Plant Trends

ORIGINAL ARTICLE

Plant Trends. 2025 Sep; 3(3): 41-52
eISSN: 3006-5658, https://doi.org/10.5455/pt.2025.05
Published by www.bsmiab.org

Chemical mutagenesis-derived potential somaclonal variations improve genetic diversity and yield in potato (*Solanum tuberosum* L.)

Md. Omor Faruk[1], Md. Robiul Auwal[1], Lamia Bintay Masud[1], Tariqul Islam[1], Ashraful Islam Rabbee[2], Md. Sarafat Ali[1,*]

[1]Department of Biotechnology and Genetic Engineering, Gopalganj Science and Technology University, Gopalganj 8100, Bangladesh
[2]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh

***Corresponding author**
Md. Sarafat Ali
Department of Biotechnology and Genetic Engineering, Gopalganj Science and Technology University, Gopalganj 8100, Bangladesh
Email: sarafatbiotech@gstu.edu.com

Academic editor
Monirul Islam, PhD
University of Massachusetts Amherst Amherst, USA

Article info
Received: 27 June 2025
Accepted: 21 September 2025
Published: 30 September 2025

Keywords
Crop improvement, Genetic variability, Potato trait, Regeneration frequency

ABSTRACT

Somaclonal variation through mutagenesis is an efficient technique for enhancing genetic variability and agricultural traits in clonally grown plant species. The aim of the study was to evaluate chemical mutagens, including methyl methane sulphonate (MMS), 5-Bromo Uracil (BU), and ethyl methane sulphonates (EMS)-derived potential somaclonal variations, and their impact on genetic diversity and yield in potato (*Solanum tuberosum* L.) cultivars Cardinal, Diamant, and Asterix. In this study, auxin (2,4-D) showed efficiency in effective callus regeneration. A concentration of 4.0 ppm 2,4-D resulted in callus formation of distance cultivars, which was also involved in distinct plantlet formation. Besides somaclonal variations, the varying concentrations of EMS, MMS, and BU showed significant abnormalities during *in vitro* plant regeneration, particularly at 3.0 ppm. The regrafted seedlings of three potato cultivars were acclimated in soil conditions. The results showed that plantlets exposed to high concentrations of mutagens showed severe abnormalities in potato plants. The plantlets exposed to 2.0 and 3.0 ppm of MMS, EMS, and BU exhibited thinning shoots and a poor growth rate. However, the Diamant cultivar exhibited more notable results compared to the other checked cultivars. The Diamant cultivar showed the highest survival rate (47.8%), morphological stability compared to Cardinal and Asterix. This insight helps to select an *in vitro* strong cultivar for further study in field conditions. Conversely, SVP 71, 91, 117, and 83 variants showed better tuber yield compared to other potato cultivars. The study uncovers that chemical mutagenesis-derived potential somaclonal variations would be a potential approach for improving genetic variation and yield in potato. These findings together might be useful to potato breeders or farmers for improving plants through a breeding program.

INTRODUCTION

The staple food crop, the potato, is the fourth most produced commodity worldwide after rice, wheat, and maize. Potato (*Solanum tuberosum L.*) is one of the vital vegetable crops in Bangladesh, utilized for both food and industrial purposes [1]. Approximately 5.21 million hectares of potatoes have been grown, which produce a yield of 20.443 metric tons per hectare in Bangladesh. As a vegetatively propagated crop, potatoes are highly susceptible to the accumulation of pathogens and genetic uniformity, which limits their adaptability to multiple environmental stresses [2]. Thus, production of disease-free or resistant, starch-enriched, and high-yielding cultivars is highly desirable for sustainable food security.

Traditional breeding methods are challenging due to the autotetraploid DNA, poor flowering, and low fertility, making genetic improvement via hybridization ineffective. Traditional breeding methods for potato improvement are often time-consuming and constrained by the autotetraploid genome and low fertility of the potato. Sustainable

Copyright: © by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution 4.0 (CC BY 4.0) International license.

farming in potato cultivation requires the urgent development of disease-resistant, starch-enriched potato varieties with high-yielding resistance characteristics. Crop improvement programs heavily depend on genetic diversity because they enable breeders to develop cultivars that yield better amounts in challenging environmental situations. Throughout the country, potato is being cultivated with a limited number of potato varieties, that reduces genetic variability [3, 4]. Tomato variety limits the creation of genetic output, and explores the variability through standard hybridization techniques because it showed poor flowering performance and vegetative reproduction [5]. Improvement of tissue culture tools, the potato would be a promising crop material for genetic variability study and yield improvement. Somaclonal variation, known as genetic modifications that occur within cells and tissues during the *in vitro* plant cultivation process [6, 7]. Plant breeders utilize this method to obtain assistance in creating diverse crop varieties that contribute to improving selection. This method has been adopted in numerous new plant varieties, including those of sugarcane and mustard rice, as well as Apium and other crops [8]. Various potato variants exhibit distinct agronomic characteristics, as well as stress responses. The red-skinned Cardinal cultivar stands out due to its desirable tubers and high dry matter content, making it a preferred choice for both table and processing purposes. Asterix shows its multiple qualities, including unique tuber shape, plant fitness, and disease tolerance, that ensure its acceptability as food and for commercial purposes. The Diamant is cultivated mostly in Asian and African farming regions [9]. Although several traits need to be improved in these cultivar prospects in Bangladesh.

The identification process encompasses factors that influence variation in both the occurrence and quantity of these events. Any variation in genetic composition, characteristics of the mutagenic agent, and explant type influences the results. The polyploid genomic structure of this species enables researchers to study somaclonal variation methods as an improvement technique. During this experiment, scientists used various chemical mutagens to generate somaclonal variation, creating valuable potato strains. A previous study with a completely Randomized Design (CRD) provides the experimental layout to study two factors (varieties and treatments) with three replications [10].

The importance of the study is to explore the limitations that can be overcome by producing disease-resistant, high-yielding, and stress-resistant potato hybrids. These traits are useful to enhance food security and sustainable plant production. The research addresses the gap because it relies on the chemical mutagenesis and tissue culture methods to generate somaclonal variation in three potato cultivars: Cardinal, Asterix, and Diamant. The cultivar selection was completed according to their distinct agronomic attributes, which included their content of dry matter, shape of the tuber, and resistance to disease. The aim of this study was to explore somaclonal variations and other morphological, physiological, and agronomic properties, thereby increasing genetic diversity and facilitating the production of disease resistance, vigour sustainable potato production.

MATERIALS AND METHODS

Plant materials and explant preparation

Three potato cultivars, Cardinal, Asterix, and Diamant, were obtained from the Tuber Crop Research Centre (TCRC) at the Bangladesh Agricultural Research Institute (BARI) in Gazipur. Fresh potato sprouts were used as the explant source. Surface sterilization of

explants was conducted by washing with a steady flow of fresh tap water, soaking in 70% ethanol, and dipping in de-ionized water before plunging them into 0.1% $HgCl_2$.

Callus induction and *in vitro* regeneration

All studies were set with a completely randomized design (CRD), where at least three individual replications were performed. Sterilized explants were cultured with the Murashige and Skoog (MS) medium (Sigma-Aldrich, St. Louis, USA). Three mutagenic chemicals such as ethyl methane sulphonate (EMS, HiMedia, Mumbai, India), methyl methane sulphonate (MMS, Sigma-Aldrich, St. Louis, USA), 5-bromo uracil (BU, Merck, Darmstadt, Germany), and 2,4-dichlorophenoxyacetic acid (2,4-D, Sigma-Aldrich, St. Louis, USA) were added to plant culture media at varying concentrations (1.0, 2.0, and 3.0 ppm)[11]. *In vitro* regeneration of somaclonal variants was cultured with MS medium supplemented with three different concentrations of chemical mutagens. Experimental works were conducted in laminar airflow control environments to prevent contamination. A 16/8 h light and dark period was maintained under 2,500-3,000 lux of light intensity, and culture room temperature 24±1 °C. Phenotypic variations were observed among the regenerated plantlets, suggesting successful induction of genetic variability, which may serve as a potential source for trait enhancement and further molecular characterization in mutation breeding programs.

E*x-vitro* acclimatization and field-based phenotypic profiling

After mutagen treatment, the somaclonal variants were transplanted for field-based cultivation. The field-based cultivation took place after the mutagen treatment of somaclonal variants. The regenerated somaclonal variants were initially cultured in trays under controlled conditions to monitor early growth responses. This setup enabled the precise assessment of phenotypic deviations during the initial stages of development within 15 days. Mosquito nets covered the experimental materials to protect them from insect-borne viral infection. Our team conducted intercultural tasks according to their requirements. Farming specialists carefully managed plant growth to produce small potato offspring from each SVP strain. We monitored plant metrics: height, stem shade, leaf hue, leaf form, snub count per plant, and tuber details.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the IBM SPSS program. Data means were compared using the least significant difference (LSD) at $p \leq 0.0$. Data were presented as mean values ± standard error (not shown).

RESULTS

Chemical mutagenesis and somaclonal variations

Three potato cultivars, Cardinal, Asterix, and Diamant, were selected as study material (Figure 1). Different concentrations (1, 2, and 3 PPM) of three mutagens, EMS, MMS, and 5-BU, were applied, respectively, to induce somaclonal variation in potato. The growth hormone 2,4-D showed effective callus formation from the sprout of the potato. An efficient approach for plant regeneration has been used on plants previously [12]. Callus-derived successful plant regeneration was shown in *in vitro* conditions.

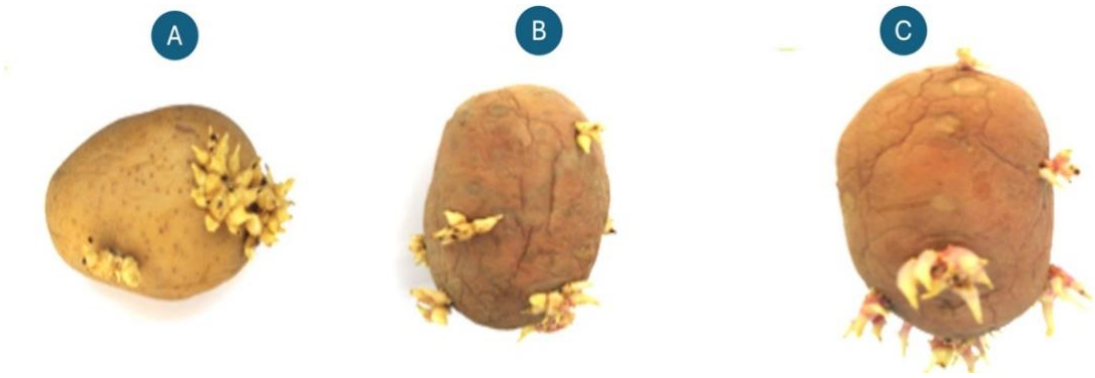

Figure 1. Three distinct potato cultivars were used in the study. A) The cultivar Cardinal; B) Cultivar Asterix; and C) Cultivar Diamant.

In vitro callogenesis in potato

Table 1 presents the parameters that describe *in vitro* plantlet regeneration, and Table 2 summarizes mutagen-induced both normal and abnormal shoot regeneration in three potato cultivars. The different varieties examined in this study. The combination of MS media with 3.0 ppm of 2, 4-D induced high calli formation in all studied genotypes. Following 10 days, Asterix showed shoot initiation using the treatment MS+1.0 ppm of 2,4-D. Cardinal, Diamant, and Asterix varieties displayed the largest callus formation with 2.2 cm, which developed 45 days after initiation (Figure 2). All three varieties displayed vigorous shoot initiation within MS+2.0 ppm 2,4-D media, as illustrated in Figure 3. Two five-day-old Petri dishes contained calli with equal dimensions. Cardinal and Asterix show a minimal distinction from each other regarding their final 45-day calli weights.

Table 2 presents shoot initiation, root initiation, and the results of normal and abnormal variant regeneration efficiency. The number of shoots was higher in plantlets across 30-day and 45-day measurements. At 45 days, the treatment MS+3.0 ppm 2, 4-D generated the highest number of 18.69 shots in the variety Diamant. The treatment combinations produced a maximum number of 12.47 roots per variant for Diamant. The plantlet root length directly aligns with the number of plantlets forming shoots.

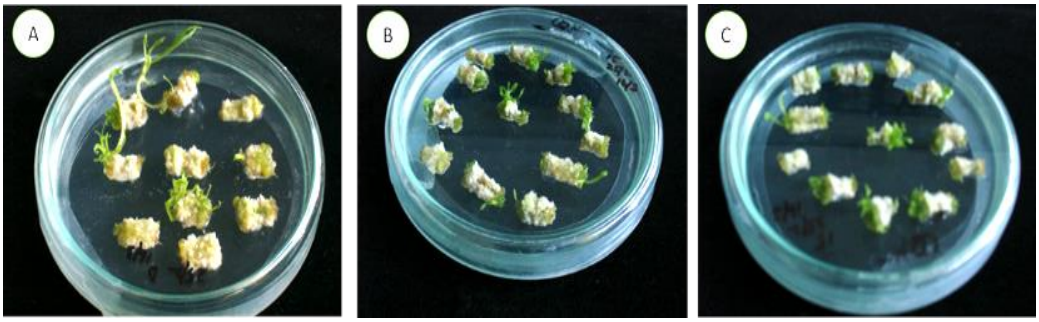

Figure 2. Potato sprout explant-derived rapid callus formation. Three peri-plates show contamination-free vigour callus of three different potato cultivars. A) *Solanum tuberosum* cv. Cardinal; B) *S. tuberosum* cv. Asterix, and C) *S. tuberosum* cv. Diamant.

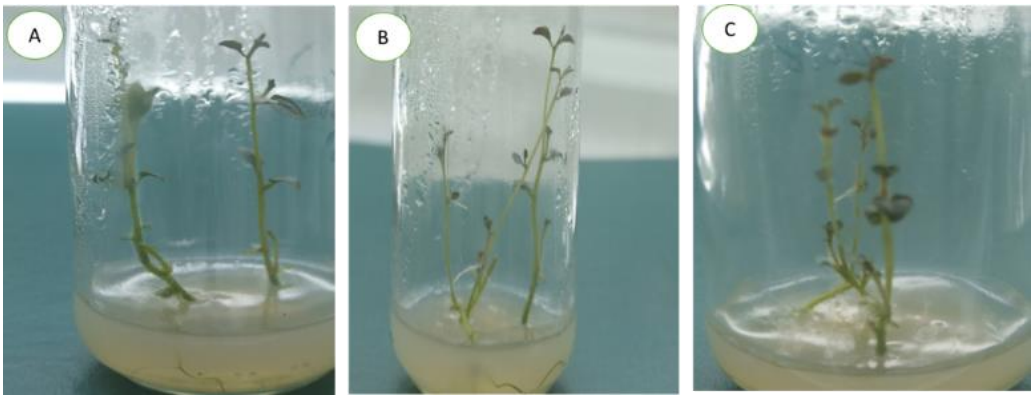

Figure 3. Callus tissue-derived *in vitro* plantlet formation of three distinct potato cultivars. A) Potato cultivar Cardinal; B) Potato cultivar Asterix; and C) Potato cultivar Diamant.

Table 1. Effects of different MS media on *in vitro* plantlet formation in different potato cultivars.

Variety	Treatments (ppm)	Shoot per plantlet			Root per plantlet		
		15 days	30 days	45 days	15 days	30 days	45 days
Cardinal	T1= Normal MS	1.1	1.6	2.8	2.45	1.97	5.3
	T2=MS+1	1.31	2.6	4.6	3.39	1.85	3.6
	T3=MS+2	1.5	3.1	8.6	5.04	2.12	4.1
	T4=MS+3	2.1	5.5	9.1	6.7	4.9	3.8
Asterix	T1= Normal MS	1.5	2.5	2.8	1.8	2	4
	T2=MS+1	2.6	2.95	3.5	2.78	1.99	4.2
	T3=MS+2	3.95	6.8	6.1	4.1	3.71	3.7
	T4=MS+3	2	9.5	9.8	3	6	5
Diamant	T1= Normal MS	2.81	2.9	3.9	2.9	2.23	6
	T2=MS+1	3.5	3.9	5.8	3.854	2.15	7.6
	T3=MS+2	3.5	9.2	13.1	5.3	3.85	9
	T4=MS+3	8.8	11.1	16	6.1	4.00	3.18

Table 2. Role of 2,4-D combined with mutagens on both normal and abnormal shoot regeneration in three potato cultivars.

Selected Variety	Treatments (ppm)	Days to shoot initiation	Number of shoots per plantlet (average)			Length of shoot per plantlet (cm)		
			15-days	30-days	45-days	15-days	30-days	45-days
Cardinal	T1= Simple MS	0	0	0	0	0	0	0
	T2=MS+1.0 EMS	3.05	1.19	3.35(AB)	4.29	1.17	3.92	10.05
	T3=MS+2.0 EMS	7.03	3.04	3.01	5.09	2.09	7.71	9.39
	T4=MS+3.0 EMS	8.08	2.09	3.37	2.99	1.80	5.13	8.19
	T5=MS+1.0 MMS	10.07	2.31	4.83	5.6	2.10	7	11.46
	T6=MS+2.0 MMS	8	1.89	3.95 (AB)	0	2.05	5.93	4.08
	T7=MS+3.0 MMS	9.09	2.01	6.5	13.2	2	7.01	12.21
	T8=MS+1.0 BU	9.06	1.01	5.07	4.91	2.08	7.29	10.59
	T9=MS+2.0 BU	10.04	2.19	5.93	0	1.90	8.12	9.41
	T10=MS+3.0 BU	7.23	2.34	2.99	0	2.10	7.29	6.89
Asterix	T1= Simple MS	0	0	0	0	0	0	0
	T2=MS+1.0 EMS	7.05	2.07	3.19	4.60	1.70	5.95	9.07
	T3=MS+2.0 EMS	6.25	2.31(AB)	3.12	9.69	2.08	4.35	9.61
	T4=MS+3.0 EMS	9.01	2.03	7.35(AB)	14.78	1.81	4.59	9.37
	T5=MS+1.0 MMS	8.02	2.06	4.28	4.91	1.60	7.51	11.97
	T6=MS+2.0 MMS	9.03	1.07	3.25	15	2.10	7.05	12
	T7=MS+3.0 MMS	10.14(AB)	1.08	13.06	14.8	3.10	6.37	10.57
	T8=MS+1.0 BU	11.01	1.27	3.92	4.19	1.70	5.22	11.02
	T9=MS+2.0 BU	12	1	12.80	14.4	1.08	5.21	5.32
	T10=MS+3.0 BU	13	2.13	11.01	5.09	1.39	5.36	8.45

Diamant	T1= Simple MS	0	0	0	0	0	0	0
	T2=MS+1.0 EMS	6.19	1.49(AB)	2.45	3.85	2.13	7.03	10.15
	T3=MS+2.0 EMS	8.32	3.08	11.39	4.39	2.1	6.11	12.47
	T4=MS+4.0 EMS	10.37	2.19	2.63	3.5	2.7	6.2	9.17
	T5=MS+1.0 MMS	8.37	1.31	2.29	5.39	1.7	5.9	9.73
	T6=MS+2.0 MMS	9.05	1.09	5.26	8	2.05	6.3	11.52
	T7=MS+3.0 MMS	9.29	3.01	13.02	18.69	3.01	4.7	8.99
	T8=MS+1.0 BU	7.09	2.39	5.61	8.03	1.3	4.8	8.09
	T9=MS+2.0 BU	8	3.17	6.84(AB)	10	1.9	3.49	10.2
	T10=MS+4.0 BU	9.51	2	5.91	7.89	2	5.12	7.73

T, treatment; MS, Murashige and Skoog medium; EMS, Ethyl Methanesulfonate; MMS, Methyl Methanesulfonate; BU, 5-Bromouracil; AB= Abnormal shoot

Ehsanpour *et al.* induced *in vitro* callus formations from potato leaf segments on media bases containing MS solution plus NAA, Kinetin, yeast extract, and 2, 4-D [13]**.** The study suggests a change in DNA patterns as the source of genetic variation. They suggest that the variants create opportunities to identify potato calli with improved characteristics, including tolerance to salt and drought stress. The occurrence of somaclonal variation in potato meristem cultures was reported, whereas it showed differences among meristem clones based on yield, tuber induction, and tolerance to late-blight disease [14]. However, this current study showed coherence with that study. In a previous study, a combination of picloram at 1.65 mM and 2,4-D at 11.5 mM induced somaclonal variation in potato plants, according to Patricia *et al.* [15].

Impact of chemical mutagen on *in vitro* regeneration efficiency in potato

Chemical mutagen effects on root initiation in different potato cultivars (Table 3). The explants that received mutagens resulted in direct shoot formation. Higher concentrations of mutagens led to toxic effects and delayed shooting initiation in all varieties. The newly developed plant forms exhibited mortality before the 15-day cultivation period was completed. The mutagenic effect of BU proved to be the most destructive on potato plantlets. The effects of single treatments appear in the information below. It took a maximum of 13.0 days for shoot initiation in the treatment that used the Cardinal variety with MS media combined with 3.0 ppm of BU. The combination of medium and EMS dose (1.0 ppm) in Diamant produced shoot formation at the shortest time (4.5 days). Asterix plants grown in MS+ 3.0 ppm MMS solution at 45 days showed the highest shoot number of 19.6. Higher doses of mutagen treatment led to the formation of numerous branches and fragile stems, indicating the creation of abnormal variants (Figure 4A-C). The longest plantlet shoots (13.06cm) appeared during the evaluation of MS+3.0 MMS in Asterix. When evaluating Cardinal cultivars, the shortest shoots (1.01 cm) measurement occurred in treatment MS+1.0 ppm BU.

The development of abnormal shoots occurred in selected treatments, although they subsequently ceased to exist. The application of MS media combined with 3.0 and 2.0 ppm of EMS MMS and BU had a negative impact on shoot development, as shown in Table 4. Under the above conditions, experimental materials either died in 30 or 45 days of culture, which showed that chemical mutagens (EMS, MMS, and BU) have mutagenic toxicity on potato genotypes.

Table 3. 2,4-D combined with mutagens-induced root initiation in potato cultivars.

Variety	Treatments (ppm)	Days to root initiation (average)	Number of roots per plantlet (average)		
			15 days	30 days	45 days
Cardinal	T1= Normal MS	5.19	0	5.63	7.2
	T2=MS+1.0 EMS	7.25	2.29	7.35	18.9
	T3=MS+2.0 EMS	8.09	4.34	7.46	10.9
	T4=MS+3.0 EMS	11.03	6.28	0	0
	T5=MS+1.0 MMS	7.29	0	6.55	8.3
	T6=MS+2.0 MMS	10.03	1.27	7.21	0
	T7=MS+3.0 MMS	7.19	3.32	7.62	10.20
	T8=MS+1.0 BU	11.95	0	5.35	7.06
	T9=MS+2.0 BU	11.19	2.65	0	5.39
	T10=MS+3.0 BU	7.09	0	7.15	0
Asterix	T_1= Normal MS	10.01	2.45	3.08	7.29
	T2=MS+1.0 EMS	8.06	2.81	6.19	8.43
	T3=MS+2.0 EMS	9.71	1.95	5.97	9.45
	T4=MS+3.0 EMS	10.05	2.25	7.95	10.86
	T5=MS+1.0 MMS	12.06	1.71	5.72	9.91
	T6=MS+2.0 MMS	14.02	2.39	9.75	8.83
	T7=MS+3.0 MMS	17.02	3.51	7.65	10.78
	T8=MS+1.0 BU	16.06	2.65	5.82	9.72
	T9=MS+2.0 BU	10.01	3.91	6.85	8.57
	T10=MS+3.0 BU	11.09	0	0	0
Diamant	T1= Normal MS	13	2.29	3.75	5.65
	T2=MS+1.0 EMS	9.10	2.08	3.53	7.88
	T3=MS+2.0 EMS	12.1	2.11	5.51	8.23
	T4=MS+3.0 EMS	10.5	2.81	6.36	0
	T5=MS+1.0 MMS	6.5	1.13	4.47	6.41
	T_6=MS+2.0 MMS	8.7	3.32	5.75	7.51
	T7=MS+3.0 MMS	9.1	4.18	5.03	8.09
	T8=MS+1.0 BU	13.2	2.06	5.53	7.21
	T9=MS+2.0 BU	14.8	2.93	4.34	8.04
	T10=MS+3.0 BU	17.2	3.17	0	4.72

T, treatment; MS, Murashige and Skoog medium with 2,4-D; EMS, Ethyl Methanesulfonate; MMS, Methyl Methanesulfonate; BU, 5-Bromouracil

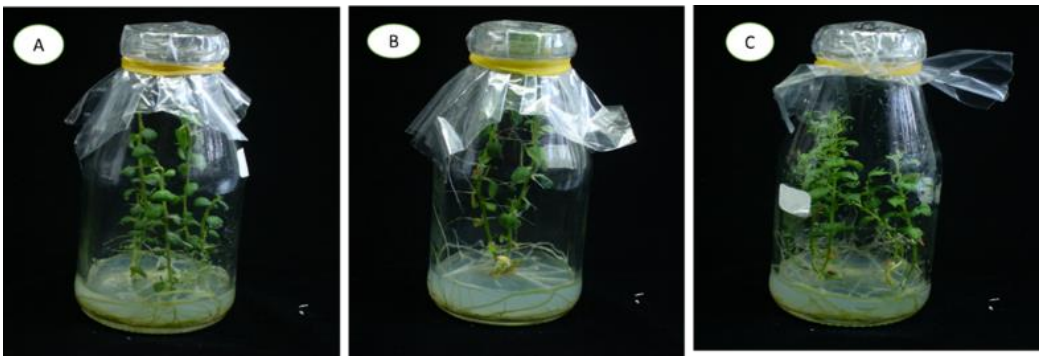

Figure 4. Responses of shoot generation in three different potato cultivars in response to distinct chemical mutagens. A) EMS-induced shoot development in the potato Cardinal cultivar; B) MMS-induced new type of shoot generation in the potato Asterix cultivar; C) BU-induced viable growth pattern and shoot development in the potato Diamant cultivar.

Table 4. Mutagen-induced variation in root initiation of potato explants in different time intervals.

Variety	Treatments (PPM)	Days to root initiation (average)	Number of roots per plantlet (average)		
			15 days	30 days	45 days
Cardinal	T1= Simple MS	9.13	2.23	5.11	7.32
	T2=MS+1.0 EMS	10.5	2.93	7.35	17.49
	T3=MS+2.0 EMS	11.02	4.23	7.36	9.94
	T4=MS+3.0 EMS	12.13	4.18	0	0
	T5=MS+1.0 MMS	10.52	0	6.35	8.43
	T6=MS+2.0 MMS	7.91	7.17	0	0
	T7=MS+3.0 MMS	7.19	3.29	7.42	10.22
	T8=MS+1.0 BU	11.75	0	5.25	7.36
	T9=MS+2.0 BU	10.91	3.35	0	0
	T10=MS+3.0 BU	8.09	0	0	0
Asterix	T_1= Normal MS	10.71	2.45	4.78	6.79
	T2=MS+1.0 EMS	9.56	2.61	5.61	8.13
	T3=MS+2.0 EMS	8.78	3.75	7.67	12.45
	T4=MS+3.0 EMS	9.56	1.85	6.65	9.65
	T5=MS+1.0 MMS	13.68	4.01	5.62	8.91
	T6=MS+2.0 MMS	16.62	3.69	6.65	10.53
	T7=MS+3.0 MMS	15.69	5.11	6.35	10.87
	T8=MS+1.0 BU	17.62	1.55	4.22	7.29
	T9=MS+2.0 BU	9.18	2.10	4.95	9.23
	T10=MS+3.0 BU	10.93	0	0	0
Diamant	T1= Normal MS	12.21	1.87	3.75	5.85
	T2=MS+1.0 EMS	13.21	1.82	3.63	7.58
	T3=MS+2.0 EMS	11.18	3.23	5.51	8.42
	T4=MS+3.0 EMS	13.54	2.43	6.46	0
	T5=MS+1.0 MMS	7.25	1.78	4.37	6.61
	T_6=MS+2.0 MMS	9.27	3.69	5.37	7.91
	T7=MS+3.0 MMS	9.19	4.73	5.12	8.53
	T8=MS+1.0 BU	12.27	2.57	5.32	7.62
	T9=MS+2.0 BU	13.89	2.39	4.14	8.21
	T10=MS+3.0 BU	16.69	3.31	0	4.29

T, treatment; MS, Murashige and Skoog normal medium; EMS, Ethyl Methanesulfonate; MMS, Methyl Methanesulfonate; BU, 5-Bromouracil.

Post-regeneration acclimatization efficiency of somaclonal lines

Survival percentage (%) of transplants of potato variants is shown in Table 5. We monitored the acclimatization success rate of the variants at their regular outdoor field locations. We transferred 1625 variants into the plastic trays for hardening purposes. The mean survival rate (%) of plantlets during their initial phase in plastic trays was 18.37%, but cardinal showed the highest rate at 19.5% (Table 5). The cultivated field received the transferred well-developed variants, while the main field received 438 well-established variants for sowing. The research revealed that the highest survival rate in field conditions was achieved by Diamant at 47.80%, with an average of 34.57% of variants surviving through field conditions (Figure 5).

Table 5. Post-transplantation survival rates of regenerated plantlets in potato.

Variety Name	Acclimatization	Number of Transplanted Plantlets	Number of Survived Plantlets (average)	Percentage (%) of Survival
Cardinal	In a Small Plastic Tray	821.00	160.15	19.50%
Cardinal	Under netting	221.00	53.15	24.04%
Asterix	In a Small Plastic Tray	411.00	79.13	19.25%
Asterix	Under netting	76.00	24.23	31.88%
Diamant	In a Small Plastic Tray	923.00	151.18	16.37%
Diamant	Under netting	141.00	67.41	47.80%

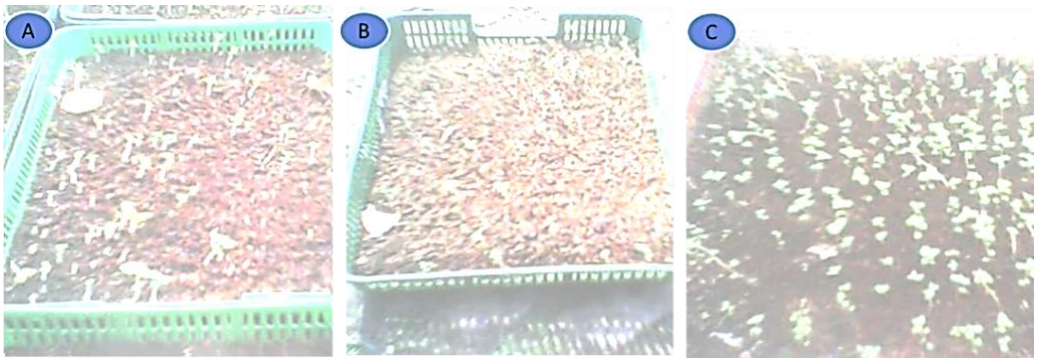

Figure 5. Acclimatization of somaclonal potato variants to soil conditions**.** Growing newly generated potato seedlings in a plastic tray containing nursery soil medium. A) Potato cultivar Cardinal; B) Potato cultivar Asterix; and C) Potato cultivar Diamant.

Agronomic characterization and yield assessment of promising potato clones

A specific approach was used to care for each remaining variant. Good crop production remained the guiding principle in conducting all management procedures. The agronomic performance of all variants was inferior, while SVP21 was promising. A total of 21 promising variants were picked for advanced examination among the surviving candidates. Table 6 displays phenotype characteristics and yield-related data of the selected potato genotypes. Each somaclonal variant had inferior outcomes in terms of leaf numbers and plant height compared to the check variety. SVP 71, SVP 91, SVP 117, and SVP 83 produced the highest number of tubers per plant and the highest average weight compared to other variants. Check variety Diamant had the highest mini tuber weight of 45.57g, then Asterix 39.13 g, and Cardinal check variety 35.93 g. The maximum potential of plant tubers was 21.0 in Asterix, while Check-2, viz. Diamant Check-3 (20.0) and Cardinal Check-1 (15.0) followed alongside SVP-83 (15.0), SVP 117 (14.0), and SVP 71,91 (13.0). The yield results from check variety Diamant (Di-Ch-3) exceeded both check varieties, including Cardinal and Asterix, regarding tuber count per plant.

Table 6. Field evaluation of key agronomic traits and yield performance of selected potato variants.

Name of Variants	Plant height (cm)	Number of leaves per plant	Stem colour	Number of tubers per plant	Average weight of tubers (gm)	Yield per plant (kg)	Tuber colour	Tuber size
SVP-2	29.4300	13.0000	Light Green (Li)	8.00	10.8000	0.0500	Brown	Small
SVP-05	32.1300	11.0000	Light Green (Li)	11.00	11.0300	0.0900	Red	Medium
SVP-10	34.3900	17.0000	Green (Gr)	7.00	13.0600	0.0400	Brown	Small
SVP-21	29.9700	19.0000	Light Green (Li)	12.00	9.1700	0.4700	Brown	Medium
SVP-29	41.3200	12.0000	Light Green (Li)	11.00	13.0800	0.0800	Brown	Small
SVP-54	43.2900	16.0000	Red (Re)	8.00	11.0500	0.0800	Brown	Small
SVP-69	37.7900	9.0000	Light Green (Li)	9.00	9.9100	0.2100	Off White	Medium
SVP-71	47.1200	10.0000	Green (Gr)	13.00	20.0800	0.0300	Brown	Small
SVP-83	39.3500	7.0000	Green (Gr)	15.00	15.0900	0.0900	Off White	Medium
SVP-91	50.0900	11.0000	Light Green (Li)	13.00	9.8300	0.0800	Red	Small
SVP-99	47.3900	10.0000	Green (Gr)	10.00	15.0100	0.4300	Off White	Medium
SVP-113	39.3300	9.0000	Light Green (Li)	12.00	17.6300	0.0700	Brown	Small
SVP-117	43.8900	13.0000	Purple (Pu)	14.00	10.2900	0.0200	Brown	Small
SVP-128	49.1900	15.0000	Light Green (Li)	11.00	16.5900	0.0400	Brown	Small
SVP-152	52.2100	13.0000	Green (Gr)	9.00	11.2300	0.0600	Off White	Medium
SVP-167	49.6800	17.0000	Red (Re)	12.00	13.3900	0.1100	Red	Medium
SVP-171	39.9800	11.0000	Light Green (Li)	8.00	10.9100	0.0900	Red	Small
Ca_Ch-1	53.1900	30.0000	Light Green (Li)	15.00	35.9300	0.6300	Brown	Big
As_Ch-2	51.3900	33.0000	Green (Gr)	21.00	39.1300	0.7900	Brown	Big
Di-Ch-3	59.3100	39.0000	Green (Gr)	20.00	45.5700	0.8300	Brown	Big

DISCUSSION

This study explores the potential action of chemical mutagens—EMS, MMS, 5-BU, and 2,4-D—to trigger somaclonal variation in three potato cultivars: Cardinal, Asterix, and Diamant. Among the tested mutagens, only 2,4-D consistently generated callus formation and successful regeneration in all cultivars. This confirms its efficiency in the in vitro dedifferentiation-promoting and development of plantlets [16]. Conversely, higher concentrations of EMS, MMS, and 5-BU led to stunted shoots, malformed leaves, fragile stems, delayed root initiation, and, in some cases, plantlet mortality. Outcomes of this research are consistent with previous reports showing that auxin-type compounds, such as 2,4-D, enhance callogenesis [17]. Whereas alkylating agents or base analogues can be cytotoxic at high doses [18]. This limits their practical application for somaclonal variation.

Quantitative studies confirmed the significant mutagen-induced phenotypic changes. Paired sample t-tests and MOD_1 standardized trait assessment presented notable changes in growth and yield-related traits. Tukey's fractional rank estimation highlighted correlated responses among plant height, shoot number, and tuber characteristics. This indicates that the effects of mutagenic may act on linked genetic loci or regulatory pathways [19, 20]. These statistical validations aid in the identification of true genetic or phenotypic variation. As opposed to random experimental variation, consistent with prior studies present that somaclonal variation can affect multiple linked traits simultaneously [21, 22]. Post-regeneration acclimatization and field evaluation further emphasized cultivar-specific responses. Diamant displayed the highest survival (47.8%) with morphological stability, while other cultivars showed lower survival rates. Although most somaclonal variants had lesser vegetative growth compared to check varieties, selected variants such as SVP 71, SVP 91, SVP 117, and SVP 83 presented superior tuber yield, number, and weight. This shows that when somaclonal variation is carefully screened, it can generate traits with agronomically advantageous [23, 24], despite occasional negative effects on vegetative growth. Overall, these results demonstrate that controlled *in vitro* mutagenesis using 2,4-D, combined with systematic

selection, is an effective strategy for potato variants that increase yield and stress tolerance. This insight might be useful for future potato breeding programs.

CONCLUSIONS

This study uncovers a potential tool related to chemical mutagenesis-derived somaclonal variations that enhance genetic diversity and yield in potato. Three mutagenic chemicals, including EMS, MMS, and BU, respectively, combined with 2,4-D led to induced somaclonal variations. The potato tissue cells suffered from high doses of mutagenic substances, suggesting that an optimum dose of mutagens is effective in somaclonal variation. The study further suggests that somaclonal variants, such as SVP 71, SVP 91, SVP 117, and SVP 83, represent a higher yield of tuber and slightly lower growth of the vegetative part. However, the cultivar Diamant showed an opposite result, that is morphologically stable and able to survive in field conditions. The material can serve as a potential candidate for selection in future generations of potato's varietal improvement. These results also suggest that controlling *in vitro* mutagenesis with 2,4-D-assisted could be a successful technique for developing agronomically valuable potato varieties, as well as improving potato traits through breeding programs.

ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission (UGC), Bangladesh, for providing support related to resources and expenses of this study. We would like to thank the authority of biotechnology and tissue culture laboratories of Gopalganj Science and Technology University for their technical support.

AUTHOR CONTRIBUTIONS

MOF and MSA: conceptualization and experimental design; ARA and LBM: data curation and visualization; TI and AIR: performed draft editing and modification; MOF: wrote the initial draft; MSA: reviewing, editing, funding, and supervision of the whole project. All the authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1] Wijesinha-Bettoni R, Mouillé B. The contribution of potatoes to global food security, nutrition and healthy diets. American Journal of Potato Research. 2019;96:139-49.
[2] Abdullah-Al-Mahmud HM, Akhter S, et al. Screening of cip potato clones for salinity tolerance in pot and field condition. Advances in Plant and Agriculture Research. 2018;8:573-80.
[3] Jansky SH, Spooner DM. The evolution of potato breeding. Plant breeding reviews2018. p. 169-214.
[4] Swarup S, Cargill EJ, et al. Genetic diversity is indispensable for plant breeding to improve crops. Crop Science. 2021;61:839-52.
[5] Krishna H, Alizadeh M, et al. Somaclonal variations and their applications in horticultural crops improvement. 3 Biotech. 2016;6:54.
[6] Karp A. Somaclonal variation as a tool for crop improvement. Euphytica. 1995;85:295-302.

[7] Bairu MW, Aremu AO, et al. Somaclonal variation in plants: Causes and detection methods. Plant Growth Regulation. 2011;63:147-73.
[8] Larkin PJ, Scowcroft WR. Somaclonal variation — a novel source of variability from cell cultures for plant improvement. Theoretical and Applied Genetics. 1981;60:197-214.
[9] Eaton TE, Azad AK, et al. Evaluation of six modern varieties of potatoes for yield, plant growth parameters and resistance to insects and diseases. Agricultural sciences. 2017;8:1315.
[10] Rodríguez NV, Kowalski B, et al. In vitro and ex vitro selection of potato plantlets for resistance to early blight. Journal of Phytopathology. 2007;155:582-6.
[11] Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 1962;15:473-97.
[12] Bhajan SK, Hasan MM, et al. An efficient approach of in vitro plant regeneration and propagation of mungbean [vigna radiata (l.) wilczek]. Plant Trends. 2024;2:46-56.
[13] Ehsanpour A, Madani S, et al. Detection of somaclonal variation in potato callus induced by uv-c radiation using rapd-pcr. Gen Appl Plant Physiol. 2007;33:3-11.
[14] Rosenberg V, Tsahkna A, et al. Somaclonal variation in potato meristem culture and possibility to use this phenomenon in seed potato production and breeding. Estonian Research Institute of Agriculture; 2010. p. 697–704.
[15] Bordallo PN, Silva DH, et al. Somaclonal variation on in vitro callus culture potato cultivars. Horticultura Brasileira. 2004;22:300-4.
[16] Phua QY, Chin CK, et al. The callugenic effects of 2, 4-dichlorophenoxy acetic acid (2, 4-d) on leaf explants of sabah snake grass (clinacanthus nutans). Pakistan Journal of Botany. 2016;48:561-6.
[17] Rajaram K, Moushmi M, et al. Comparative bioactive studies between wild plant and callus culture of tephrosia tinctoria pers. Appl Biochem Biotechnol. 2013;171:2105-20.
[18] Hoque M, Morshad M. Somaclonal variation in potato (solanum tuberosum l.) using chemical mutagens. The Agriculturists. 2014;12:15-25.
[19] Sikora P, Chawade A, et al. Mutagenesis as a tool in plant genetics, functional genomics, and breeding. Int J Plant Genomics. 2011;2011:314829.
[20] Xu L, Najeeb U, et al. In vitro mutagenesis and genetic improvement. In: Gupta SK, editor. Technological innovations in major world oil crops, volume 2: Perspectives. New York, NY: Springer New York; 2012. p. 151-73.
[21] Dorani E, Dehghanian Z, et al. Application of somaclonal variation in crop improvements. In: Kumar N, editor. Plant mutagenesis: Sustainable agriculture and rural landscapes. Cham: Springer Nature Switzerland; 2024. p. 93-109.
[22] Rashda Naheed RN, Muhammad Arfan MA, et al. Induction of somaclonal variation in selected drought sensitive genotype of sugarcane (saccharum officinarum). 2018.
[23] Yoo C-M, Dalid C, et al. Improving strawberry varieties by somaclonal variation: Hs1448, 10/2022. EDIS. 2022;2022.
[24] Manchanda P, Kaur A, et al. Somaclonal variation for sugarcane improvement. Biotechnologies of crop improvement, volume 1: Cellular approaches: Springer; 2018. p. 299-326.