total		331	123	37.16%

Days to root initiation and number of roots per plantlet are presented in the Table 4. It was observed that the maximum number of days (19.2) was required for root initiation in the treatment MS+4.0 mg/L of MMS Cardinal. Minimum days (6.5) to root initiation was noticed in the treatment MS+1.0 mg/L MMS in the variety Asterix. The highest number (18.9) of roots was found in the treatment MS+1.0 mg/L of EMS in Diamant at 45 days and the lowest (4.2) was in the treatment MS+2.0mg/L BU in Asterix on 45 days after culture of plantlet. *In vitro* mutagenesis and somaclonal variations were studied by Kumar *et al*. (2010). They used physical mutagen (gamma rays) and chemical mutagens *viz*. ethyl methane sulphonate (EMS) and Methyl methane sulphonate (MMS) to induce salt tolerance in a commercial citrus rootstock. The chemical mutagenesis was carried out on 45 and 60 Doc with EMS and MMS treatments at the concentrations of 0.1, 0.2, 0.3 and 10.4%. Results revealed that 0.1% chemical mutagen was the most suitable dose for 45 Doc, whereas 60 Doc did not regenerate after mutagen treatment. The results of the present investigation are similar to those reported by Kumar *et al*. (2010). In addition to this, 4.0 mg/L of EMS, MMS or BU treated cultures died within 45 days after inculcation in the present study.

3.3. Acclimatization efficiency of regenerated variants

Rate of survival of regenerated variants after transplantation are presented in Table 5. Acclimatization efficiency of the regenerated variants was recorded under natural field condition. In the first step, the plantlets were sown in plastic tray for hardening of the culture. A sub total of 1941 variants were transferred to the plastic trays. On an average, only 17.81% of plantlets were able to survive under primary establishment in plastic tray. The well developed variants were transfer to main field and a total of 331 well established variants were sown in the main field. It was noticed that 37.16 % of the variants were able to survive in field condition (Fig. 4).

3.4. Agronomic traits and yields of promising somaclones

Individual care was taken for each of the survived variant. All management practices were done for good crop production. It was observed that most of the variants showed vary poor agronomic performance compared to the check varieties. However, only 19 promising variants were selected for further study. The morphology and yield contributing data of those genotypes are given in Table 6. In respect of plant height and leaf per plant all the somaclonal variant showed lower performance than the check variety. Only three variants *viz.*, SVP 9, SVP 53 and SVP 68 gave larger number of tubers per plant and average weight of tubers. The maximum weight (45.0 g) of mini tubers was found in the check variety Asterix, which was followed by SVP-53 (43.20 g) and check variety Cardinal (40.5). Number of tubers per plant was the highest (21.0) in Cardinal which was followed by Check-2 *viz*. Cardinal (20.0), SVP53 (19.2), SVP-9 (18.0) and SVP 68 (15.20). SVP-53 showed higher yield per plant than the two check varieties, Diamant and Asterix.

Table 6. Major agronomic traits and yield of some promising somaclones under field condition

Sl. No.	Name of Variants	Plant height (cm)	No. of leaves per plant	Stem colour	Morphology and yield contributing traits			Tuber colour	Tuber size
					No. of tubers per plant	Average weight of tuber (g)	Yield per plant (kg)		
1.	SVP-02	29.00	12.0	Green	8.80	10.78	0.09	off white	Medium
2.	SVP-9	37.19	17.0	Light red	**18.00**	12.96	0.21	red	Small
3.	SVP-14	22.18	12.0	Green	8.63	4.94	0.04	Brown	Small
4.	SVP-18	34.74	13.0	Green	9.87	4.50	0.04	off white	Small
5.	SVP-20	22.97	11.0	Light green	12.47	7.83	0.09	off white	Small
6.	SVP-21	41.00	14.0	Green	9.54	3.90	0.03	Brown	Small
7.	SVP-22	23.45	7.0	Green	10.79	5.02	0.05	Brown	Small
8.	SVP-25	32.15	14.0	Green	7.0	2.90	0.02	Brown	Small
9.	SVP-33	20.17	11.0	Green	10.50	5.42	0.05	Brown	Medium
10.	SVP-46	30.00	12.0	Green	10.33	8.34	0.08	Brown	Medium
11.	SVP-53	32.00	11.0	Green	**19.20**	**43.20**	**0.83**	Brown	Medium
12.	SVP-56	32.63	11.0	Green	9.38	5.83	0.05	Brown	Medium
13.	SVP-59	28.93	13.0	Light green	8.44	8.17	0.06	Brown	Small
14.	SVP-68	27.00	9.0	Green	**15.12**	24.18	0.36	Brown	Small
15.	SVP-74	28.35	12.0	Red	9.10	6.21	0.05	Red	Small
16.	SVP-77	19.18	9.0	Light green	10.10	7.36	0.07	Brown	Medium
17.	SVP-86	27.03	11.0	Green	11.85	11.59	0.02	Brown	Medium
18.	SVP-88	35.29	15.0	Green	16.48	8.36	0.41	Brown	Small
19.	SVP-92	24.72	13.0	Red	8.00	3.33	0.02	Red	Small
21.	Cardinal (Ch-1)	52.15	32.0	Green	**21.00**	40.5	**0.85**	Red	Big
22.	Diamant (Ch-2)	49.80	29.00	Green	20.10	35.90	0.72	Brown	Big
23.	Asterix (Ch-3)	57.41	35.00	Light redish	14.5	**45.0**	0.65	Red	Big

4. Conclusions

Somaclonal variation is an enabling technology which can be used in potato improvement. The mutagenic chemicals EMS, MMS, BU and 2, 4-D were used at three concentrations (1.0, 2.0 and 4.0 mg/L) to create somaclonal variations in potato. All the mutagens had negative effect on tissue culture in potato. Cases of death occurred in higher dose. Abnormal shoot development and less leaf formation was observed in the evaluated materials. Survival rates of newly created somaclones were only 17.18% at plastic tray and 37.16% at field condition. One hundred and twenty three somaclonal variants were evaluated under field condition with three check varieties in respect of agronomic traits and yield potentiality. Only one SVP 53 gave higher yield than the two check varieties. Hence, it can be evaluated in subsequent generation for varietal development of potato.

5. Acknowledgement

The authors express their gratefulness and thanks to Bangladesh Academy of Sciences (BAS) and USDA for financial assistance to conduct the research work.

References

BBS, 2012. Statistical Year Book. Bangladesh Bureau of Statistics, Ministry of Planning. The People's Republic of Bangladesh, Dhaka.

Larkin, P. J. and Scowcroft, W. P. 1981. Somaclonal variation- a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics*, 60: 197-214.

Karp, A. 1995. Somaclonal variation as a tool for crop improvement. *Euphytica*, 85: 295-302.

Ehsanpour, A. A. Madani, S. and Hoseini, M. 2007. Detection of somaclonal variation in potato callus induced by UV-C radiation using RAPD-PCR. *General and Applied Plant Physiology*, 33 (1-2): 3-11.

Rosenberg, V., Tsahkna, A., Kotkas, K., Tahtgarv, T., Sarekanno, M. and Liiv, K. 2010. Somaclonal variation in potato meristem culture and possiblity to use this phenomenon in seed potato production and breedng. *Agronomy Research* (Special Issue III), 697-704 pp.

Kumar, K., Gill, M. I. S., Kaur, H., Choudhary, O. P. and Gosal, S. S. 2010. *In vitro* mutagenesis and somaclonal variation assisted salt tolerance in Rough lemon (*Citrus jambhiri* Lush.) *European Journal of Horticultural Science*, 75(6): 233-238.

Patricia, N. B., Silva, D. H., Maria, J., Cruz, C. D. and Fontes, E. P. 2004. Somaclonal variation on *in vitro* callus culture potato cultivar. *Horticultura Brasileira*. 22 (2). http://dx.doi.org/10.1590/S0102-05362004000200027.

Murashige, T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473-497.