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Effect of Ethyl methanesulfonate (EMS) mutagen on genetic variability, growth characters and yield of potato

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ABSTRACT

This work was carried out at Faculty of Agriculture, Cairo University, to study the efficiency of three concentrations (20, 30 and 40 mM) of Ethyl methanesulfonate (EMS) mutagen on three potato cultivars, Atlas, Nicola and Simon. Also, we explored the induced genetic variability for the resulted potato mutants in terms of yield and its component. The gradual increase in EMS concentrations resulted in a significant steady decreases in survival percentage, number of stems/plant and growth vigor. The highest concentration of EMS (40 mM) showed the most inhibitory effect on the measured vegetative traits. Concerning tuber yield and its component, the number of tubers/plant, and total tubers weight/plant were decreasing by the increasing of EMS concentrations. However, significant variations between EMS treatment concentrations within each cultivar and between cultivars for the vegetative traits and yield and its component characteristics were noticed. Selection for yield and quality improvement was began in the M_1V_2. Thirty-one clones, with improved characteristics in the M_1V_3, from the three cultivars; twelve plants from cv. Atlas, eleven plants from cv. Nicola, eight plants from cv. Simon were selected. Still further studies are needed to investigate those clones in order to detect the stability of vegetative traits, yield and its component under field conditions.

Key words: *Solanum tuberosum,* EMS, mutant, growth, yield

Introduction

Potato (*Solanum tuberosum* L.) is a major crop in Egypt that produces 2,760,460 Mt (Metric ton) annually used as a source of modified starches for food and industrial processes. However, main potato cultivars generally lack at least some agro-economically important characters like disease and pest resistance as well as environmental stress tolerance (Shin *et al.,* 2011 and Bouaziz *et al.,* 2012). However, genetic variation is the starting point of any breeding programme (Novak and Brunner, 1992). Therefore, induction of mutation in potato and other crops have been used to induce favorable variability for biotic and abiotic stresses (Lestari, 2006). This technology is useful in the improvement of vegetative propagated plants in which there is no seed set or the seed progenies are highly heterogeneous and do not reproduce true type (Neto *et al.,* 1998 and Ahloowalia *et al.,* 2004). The mutagenic agent Ethyl methanesulphonate (EMS) can be used to make mutations at a higher frequency and generate genetic variation from which desired mutants may be selected (Asbah, 2007, Ibrahim, 2008 and Talebi *et al.*, 2012). EMS induces chemical modification of nucleotides, which results in mispairing and base changes. Strong, biased alkylation of guanine (G) residues results, forming O6-ethylguanine, which can pair with thymine (T) but not with cytosine (C). Through subsequent DNA repair, the original G/C pair can then be replaced with A (adenine)/T.

Mostly, EMS induces C-to-T changes resulting in C/G to T/A substitutions (Kim *et al*., 2007). In this respect, Li and Chao (1994) treated potato axillary buds with EMS, SA and gamma irradiation. The highest concentrations only (1.0% EMS, 0.75 mM SA and 15 Kr gamma rays) caused M1 changes in major features such as plant height and weight of aerial parts. Dwarf mutants and plants with small tubers on the stems were found in the M1. Shahien (2005) treated cowpea seeds with EMS (0.0, 0.2, 0.4, 0.6, 0.8 %) and gamma-rays (10, 20, 30 and 40 Kr). The author selected many mutation types: dwarf, early flowering, high yield, heavy branching and large seeds. Meanwhile, Dhanavel *et al*. (2008) applied mutation breeding in the cowpea variety CO-6 to assess the efficiency and

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effectiveness of different chemical mutagens i.e., Ethyl methanesulphonate (EMS), Diethyl sulphate (DES) and sodium azide (SA). Clearly, EMS was highly effective than SA and DES. In another study, seeds of the potato cross H21×H289 were incubated for 16 h in EMS at concentrations varying in the range 0.5%-2.0%. From a population of 2748 EMS-mutagenized and tuber forming hybrids from dihaploid potato clones, *waxy* DNA from 864 clones (E289–E1152) was sequenced. Nineteen mutations were detected and confirmed by sequencing, representing a density of one mutation every 91 kb. All the mutations were G:C to A:T transitions, which is typical for EMS-induced mutations as a result of the alkylation of guanidine residues (Muth *et al*., 2008). Talebi *et al.* (2012) found that increasing EMS concentrations decreased germination, seedling height, root length and emergence of rice under field conditions in M1 generation as compared with the non-treatment control. However, the mutagen dose can be either high or low causing mutation frequency (Jain, 2010).

This work aims to explore the ability of three EMS concentrations (20, 30 and 40 mM) to induce genetic variability in potato and select the better mutants to improve yield and quality of three potato cultivars, Atlas, Nicola and Simon.

Material and methods

Sets of successful experiments were carried out at the Faculty of Agriculture, Cairo University, during 2006 and 2007 growing seasons. Three potato (*Solanum tuberosum* L.) cultivars: Atlas, Nicola and Simon were used. At the beginning, tubers of the selected cultivars were washed and stored in dark at room temperature 25±1ºC for two weeks till sprouting.

Ethyl Methane Sulphonate treatment

Four concentrations of EMS were used. Sprouted tubers were soaked in 0, 20, 30 and 40 mM water solution of EMS for 3 hours, respectively, while water was used as mock treatment. Tubers treated with EMS were sown in summer growing season of January 2006 (M_0V_1). For the next evaluation and selection seasons, tubers were sown in fall growing season of September 2006 (M_1V_2) and in summer growing season of January 2007 (M_1V_3). Survival percentage was estimated at 45 days after planting (DAP); while stem diameter, growth vigor and number of stems per plant were recorded at 75 DAP. In addition, number of tubers /plant, total tubers weight/plant and average tuber weight were calculated. Growth vigor was measured on five levels scale, 1: Very poor, 3: Poor, 5: Average, 7: Good and 9: Very good.

Experimental design

Randomized complete block design was used for all experiments with three replicates. All resulted data were tested by analysis of variance, while Duncan's multiple range test was used for the comparisons among the means of treatments (Waller and Duncan, 1969).

Results and discussion

1. Effect of the mutagene EMS on the growth and development of potato plants

Vegetative traits, yield and its components of the three cultivars treated by EMS were presented in (Table 1). Increasing EMS concentrations resulted in a significant gradual decreases in the survival percentage, number of stems/plant and growth vigor. The highest concentration of EMS (40 mM) exhibited lower survival percentages 93.3, 90, and 93.3% for the three tested cultivars Atlas, Nicola and Simon, respectively, while 100% of survival rate was obtained with their control. On contrast, different EMS concentrations did not affect stem diameter of the three treated potato cultivars. Concerning yield and its component, data show that number of tubers/plant, and total tubers weight/plant were decreasing significantly by increasing the EMS concentrations. However, 40 mM of EMS resulted the lowest values of number of tubers/plant and total tubers weight/plant in the three cultivars. Meanwhile, 20 mM of EMS produced the highest values of the number of tubers/plant, and total tubers weight/plant. Concerning the differences among the three cultivars, data showed

significant variations between EMS treatment within each cultivar and among cultivars in terms of vegetative traits, yield and its component. These results more or little are in agreement with those obtained by other workers among them Das *et al*. (2002) and Badawi *et al.* (2006) on potato, Asbah, (2007) on squash and Ibrahim, (2008) on strawberry.

2. Induced genetic variability using EMS mutagen for selecting mutants with improved yield and quality of potato tubers

Potato plants originating from the tubers treated with EMS as well as tubers produced by those plants was designated mutant clonal generation one (M_0V_1). Selection for yield and quality improvement began in the M_1V_2. In the M_0V_1 tubers harvested from each plant were evaluated as a separate entity, to segregate the majority of sectorial chimeric events. Evaluation and selection were done depending on single plant selection in M_1V_2. Meanwhile, for next generation, evaluation and selection were done depending on clonal evaluation and selection.

Table 1: Effect of mutagen treatments (EMS) on growth, yield and its component in V0M1 generation of the three potato cultivars: Atlas, Nicola and Simon.

Cultivar	EMS (mM)	Survival (%)	No. of Stems /plant	Stem diameter (cm)	Growth vigor	No. of Tubers /plant	Tuber weight (g)	Yield /plant (g)
Atlas	0	100 a	4.2 a	1.4 a	8.3 a	7.1 a	65.7 a	469ab
	20	100 a	4.3 a	1.37 a	7.5 bc	7.2 a	66.1 a	493a
	30	96.7 a	3.7 b	1.37 a	7.8 ab	6.6 b	62.6 a	411b
	40	93.3 a	3.4 b	1.3 a	7.1 c	6.4 b	67.3 a	430b
Nicola	0	100 a	4.2 b	1.3 a	8.1 a	7.1 a	70.6 a	476a
	20	96.7 ab	4.6 a	1.3 a	7.9 a	6.9 ab	70.3 a	480a
	30	96.7 ab	3.7 c	1.3 a	6.7 b	6.3 b	73.3 a	453ab
	40	90.0 b	3.6 c	1.26 a	6.5 b	6.1 b	67.2 a	411b
Simon	0	100 a	3.9 ab	1.3 b	8.4 a	6.9 ab	64.5 b	448ab
	20	100 a	4.1 a	1.3 b	8.1 b	7.1 a	68.3 b	486a
	30	96.7 a	3.7 b	1.3 b	7.8 b	6.7 b	66.3 b	443b
	40	93.3 a	3.2 c	1.4 a	7.2 c	6.1 c	70.5 a	427b

Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

a. M_1V_2

One thousand three hundred and twenty eight plants of potato cvs Atlas, Nicola and Simon, were produced from the four used concentrations (0, 20, 30, and 40 mM) of EMS. Each cultivar was evaluated for its vegetative traits, yield and its component along with its original cultivar.

1. Number of stems/plant

Mean values of number of stems/plant for EMS treatments were higher than control in plants derived from potato cvs Nicola and Simon at 75 DAP (Table 2). Meanwhile, higher values were obtained with control and 20 mM EMS compared to other treatments in cv. Atlas. The lowest value (3.9) was obtained with 30 and 40 mM EMS treatments for plants derived from cv. Atlas. While, the highest value was obtained with 30 and 40 mM EMS for plants derived from cv. Simon (4.6 each), respectively. The range of distribution in plants derived from potato cvs Nicola and Simon was wider with all EMS treatments than control and cv. Atlas. Coefficients of variance (CV %) values in the three potato cvs for all EMS treatments were higher than the control. The maximum increase in variability was obtained with 30 mM of EMS treatment for potato cv. Atlas (20.1%) and control (12%) plants. Meanwhile, Nicola (25%) and Simon (30%) showed the maximum variability increase with 40 mM EMS treatment.

Table 2: Statistical parameters for number of stems/plant at 75 DAP in M_1V_2 generation for potato cvs Atlas, Nicola and Simon.

Cultivar	EMS (mM)	No. of plants	Class of number of stems/plant				Range	X ± SE	CV%
			2-3	4-5	6-7	8-9			
Atlas	0	100	10	89	1		3-6	4.1 ± 0.048	12.0
	20	120	15	102	3		2-7	4.1 ± 0.065	17.1
	30	112	24	85	3		2-7	3.9 ± 0.075	20.1
	40	108	20	87	1		2-6	3.9 ± 0.063	16.6
Nicola	0	100	1	99			3-5	4.2 ± 0.041	9.7
	20	116	5	105	5	1	3-8	4.5 ± 0.072	17.33
	30	120	4	107	6	3	3-9	4.5 ± 0.085	20.7
	40	115	8	95	6	6	3-9	4.5 ± 0.105	25.0
Simon	0	100	28	71	1		3-6	3.8 ± 0.061	16.0
	20	115	21	80	10	4	3-9	4.3 ± 0.108	27.1
	30	114	6	87	18	3	3-9	4.6 ± 0.104	24.5
	40	108	14	74	12	8	3-9	4.6 ± 0.132	30

DAP: Days after planting X: Mean SE: Standard error CV: Coefficient of variance

2. Stem diameter

No significant differences in mean of stem diameter among EMS treatments and their controls in potato cvs Atlas and Simon, while there were significant differences between the control and EMS treatments for this trait in cv. Nicola (Table 3). The range of distribution in treatments of EMS in plants derived from all cultivars was slightly wider than that of the control within each cultivar. Meanwhile, coefficient of variance values for stem diameter in the three potato cvs for all EMS treatments were higher than the control within each cultivar. The maximum increase in variability was obtained from treatments 20 and 30 mM EMS for plants derived from Atlas cv. These values were higher (15.8 and 14.7%) for the two treatments, respectively) than control (10.1%).

Table 3: Statistical parameters for stem diameter at 75 DAP in M1V2 generation for potato cvs Atlas, Nicola and Simon.

Cultivar	EMS (mM)	No. of plants	Class of stem diameter (cm)				Range	X ± SE	CV%
			0.9-1.1	1.2-1.4	1.5-1.7	1.8-2.0			
Atlas	0	100	5	75	20		1-1.6	1.34 ± 0.014	10.1
	20	120	22	63	30	5	0.9-1.9	1.35 ± 0.019	15.8
	30	112	16	57	35	4	0.9-1.9	1.37 ± 0.019	14.7
	40	108	17	67	22	2	0.9-1.9	1.34 ± 0.016	12.2
Nicola	0	100	18	79	3		1-1.5	1.27 ± 0.013	9.8
	20	116	11	87	18		1-1.7	1.34 ± 0.012	9.8
	30	120	10	83	27		0.9-1.7	1.36 ± 0.013	10.6
	40	115	12	81	22		1-1.7	1.34 ± 0.013	10.7
Simon	0	100	7	89	4		1-1.5	1.30 ± 0.009	7.4
	20	115	8	88	19		1.1-1.7	1.35 ± 0.012	9.7
	30	114	8	82	24		1.1-1.6	1.35 ± 0.012	9.7
	40	108	11	89	6	2	1-1.9	1.31 ± 0.013	10.4

DAP: Days after planting X: Mean SE: Standard error CV: Coefficient of variance

3. Growth vigor

Data presented in Table (4) shows significant differences among all EMS treatments and their control in the mean growth vigor at 75 DAP. The highest values resulted from the control plants of these two cultivars was (8) with cv. Atlas and (8.4) with cv. Simon. The lowest values of growth vigor

were 7.4 and 7.5 for 30 mM EMS treatment in Atlas and Nicola cvs., respectively, and 7.9 for 20 mM EMS treatment at plants derived from Simon cultivar. Coefficient of variance values was higher in all EMS treatments compared to the control in the two potato cvs Atlas and Simon. The maximum increase in variability was recorded at 30 mM EMS treatment for cv. Atlas (19.5%) while it was 14% with control. Meanwhile, cv. Simon presented the maximum increase in variability at 20 mM EMS treatment (14.3%) and (11.6%) for the control.

Table 4: Statistical parameters for growth vigor at 75 DAP in M1V2 generation for potato cvs Atlas, Nicola and Simon.

Cultivar	EMS (mM)	No. of plants	Class of growth vigor			Range	X ± SE	CV%
			5	7	9			
Atlas	0	100	3	44	53	5-9	8 ± 0.11	14.0
	20	120	13	51	56	5-9	7.7 ± 0.12	17.4
	30	112	20	50	42	5-9	7.4 ± 0.14	19.5
	40	108	10	45	53	5-9	7.8 ± 0.13	16.8
Nicola	0	100	10	58	32	5-9	7.4 ± 0.12	16.5
	20	116	9	66	41	5-9	7.6 ± 0.11	15.9
	30	120	9	74	37	5-9	7.5 ± 0.11	15.4
	40	115	8	67	40	5-9	7.6 ± 0.10	15.5
Simon	0	100	1	29	70	5-9	8.4 ± 0.10	11.6
	20	115	4	57	54	5-9	7.9 ± 0.11	14.3
	30	114	2	37	75	5-9	8.3 ± 0.10	12.5
	40	108	2	42	64	5-9	8.2 ± 0.10	13.1

DAP: Days after planting X: Mean SE: Standard error CV: Coefficient of variance

4. Number of tubers/plant

Significant differences for number of tubers/plant among 20 and 40 mM EMS treatments and the control in Simon cv (Table 5). Also significant differences were detected among EMS treatments 20 and 30 mM and the control in Nicola and Simon cvs. Lower value (6.9) was obtained with 40 mM EMS for plants derived from cv. Simon. While, the highest value (7.6) was got at 20 mM EMS for plants derived from cv. Atlas if compared to the control of Atlas (7.3), Nicola (7.0) and Simon (6.9), respectively.

Table 5: Statistical parameters for number of tubers/plant in M1V2 generation for potato cvs Atlas, Nicola and Simon.

Cultivar	EMS (mM)	No. of plants	Class of number of tubers/plant				Range	X ± SE	CV%
			5-7	8-10	11-13	14-16			
Atlas	0	100	56	44			5-10	7.3 ± 0.09	13.2
	20	120	49	68	2	1	5-14	7.6 ± 0.13	18.9
	30	112	65	44	3		5-13	7.3 ± 0.12	18.1
	40	108	58	47	3		5-12	7.4 ± 0.11	15.9
Nicola	0	100	79	21			5-10	7.1 ± 0.09	13.3
	20	116	78	35	3		5-13	7.3 ± 0.12	17.7
	30	120	72	46	2		5-12	7.3 ± 0.11	15.7
	40	115	77	36	2		5-12	7.1 ± 0.11	16.3
Simon	0	100	83	17			5-9	6.9 ± 0.09	12.9
	20	115	86	25	4		5-13	7.3 ± 0.11	16.4
	30	114	83	31			5-10	7.1 ± 0.08	12.0
	40	108	90	15	3		5-12	6.9 ± 0.11	17.3

DAP: Days after planting X: Mean SE: Standard error CV: Coefficient of variance

The widest range was at 20 mM EMS treatment for plants derived from cvs Atlas, Nicola and Simon. Co-efficients of variance values of the three potato cvs for EMS treatments were higher than the control. The maximum increase in variability was found at 20 mM EMS treatment for potato cv. Atlas (18.9%) than control (13.2%). The maximum increase in variability was obtained at 20 mM

EMS treatment for cv. Nicola and 40 mM EMS treatment for cv. Simon. These values were 17.7% and 17.3% for the two cultivars than the control (13.3% and 12.9%), respectively.

5. Tuber weight

All concentrations of EMS presented high significant tuber weight of the investigated plants (Table 6) compared to control in plants derived from the three potato cultivars. The highest tuber weight for cv. Atlas and Simon were obtained at 40 mM EMS (74.7g and 69.7g) if compared to control (64.1g). While, cv. Nicola recorded the highest tuber weight with 20 mM EMS (74g) than control (68.1g). The range of distribution for tuber weight at all EMS treatments in plants derived from the three potato cultivars was wider than the control within each cultivar. Also, meaningful differences were noticed among cultivars. The widest range was at 30 mM EMS treatment for plants derived from Atlas and Nicola cvs. While, cv. Simon showed the widest range at 20 mM EMS treatment. Coefficients of variance were higher for EMS treatments progenies compared to the control in the three potato cultivars. Maximum variability was obtained with 30 mM EMS treatment for plants derived from Atlas (31%) and Nicola (23.5%) than control (14% and 15.1%), respectively. Meanwhile, cv. Simon showed the maximum variability (24.7% and 12.50%) with 20 mM EMS and control plants, respectively.

Table 6: Statistical parameters for tuber weight in M_1V_2 generation for potato cvs Atlas, Nicola and Simon.

Cultivar	EMS (mM)	No. of plants	Class of tuber weight (g)							Range	X ± SE	CV%
			30 - 50	51 - 70	71 - 90	91 - 110	111 - 130	131 - 150	151 - 170			
Atlas	0	100	7	70	23					40-90	64.1 ± 0.9	14.0
	20	120	3	66	41	8	1	1		47-144	70.3 ± 1.29	20.1
	30	112	12	39	52	3	2	1	3	34-165	72.5 ± 2.13	31.0
	40	108	5	50	39	7	2	4	1	42-164	74.7 ± 2.08	28.8
Nicola	0	100	6	49	45					40-89	68.1 ± 1.03	15.1
	20	116	8	42	53	9	2	2		40-150	74.0 ± 1.54	22.4
	30	120	10	53	48	5	3	1		39-149	69.9 ± 1.5	23.5
	40	115	5	57	38	9	5	1		41-143	72.5 ± 1.58	23.3
Simon	0	100	3	75	22					4-85	64.1 ± 0.80	12.5
	20	115	11	67	30	3	3	1		33.3-153.9	67.6 ± 1.56	24.7
	30	114	6	71	31	5	1			35.7-119.1	68.6 ± 1.18	18.4
	40	108	4	64	33	4	2	1		46.6-132.7	69.7 ± 1.5	22.3

DAP: Days after planting X: Mean SE: Standard error CV: Coefficient of variance

6. Total tubers weight/plant

As shown in Table (7), the highest total tubers weight per plant was obtained at 40 mM EMS treatment for potato cv. Atlas (548 g) than control (463g). Meanwhile, the maximum increase was noticed at 20 mM EMS with cv. Nicola (530 g) and at 30 mM EMS for cv. Simon (487g) than control (478g and 443 g), respectively. The range of distribution for total tubers weight per plant for all EMS treatments in plants derived from potato cvs Atlas, Nicola and Simon was wider than control within each cultivar. Also, significant differences were observed for the treatments between the three cultivars. The widest range was recorded at 30 and 40 mM EMS for plants derived from cvs Atlas and Nicola. EMS at 20 mM gave the highest range of distribution for total tubers weight/plant with cv. Simon. It is clear from data presented in Table (7) that coefficient of variance values in the three potato cultivars were higher than control under all EMS treatments. The maximum increase in variability was achieved at 40 mM EMS for potato cv. Atlas (28.5%) than control (15.7%). Meanwhile, the maximum increase in variability was obtained at 30 mM EMS with cv. Nicola

(24.1%) and 20 mM EMS with cv. Simon (22.4%). These results more or less are in agreement with those obtained by other workers (El-Sahhar *et al.* (1984) on soybean, Sarkar et al., 1985 on sweet potato, Asbah, 2007 on Squash L. and Ibrahim, 2008 on strawberry). Sharma and Pandey (1996) reported that both EMS and low gamma irradiation doses up to 8 Kr gave useful mutations and led to selections of clones with better potato tuber characters, higher yield and earliness in maturity. Our results nearly agree with those obtained by other workers on garlic (Bohmova *et al.,* 1988; Shalaby *et al.,* 1983; Selvaraj *et al.,* 2001 and Metwally and Abou Shousha, 2002) whom found that in the M2-generation, after EMS treatments most of the studied characters (bulb diameter, bulb weight, number of cloves per bulb, average clove weight and total yield) showed higher values.

Table 7: Statistical parameters for total tubers weight/plant, in M_1V_2 generation for potato cvs Atlas, Nicola and Simon.

Cultivar	EMS (mM)	No. of plants	Class of total tubers weight/plant (g)						Range	X ± SE	CV%
			200 - 350	351 - 500	501 - 650	651 - 800	801 - 950	951 - 1100			
Atlas	0	100	8	32	60				246-643	463 ± 7.28	15.7
	20	120	6	34	70	7	3		247-943	528 ± 9.87	20.8
	30	112	12	45	43	5	4	3	235-1002	516 ± 13.6	27.8
	40	108	9	35	40	16	4	4	242-98	548 ± 15	28.5
Nicola	0	100	8	47	43	2			201-675	478 ± 7.67	16.0
	20	116	11	23	70	8	3		261-921	529 ± 10.8	21.9
	30	120	10	54	41	11	4		234-903	506 ± 11.1	24.1
	40	115	9	51	41	10	4		255-933	512 ± 11.3	23.6
Simon	0	100	10	72	18				242-649	443 ± 7.12	16.2
	20	115	10	58	42	3	2		213-924	484 ± 10.1	22.4
	30	114	11	53	47	2	1		236-834	487 ± 8.62	18.9
	40	108	9	65	30	3	1		238-905	473. ± 9.93	21.8

DAP: Days after planting X: Mean SE: Standard error CV: Coefficient of variance

b. M_1V_3

Potato plants with improved characteristics have been selected from each cultivar as follow: Thirty one plants from cv. Atlas; nine from treatment 20 mM EMS, eight from treatment of 30 mM EMS and fourteen from treatment 40 mM EMS. Thirty plants from cv. Nicola; eight from treatment 20 mM EMS, twelve from treatment of 30 mM EMS and ten from treatment 40 mM EMS. Twenty-three plants from cv. Simon; ten from treatment 20 mM EMS, four from treatment of 30 mM EMS and nine from treatment 40 mM EMS. Selected plants were evaluated in M_1V_3 generation depending on clonal evaluation and selection. Vegetative traits, yield and its component have been recorded. Clones with improved characteristics have been selected. Data in Tables (8-10) show the characteristics of thirty-one selected clones from the three potato cultivars as follow:

1. Atlas cultivar

Twelve plants have been selected; three from treatment 20 mM EMS, four from treatment 30 mM EMS and five plant from treatment 40 mM EMS (Table 8).

a. Number of stems/plant

The maximum number of stems/plant was recorded inclones no. 1 and 3, while; clones 6, 8 and 12 gave the lowest values. However, the number of stems/plant ranged from 3.5 to 5.8 compared to control (4).

b. Stem diameter

The maximum value of stem diameter was recorded in clone no. 4, while the lowest value was obtained with clone no. 6. The stem diameter of control plants averaged 1.4, whereas, different selected clones recorded values of stem diameter ranged from 1.2 to 1.6 as shown in table (8).

Table 8: Characteristics of the selected clones from cv. Atlas and their original cultivar (M_1V_3 generation).

EMS Treat. (mM)	Selected clones	No. of Stems /plant	Stem diameter (cm)	Growth vigor	No. of tubers /plant	Tuber weight (g)	Yield (g)/plant
Control	-	4 bc	1.4 bcd	8 b	6 de	85 ef	500 h
20	1	5.8 a	1.4 bcd	9 a	10.8 a	68 g	722 def
	2	4 bc	1.3 ef	9 a	5.3 e	134 b	698 f
	3	5.8 a	1.4 bcd	9 a	9.5 b	80 fg	752 cde
30	4	4.5 b	1.6 a	9 a	6.5 cd	98 de	633 g
	5	4.8 b	1.5 ab	9 a	9.3 b	82 fg	761 bcd
	6	3.5 c	1.2 f	9 a	5.3 e	162 a	845 a
	7	4.3 bc	1.5 ab	9 a	9.8 ab	82 fg	794 b
	8	3.5 c	1.3 ef	9 a	6.8 cd	118 c	790 bc
40	9	4.3 bc	1.5 ab	9 a	7 cd	111 cd	766 bc
	10	4.3 bc	1.5 ab	9 a	7.3 c	109 cd	784 bc
	11	4.3 bc	1.5 ab	9 a	7 cd	108 cd	759 bcd
	12	3.5 c	1.4 bcd	9 a	8.75 b	83 fg	718 ef

Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

c. Growth vigor

There were no significant differences among different clones in growth vigor under all EMS treatments. But, the selected clones showed high growth vigor compared to control.

d. Number of tubers/plant

The selected clones no. 1, 3, 5, 7 and 12 showed significant higher values than the control. The maximum value of number of tubers/plant was recorded in clone no. 1 (10.8) while the lowest was obtained by the clones no. 2 and 6 (5.3 each).

e. Tuber weight

Plants of the selected clones no. 6, 2, 8, 9, 10 and 11, in decreasing order, recorded significant high values of tuber weights. On the other hand, the lowest values were detected with clone no. 1, compared to the control. Such observation was stated before in the cases of stem number per plant, stem diameter and tuber number per plant.

f. Total tubers weight/plant

The different selected clones resulted a wide range of total tubers weight/plant (63 to 845 g). The maximum value was recorded in clone no. 6, while clone no. 4 showed the lowest value if compared to the control (500 g).

2. Nicola cultivar

Eleven plants from cv. Nicola have been selected; three from treatment 20 mM EMS, five from treatment 30 mM EMS and three plant from treatment 40 mM EMS (Table 9).

a. Number of stems/plant

Data presented in Table (9) revealed that the number of stems/plant value of control plants was 3.8, whereas, different selected clones recorded values of stem number per plant ranged from 4.3 to 6.8. The maximum value of stem number per plant was recorded in clones no. 1, 3 and 7, while clones no. 5 and 11 recorded the lowest value.

b. Stem diameter

The stem diameter value of control plants was 1.3, whereas, different selected clones recorded values ranged from 1.2 to 1.5. The maximum value of stem diameter was recorded in clone no. 1, 2 and 9, while clone no. 7 resulted the lowest value.

c. Growth vigor

There was no significant difference among the clones and the control in growth vigor.

d. Number of tubers/plant

Presented data showed that some selected clones particularly no. 1, 2, 3, 6, 7 and 8, significantly recorded higher values than the control (Table 9). The maximum value of tuber number per plant was recorded in clone no. 3 (9.8), while the lowest one (6.3) was obtained by clones no. 5 and 6.

Table 9: Characteristics of the selected clones from cv. Nicola and their original cultivar (M1V3-generation).

EMS (mM)	Selected clones	No. of Stems /plant	Stem diameter (cm)	Growth vigor	No. of tubers /plant	Tuber weight (g)	Yield (g)/plant
Control	-	3.8 d	1.3 ef	8 a	6.5 e	74 fgh	478 g
	1	6.8 a	1.5 a	9 a	8.5 b	86 def	730 bc
20	2	5.8 ab	1.5 a	8.5 a	9.5 a	70 gh	661 d
	3	6.8 a	1.4 bc	9 a	9.8 a	64 h	617 e
	4	6 ab	1.4 bc	9 a	7 de	104 bc	726 bc
	5	4.3 cd	1.4 bc	9 a	6.3 e	121 a	749 ab
30	6	6.5 a	1.3 de	9 a	7.5 cd	93 cde	692 cd
	7	6.8 a	1.2 f	8.5 a	8 bc	84 ef	672 d
	8	6 ab	1.4 bc	8.5 a	8 bc	76 fgh	602 ef
	9	6 ab	1.5 a	9 a	7 de	83 efg	574 f
40	10	5.3 b	1.4 b	9 a	7 de	98 cd	685 d
	11	5 bc	1.4 b	9 a	6.8 de	115 ab	769 a

Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

e. Tuber weight

Tuber weight differed among the selected clones as presented in Table (9). Plants of the selected clones no. 6, 10, 4, 11 and 5, in descending order, recorded the highest significant values. On the other hand, the lowest value was detected from clone no. 3, comparing to the control with its selected clones. Some clones gave insignificant increment in tuber weight. Such observation was mentioned before in the cases of number of stems/plant, plant height, stem diameter and number of tubers/plant .

f. Total tubers weight/plant

Significant differences between the selected clones and the control plants in this trait were noticed. Different selected clones recorded values of total tubers weight/plant ranged from 574 g to 787 g. The maximum value of total tubers weight/plant was recorded in clone no. 11; and the lowest

value was obtained by the clone no 9, while, the value of total tubers weight/plant for the control was 479 g.

3. Simon cultivar

Eight plants from cv. Simon have been selected; four from treatment 20 mM EMS, one from treatment 30 mM EMS and three plant from treatment 40 mM EMS (Table 10).

a. Number of stems/plant

The number of stems/plant of control plants was 4, whereas, different selected clones recorded values of number of stems/plant ranged from 4.3 to 7.8. The maximum value of number of stems/plant was recorded in clone no. 5, while; the clone no. 2 obtained the lowest value.

b. Stem diameter

The stem diameter value of control plants average was 1.3, whereas, different selected clones recorded values of stem diameter ranged from 1.2 to 1.4. The maximum value of stem diameter was recorded in clone no. 2 and 8, while; the clone no. 6 obtained the lowest value.

c. Growth vigor

Data presented in Table (10) revealed no significant differences among the clones and the control in growth vigor.

d. Number of tubers/plant

Clones no. 3, 5 and 6 showed significant higher values than the control. The maximum value of number of tubers/plant was recorded in clone no. 3(10) while the lowest value was obtained by the clone no. 2 (6.3 .

e. Tuber weight

Plants of the selected clones No. 5, 10, 4, 11, 2 and 1, in descending order, significantly recorded the highest values of tuber weight. On the other hand, the lowest value was detected from clone No. 6 comparing to the control with its selected clones; some clones gave insignificant increment in tuber weight. Such observation was stated before in the cases of number of stems/plant, plant height, stem diameter and number of tubers/plant .

f. Total tubers weight/plant

Data presented in Table (10) showed significant differences between the selected clones and the control plants. Different selected clones recorded values of total tubers weight per plant ranged from 582.7 g to 743.3 g. The maximum value of total tubers weight per plant was recorded in clone no. 1; while clone no. 9 gave the lowest value than control (508 g). Our results are in concomitant with those reported by other workers (Ahloowalia, 2001; Love *et al*., 1996; Das *et al*., 2000; Badawi *et al.*, 2006 and Muth *et al*., 2008 on potato, Sarkar *et al*., (1985) on sweet potato, Asbah, (2007) on Squash and Ibrahim, 2008 on strawberry) who used mutagenesis to induce and select mutants with improved characteristics in vegetative traits, yield and its component. Our results generally are in agreement with those obtained by many researchers on garlic (Bohmova *et al*., 1988; Shalaby *et al*., 1983; Metwally and Abou Shousha, 2002; Talebi *et al.,* 2012) whom found that in the M_2-generation, most of the studied characters (bulb diameter, bulb weight, number of cloves per bulb, average clove weight and total yield) showed higher values. Furthermore, they selected mutants with improved characteristics compared to their parents. Ibrahim (2008) used gamma irradiation to induced new genetic variation in strawberry genome, which leads to select new strains. Each strain had one or more

special and good characters (fruit firmness, total soluble solids, total yield, early yield, average fruit weight, ascorbic acid, number of flowers per plant, total titratable acidity and number of days to first harvest).

Table 10: Characteristics of selected clones from cv. Simon and their original cultivar (M_1V_3 generation).

EMS (mM)	Selected clones	No. of Stems /plant	Stem diameter (cm)	Growth vigor	No. of tubers /plant	Tuber weight (g)	Yield (g)/plant
Control	-	4 f	1.3 bc	8.5 a	6.8 de	75 cd	508 d
	1	5.5 cd	1.3 bc	8.5 a	7.0 de	108 a	743 a
20	2	4.3 ef	1.4 a	8.5 a	6.3 e	94 ab	586 c
	3	6.3 bc	1.3 bc	9 a	10.0 a	72 d	720 ab
	4	5.3 cde	1.3 bc	9 a	7.8 bcd	89 bc	683 b
30	5	7.75 a	1.3 bc	9 a	8.3 bc	81 cd	670 b
	6	6.8 b	1.2 d	8.5 a	8.5 b	69 d	583 c
40	7	5.8 bcd	1.3 bc	8.5 a	6.8 de	89 bc	597 c
	8	5 def	1.4 a	9 a	7.3 cde	93 ab	672 b

Means designated with the same letter in the same column are not significantly different at 0.05 level of probability

Conclusion

In conclusion, EMS treatments induced new genetic variation in potato genome. This variation leads to new genotypes that have favorable characteristics of vegetative traits as well as tuber yield and its component of three potato cvs. Atlas, Nicola and Simon, which could qualify them to be considered as new clones.

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