EFFECT OF GAMMA RADIATION AND ETHYL METHANESULFONATE (EMS) ON POTATO SALT STRESS TOLERANCE *IN VITRO*

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ABSTRACT

An experiment was conducted in the Date Palm Research Units labs / College of Agricultural Engineering Sciences / University of Baghdad to assess the tolerance toward salinity stress in potato after two mutagens treatments *in vitro*. Potato cv. Arizona and Rivera nodal segments were irradiated with four dosages of gamma rays at 0, 10, 20, and 30 Gray and immersed in (EMS) with four concentrations included 0, 10, 20, and 30 mM. The survival rates after mutagenesis treatments were calculated and 449 lines were obtained. The lines were tested for salinity tolerance by growing in MS medium supplemented with four concentrations of NaCl at 0, 100, 150, and 200 mM and data were analyzed according to the CRD with 10 replicates and means were compared according to LSD at 5%. Out of 449 lines obtained, only 38 lines were showed to be tolerant to salinity stress depending on some vegetative characteristics. At the highest NaCl concentration of 200 mM, Almost all derived lines were higher when compared with their controls especially the lines that derived from Arizona in which line 262 gave the most significant plantlets height of 35 mm and line 180 gave the most significant number of nodes of 3.66 nodes.plant^{-1} and number of shoots of 2 shoots.plant^{-1} when compared with their control that gave 11.33 mm, 2 nodes.plant^{-1}, and 1.5 shoots.plant^{-1}, respectively. The resulted lines were analyzed at the molecular level utilizing the inter simple sequence repeats (ISSR) markers and revealed that line 69 was the much distanced from its derived Rivera cultivar while lines 551, 261, 170, 262, 459, 463 were the much genetically distanced from their derived cultivar Arizona.

Key words: mutagenesis, survival rate, salinity, ISSR, dendrogram
Part of PhD Dissertation of the 1st author

تاثير اشعة كاما والاثيل ميثان سلفونيت (EMS) في تحمل البطاطا للاجهاد الملحي خارج الجسم الحي

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المستخلص

نفذت التجربة في مختبرات وحدت ابحاث النخيل والتمور/ كلية علوم الهندسة الزراعية/ جامعة بغداد لتقدير درجة تحمل نباتات البطاطا للاجهاد الملحي بعد تعريضها الى نوعين من المطفرات خارج الجسم الحي. عرضت عقد البطاطا صنف اريزونا وريفيرا الى اربعة جرعات من اشعة كاما شملت 0 و 10 و 20 و 30 غري وغمرت في اربعة تراكيز من الاثيل ميثان سلفونيت شملت 0 و 10 و 20 و 30 ملي مولر. حسبت نسبة البقاء بعد معاملات التطفير اذ تم الحصول على 449 خط من كل المعاملات واختبرت هذه الخطوط لمعرفة درجة تحملها للاجهاد الملحي عن طريق زراعتها على وسط MS يحتوي على اربعة تراكيز من كلوريد الصوديوم تضمنت 0 و 100 و 150 و 200 ملي مولر وتم تحليل النتائج باستعمال التصميم العشوائي الكامل (CRD) بعشرة مكررات وقورنت المتوسطات باستعمال اختبار اقل فرق معنوي (LSD) عند مستوى احتمال 5%. من بين الـ 449 خط تم الحصول عليه من معاملات التطفير، تم انتخاب 38 خط فقط والتي اظهرت تحملها للاجهادات الملحية المقترحة بالاعتماد على بعض الصفات الخضرية. بينت النتائج ان اغلب الخطوط الناتجة قد تفوقت على الاصناف التي اشتقت منها عند اعلى مستوى للملح بتركيز 200 ملي مولر خاصة تلك التي اشتقت من الصنف اريزونا حيث اعطى الخط 262 اعلى قيمة لارتفاع النبيتات بلغ 35 ملم في حين اعطى الخط 180 اعلى عدد للعقد بلغ 3.66 عقدة.نبات$^{-1}$ واعلى عدد افرع بلغ 2 فرع.نبات$^{-1}$ عند مقارنته مع الصنف اريزونا الذي اعطى 11.33 ملم و 2 عقدة.نبات$^{-1}$ و 1.5 فرع.نبات$^{-1}$ ،بالتتابع. حللت الخطوط المنتخبة على المستوى الجزيئي باستخدام مؤشرات الدنا البسيطة المترادفة البينية (ISSR) حيث بينت ان الخط 69 كان الابعد وراثيا عن الصنف ريفيرا الذي اشتق منه والخطوط 551 و 261 و 170 و 262 و 459 و 463 كانت الابعد وراثيا عن الصنف اريزونا التي اشتقت منها.

الكلمات المفتاحية: التطفير، نسبة البقاء، ملوحة، ISSR، التحليل العنقودي
البحث مستل من اطروحة دكتوراه للباحث الاول

***Received:12/9/2019, Accepted:14/12/2019**

INTRODUCTION

For many centuries, plants were able to cope the continuous changing in the ecosystem through genetic variation. It is of great importance to keep such variation in plant population to ensure the continuity of desired species. Salinity tolerance is one of the most important traits plant breeders always look for especially when the desired species are cultivated in subtropical areas. It was estimated that, by 2050, about 50% of the cultivated lands will be affected by salinity if no serious measures are taken (18). Potato is one of the four major staple food including wheat, rice and corn and plays a vital role in the international food security. Induced mutations can be valuable source of variations to improve plant performance for it can rearrange genetic information in a stable manner and possibly cause for the emergence of new traits or overexpression of certain genes (21). High yielded potato mutants were obtained by Hoque and Morshad (10) when using 4 types of chemical mutagens. Yaycili and Alikamanoğlu (24) obtained potato salt-tolerant mutants by exposing nodal explants to different doses of gamma rays *in vitro*. They noticed that plant regeneration was highly affected by increasing the dosage of irradiation and more that 50% of cultured nodes failed to regenerate with the doses above 25 Gray. They also found that the average genetic distance between the mutants and control was 27.5% using RAPD technique. Alwan et al. (3) evaluated the sixth generation of mutants evolved from irradiating tomato seeds with 20 Gray of gamma rays. He reported a high broad sense heritability in the term of plant height that reached 97.43% which proven the successful changes in genetic structure due to gamma irradiation. Bado et al. (4) proposed a schematic technique to increase the efficiency of gamma application for plants improvement of different potato varieties. Their results showed that growth retardations (GR) at high gamma doses were pronounced in which the GR_{50} ranged from 9.7 to 20.6 Gray depending on the genotypes used which showed to have a significant effect on the irradiation sensitivity. Afrasiab and Iqbal (1) tested a produced somaclones and mutants on the molecular level to investigate their genetic variation using RAPD technique. They found that all mutants exhibited polymorphism at the molecular level when compared with the control. The aim of this study was utilizing some physical and chemical mutagens to produce sufficient stimulant population for salt-tolerance selection and producing salt tolerant potato mutant lines in the future in Iraq.

MATERIALS AND METHODS

Plant materials and mutagenesis

The experiment was conducted at the Date Palm Research Unit labs / College of Agricultural Engineering Sciences / University of Baghdad in 2018. Nodal segments with detached leaf of two commercial potato (*Solanum tuberosum* cv. Arizona and Rivera) cultivars were irradiated with four doses of gamma rays (0, 10, 20, and 30 Gray) and immersed in EMS (Sigma-Aldrich, USA) with four concentrations included (0, 10, 20, and 30 mM). The experiment includes 800 explants came from (50 nodes* 4dosages* 2mutagens*2cultivars). Two weeks after the mutagenesis application, participated explants were screened according to the mortality rates, plant height, and number of nodes to examine the initial effect of mutagenesis on the resulted plantlets. Afterwards, resulted lines (MV0) were grown on MS medium (16) supplemented with four NaCl concentrations of (0, 100, 150, and 200 mM) designated as (S0, S1, S2, and S3, respectively) to examine their tolerance to different salt levels. The selection criteria for simulated salt stresses included plant height, number of shoots, and number of nodes. The initial effect of mutagenesis and simulated salt stress experiments were designed according to the Completely Randomized Design (CRD) with 10 replications and means were compared according to (LSD) test at 5% level of significance.

DNA extraction and ISSR marker-assisted selection for potential MV0 salt tolerant lines

Potato genomic DNA was extracted following the protocol established by Chaudhry et al. (7). Five ISSR primers were selected for the marker-assisted selection of MV0 lines obtained from (6) and (12) (Table 1).

Primers annealing temperatures were optimized prior to the PCR selection reaction as shown in Figure1 and Table 1. The PCR was performed with reaction conditions presented in Table 2. The Dendrograms were constructed utilizing the SPSS computer statistical program according to Nei and Lei (17).

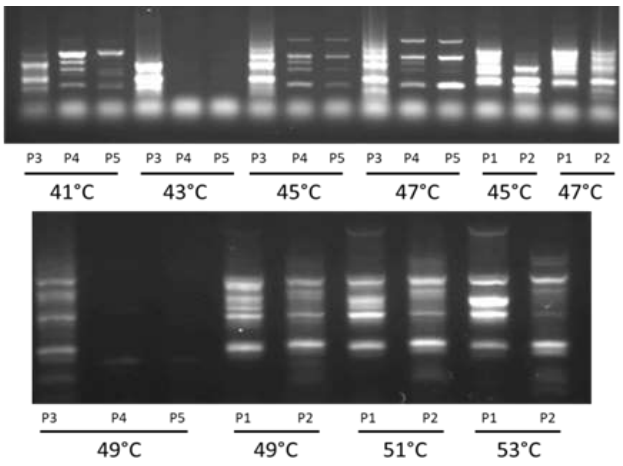

Figure1. Primers annealing temperature optimizations prior to the ISSR marker-assisted selection. P = primer

Table 1. Suggested primers sequences for the inter-simple sequence repeats (ISSR) and their optimized annealing temperatures

Primer #	Reference	Sequence	Annealing temp.
1	Bornet et al. (6)	5'-GAGCAACAACAACAACAA-3'	51°C
2	Bornet et al. (6)	5'- CTGAGAGAGAGAGAGAG-3'	51°C
3	Bornet et al. (6)	5'-AGAGAGAGAGAGAG-3'	47°C
4	Mahgoub et al. (12)	5'-CGCGATAGATAGATAGATA-3'	47°C
5	Mahgoub et al. (12)	5'-GACGATAGATAGATAGATA-3'	47°C

Table 2. PCR reaction conditions and number of cycles for the suggested ISSR primers

Preheating 95°C	Number of cycles = 34			Post extension 72°C	Holding 4°C
	Denaturation 95°C	Annealing 51 or 47°C	Extension 72°C		
5 min	1 min	1 min	2 min	10 min	∞

RESULTS AND DISCUSSION

Mortality rates after mutagenesis treatments

Results in Table 3 shown that mortality rates had increased by increasing the mutagen doses. For gamma applications at 10 and 20 Gray, all explants were survived for both participated cultivars. Increasing the gamma dosage to 30 Gray, 74% and 80% of the irradiated explants were able to grow for Rivera and Arizona, respectively. As for the EMS application, it was noticed a relatively close mortality rates between both cultivars. At 10 mM EMS, 70% and 68% of the explants survived for Rivera and Arizona, respectively. At 20 mM EMS, the survival rates decrease to 52% and 60% for Rivera and Arizona, respectively. Finally at 30 mM EMS, the rates were below the 50% lethal dose (LD_{50}) and gave 46% and 48% for both Rivera and Arizona cultivars, respectively. However, the rates at 30 mM EMS were considered acceptable and therefore accounted for the further experiments. Out of 600 (control excluded) explants participated in the mutagenesis experiments, only 449 explants were able to survive and subsequently included in a series of salt selection episodes to determine their ability to withstand different levels of salinity stress and prove to be salt tolerant lines. These results were in agreement with many other researchers results where they found that mutagenesis treatments had a significant effect on survival rates in different plant crops including potato (1), eggplant (20), tomato(22), and pepper (14). However, the survival rates varied depending on the genotypes which agreed with the results of (24) and (4).

Assessment of plant performance after mutagenesis treatments

The initial effect of mutagenesis treatments on 2 vegetative traits after 2 weeks of reculture was shown in Table 3. It is very noticeable that the increased dosages of mutagens significantly affect plant growth; however, the effect of EMS was more extreme than gamma rays.

Table 3. Initial effect of mutagenesis treatments of gamma (G) rays at (10, 20, and 30 Gray) and EMS at (10, 20, and 30 mM) on the survival rates, plant height, and number of nodes after 2 weeks of the treatments. R=Rivera cultivar. A=Arizona cultivar

Treatment	Survival rate	Plant height Mm	Nodes #	Treatment	Survival rate	Plant height Mm	Nodes #
R10G	100%	114.9	9.4	R10EMS	70%	66.90	7.5
R20G	100%	79.20	7.6	R20EMS	52%	45.20	5.9
R30G	74%	37.90	6.2	R30EMS	46%	31.40	5.1
A10G	100%	85.40	8.3	A10EMS	68%	73.50	6.1
A20G	100%	44.50	6.4	A20EMS	60%	43.90	5.3
A30G	80%	47.60	5.5	A30EMS	48%	30.80	4.5
R0	100%	118.1	10.6	A0	100%	111.3	9.4
Plant height LSD= 6.48				Nodes # LSD= 0.86			

The highest plant height among mutants was observed in the Rivera plantlets generated from 10 Gray gamma exposure and gave 114.9 mm.plant^{-1} which did not significantly differ from Rivera control that gave 118.1 mm.plant^{-1}. Moreover, the lowest value of plant height was exhibited in the Arizona treated with 30 mM EMS and gave 30.80 mm.plant^{-1}. Similarly, the number of nodes also decreased with the elevated dosages of mutagens where Rivera control gave the most significant number of nodes of 10.6 nodes.plant^{-1} while Arizona treated with 30 mM EMS gave the least significant value of 4.5 node.plant^{-1}. The differences between genotypes can be considered a key factor that effect plant response to different kinds and levels of mutagenesis treatments. The prolonged exposure to certain kinds of mutagens can cause chromosome abnormality and might inhibit mitosis which in result will severely affect plant regeneration and development. Hence, the choice of appropriate dosages is vital in mutation studies. Plant height and its correlated number of nodes showed a negative correlation with elevated mutagens dosages. Plant height is mainly depends on cell division and cell extension and can be used as an indicator of mutagenic treatment effect (11). The decreased plant height due to increase mutagenic dosages could be attributed to some physical and biochemical characteristics of the plant tissue including the inhibition or down regulation of protein synthesis in addition to the disturbance of plant hormones production. Table 4 represents the different naming designated to the potential salt tolerant lines to be distinguished from each other.

Table 4. Designated names of the survived explants from both Rivera (R) and Arizona (A) nodal segments exposed to different dosages of gamma radiation and EMS

Naming	Treatments
(1 - 50)	R , 10 Gy Gamma
(51 - 100)	R , 20 Gy Gamma
(101 - 150)	R , 30 Gy Gamma
(151 - 200)	A , 10 Gy Gamma
(201 - 250)	A , 20 Gy Gamma
(251 - 300)	A , 30 Gy Gamma
(301 - 350)	R , 10 mM EMS
(351 - 400)	R , 20 mM EMS
(401 - 450)	R , 30 mM EMS
(451 - 500)	A , 10 mM EMS
(501 - 550)	A , 20 mM EMS
(551 - 600)	A , 30 mM EMS

Simulated salt stress of MV0 lines

After the initial salt screening, only 38 out of 449 lines were able to survive. The survived lines along with the two control cultivars were subjected to *in vitro* salt stress by growing them on MS medium supplemented with four concentrations of NaCl at (0, 100, 150, and 200 mM). The results in Table (5) shown that in the term of plantlet height, 5, 6, 8, and all Rivera derived lines developed from gamma ray exposures were significantly higher than the control at S0, S1, S2, and S3, respectively with the highest significant values in lines 9, 10, 102, and 102 which gave 118.8, 63.00, 26.00, and 28.50 mm.plantlet^{-1}, respectively compared with the control that gave 107.8, 35.00, 14.00, and 9.67 mm.plantlet^{-1} for the S0, S1, S2, and S3, respectively. As for the Rivera lines developed by EMS treatments, the results shown that 5, 4, all, and all lines were significantly higher than their control at the same salt levels where the highest values were in lines 313, 321, 321, and 334 and gave 122.5, 65.83, 25.33, and 18.00 mm.plantlet^{-1} at S0, S1, S2, and S3, respectively compared to the control that gave 107.8, 35.00, 14.00, and 9.67 mm.plantlet^{-1}, respectively. In the term of number of nodes, 2, 4, 2, and 1 gamma

developed Rivera lines at S0, S1, S2, and S3 were significantly higher than the control and gave 10.66, 9.00, 3.50, and 3.33 nodes.plantlet^{-1} in lines 4, 10, 68, and 102, respectively compared to 9.00, 4.67, 2.17, and 2.00, respectively in the control cultivar. Nonetheless, the effect of augmented salt levels on EMS developed lines were also shown in Table (5) in which 3, 2, and 3 lines were significantly higher at S0, S1, and S2, respectively and gave values of 10.83, 10.00, and 4.17 nodes.plantlet^{-1} in lines 334, 321, and 360, respectively. In addition, 2 and 1 lines were significantly higher than the control at S1 and S2, respectively and gave 3.50 and 2.33 shoots.plantlet^{-1} in lines 4 and 115, respectively when compared with the control that gave 1.67 and 1.00 shoots.plantlet^{-1}, respectively. For EMS developed lines, different outcomes were noticed at S1 and S2 where 5 and 2 lines mostly from low EMS dosage at were significantly higher than the control and gave values of 3.50 and 2.17 shoots.plantlet^{-1} in lines 305 and 360, respectively compared with the control that gave 1.67 and 1.00 shoots.plantlet^{-1} at S1 and S2, respectively. An obvious superiority was noticed at the relatively high NaCl concentrations in Arizona derived lines developed from gamma ray applications as presented in Table (6). Accordingly, 6, 2, 11, and 11 lines were significantly higher in plant height than the control in S0, S1, S2, and S3, respectively and gave highest values of 121.5, 63.50, 34.33, and 35.00 mm.plantlet^{-1}, respectively compared to 109.6, 28.50, 12.50, and 11.33 mm.plantlet^{-1}, respectively. For EMS treatments, 2, all, and 3 lines were significantly higher than the control and gave values of 121.1, 29.67, and 31.17 mm.plantlet^{-1} at S0, S2, and S3, respectively.

Table 5. Effect of the interaction between Rivera (R) derived potato lines and NaCl concentrations *in vitro* reflected by plantlets height (upper value), number of nodes.plant^{-1} (middle value), and number of shoots.plant^{-1} (lower value)

Line#	NaCl (mM)				Line#	NaCl (mM)			
	0	100	150	200		0	100	150	200
Gamma developed lines					EMS developed lines				
4	111.3	51.17	16.83	16.33	305	119.1	54.17	18.83	9.17
	10.66	6.33	2.17	2.17		10.17	6.67	3.33	2.00
	2.83	3.50	1.33	1.00		3.83	3.50	1.50	1.00
7	117.6	54.83	23.67	18.33	313	122.5	41.00	24.50	12.00
	10.66	7.83	2.50	2.17		10.33	4.17	3.00	2.00
	2.17	2.50	1.33	1.17		3.83	3.33	1.67	1.00
9	118.8	53.50	17.83	17.83	321	113.8	65.83	25.33	14.67
	9.83	7.67	2.67	2.00		9.33	10.00	3.67	2.17
	2.33	2.67	1.67	1.50		3.67	2.50	1.83	1.17
10	114.5	63.00	20.50	18.17	334	117.3	37.00	19.67	18.00
	9.67	9.00	2.67	2.33		10.83	4.00	2.83	2.33
	4.00	2.50	1.83	1.33		4.50	3.17	1.50	1.00
68	114.3	34.33	24.50	17.67	349	118.5	38.50	18.50	16.67
	9.50	5.67	3.50	2.50		9.83	3.83	2.50	2.17
	3.33	2.50	1.83	1.33		3.83	2.83	1.00	1.00
69	113.1	43.33	23.33	15.50	360	108.1	33.17	23.83	15.83
	9.17	5.17	2.67	2.33		8.67	5.33	4.17	2.17
	3.67	2.33	1.67	1.50		4.00	2.17	2.17	1.67
77	106.5	54.83	20.17	20.33	373	111.8	39.83	23.33	14.83
	10.00	4.67	2.33	2.33		9.33	5.17	2.83	2.17
	2.17	2.00	1.00	1.17		3.83	3.50	1.67	1.17
102	108.1	31.17	26.00	28.50	423	97.33	33.33	22.33	17.33
	9.33	4.50	3.17	3.33		7.17	5.17	3.00	2.33
	2.50	1.83	1.67	1.33		3.17	2.17	1.33	1.33
114	105.1	34.17	22.83	20.67	$LSD_{0.05}$			4.43	
	8.50	4.00	2.83	2.50				0.93	
	2.00	2.17	1.17	1.00				0.71	
115	105.1	35.33	23.50	20.50	R	107.8	35.00	14.00	9.67
	8.17	4.33	3.50	2.83		9.00	4.67	2.17	2.00
	2.00	2.50	2.33	1.00		3.83	1.67	1.00	1.00
$LSD_{0.05}$			5.14						
			1.12						
			0.92						

Similar growth status was observed in the term of number of nodes where 9, 2, 10, 7 gamma developed Arizona derived lines were significantly higher than their control at S0, S1, S2, and S3, respectively and gave highest values of 10.83, 8.17, 4.50, and 3.67 nodes.plantlet^{-1} compared to 8.17, 4.00, 2.17, and 2.00 nodes.plantlet^{-1} in the control at the

same concentrations, respectively. All Arizona derived lines obtained from EMS treatments were significantly higher than the control at S2 in which the highest value was exhibited in line 551 which gave 4.33 nodes.plantlet^{-1} compared to the control that gave 2.17 nodes.plantlet^{-1}. For the number of shoots in gamma developed Arizona lines, 13, 2, and 4 lines significantly surpassed the control at S0, S1, and S2, respectively and gave values of 4.33, 3.50, and 2.33 shoot.plantlet^{-1}, respectively in lines 165, 153, and 180, respectively compared to the control that gave 1.83, 1.50, and 1.17 shoot.plantlet^{-1}, respectively. The results also shown that all and 3 EMS developed lines gave significant increases in the number of shoots at S0 and S2 which gave 3.83 and 2.50 shoot.plantlet^{-1}, respectively in lines 459 and 551, respectively compared to 1.83 and 1.17 shoots.plantlet^{-1}, respectively in the control. Salt stress *in vitro* mimics to some extent its effect under field condition which might cause water withholding, nutrient imbalance, and accumulate toxic elements inside plant body. In addition, high salinity levels can damage the chloroplast and hence affect photosynthesis which eventually reduces plant growth (23). Survival and growth behavior was measured for it can reflect the up and down regulation of some physiological mechanisms occurred in the plants. The results showed that most of the produced mutagenic lines were able to tolerate relatively high levels of salinity when compared to their control counterparts. Similar results were previously published by Al-Safadi and Arabi (2) and Mtawj (13) were they found that potato plants showed more ability to tolerate salinity *in vitro* when treated with different chemical and physical mutagens.

Table 6. Effect of the interaction between Arizona (A) derived potato lines and NaCl concentrations *in vitro* reflected by plantlets height (upper value), number of nodes.plant^{-1} (middle value), and number of shoots.plant^{-1} (lower value)

Line#	NaCl (mM)				Line#	NaCl (mM)			
	0	100	150	200		0	100	150	200
Gamma developed lines									
153	117.8	63.50	23.17	20.83	257	100.0	22.17	16.00	15.00
	10.50	8.17	3.33	2.67		7.50	3.50	2.00	2.50
	3.50	3.17	1.17	1.33		3.33	1.67	1.17	1.00
154	121.5	52.17	34.33	13.33	261	98.83	27.17	28.50	25.33
	10.83	6.17	3.67	2.33		8.00	3.50	3.33	2.50
	4.17	3.50	1.67	1.50		3.83	1.83	2.33	1.50
162	115.3	24.00	14.50	11.00	262	98.50	27.33	30.00	35.00
	10.16	3.00	3.33	2.00		8.17	4.67	4.17	3.33
	3.50	1.67	1.17	1.00		3.67	2.00	1.67	1.50
165	119.8	24.00	29.00	27.50	270	95.50	26.67	26.83	25.17
	10.33	3.33	4.00	3.00		8.33	4.50	3.17	3.00
	4.33	1.33	2.17	1.00		2.50	1.83	1.33	1.17
166	121.0	25.33	14.33	13.33	$LSD_{0.05}$		4.10		
	10.50	3.17	2.33	2.00			0.75		
	2.83	1.33	1.33	1.00			0.73		
170	107.1	24.50	21.83	18.00	EMS developed lines				
	7.83	3.17	2.33	3.17	459	121.1	26.00	27.50	14.17
	3.17	1.33	1.33	1.50		9.67	3.50	3.67	2.33
180	114.8	28.50	34.00	27.67		3.83	1.33	2.17	1.33
	10.33	3.00	4.50	3.67	463	109.0	27.17	25.67	26.33
	2.17	1.33	2.33	2.00		9.67	3.67	3.17	3.33
205	104.6	24.67	15.83	12.17		2.50	1.50	1.17	1.50
	8.17	3.17	2.17	2.17	474	117.3	24.83	26.17	28.17
	3.50	1.00	1.17	1.00		9.83	3.17	3.33	3.33
207	105.5	23.33	27.67	23.50		3.33	1.67	2.00	1.50
	9.33	3.00	3.33	2.50	551	94.83	26.17	29.67	31.17
	2.50	1.50	2.00	1.17		7.83	3.17	4.33	3.33
209	108.3	28.33	12.83	17.67		2.83	1.83	2.50	1.33
	9.50	4.33	2.00	2.17	$LSD_{0.05}$		4.39		
	3.83	2.17	1.00	1.00			0.86		
222	110.6	24.17	24.33	29.00			0.67		
	9.00	2.83	3.00	3.67	A	109.6	28.50	12.50	11.33
	4.17	1.67	1.17	1.50		8.17	4.00	2.17	2.00
254	99.50	28.33	25.50	26.83		1.83	1.50	1.17	1.50
	7.83	3.50	2.67	3.67					
	3.67	2.17	1.50	1.67					

Molecular analyses of MV0 lines

Figures 2A and 2B exhibited the different banding patterns among derived lines and their controls. The figure clearly showed that the size of the bands differentiates while using the different sets of ISSR primers which indicates the differences in their genetic makeup as resulted from mutagenesis treatments. A further Dendrogram trees were generated to facilitate the separation of produced mutagenic lines and better understand their genetic differences. The dendrogram analysis exhibited in Figure 3A reveals that three main clusters were produced with the highest genetic distance was between Rivera cultivar and line 69 while the closest genetic distance was between lines 334 and 321. Moreover, Figure 3B. shows the segregation of Arizona cultivar and its derived mutagenic lines in two cluster groups following the genetic distances resulted from ISSR markers.

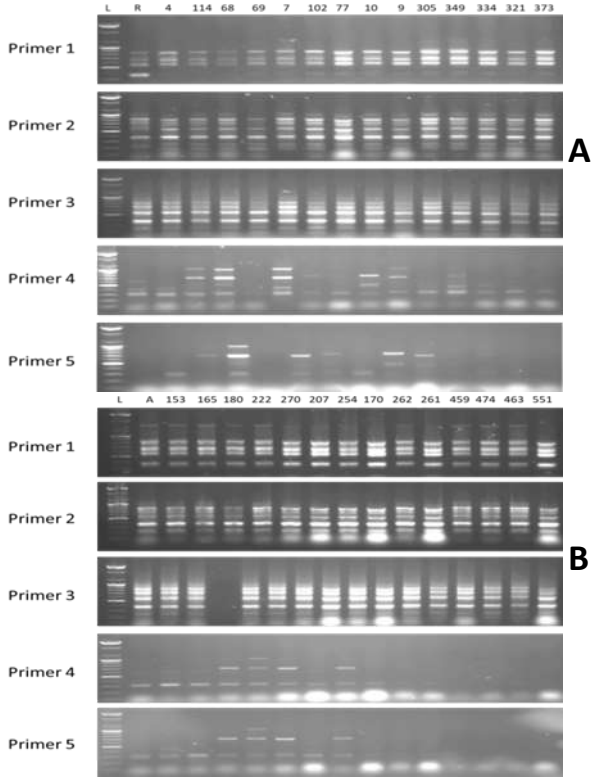

Figure 2. Amplification patterns of potato mutagenic lines derived from Rivera (A) and Arizona (B) cultivars resulted from 5 ISSR primers. L= DNA ladder, R= Rivera, A= Arizona

In accordance, Arizona cultivar was consisted in one of the main groups and was genetically close from line 153 but much distanced from lines 551, 261, 170, 262, 459, 463, and 474. The salinity-tolerance mechanisms are still under investigation in spite of what have been achieved in this regard in the past years.

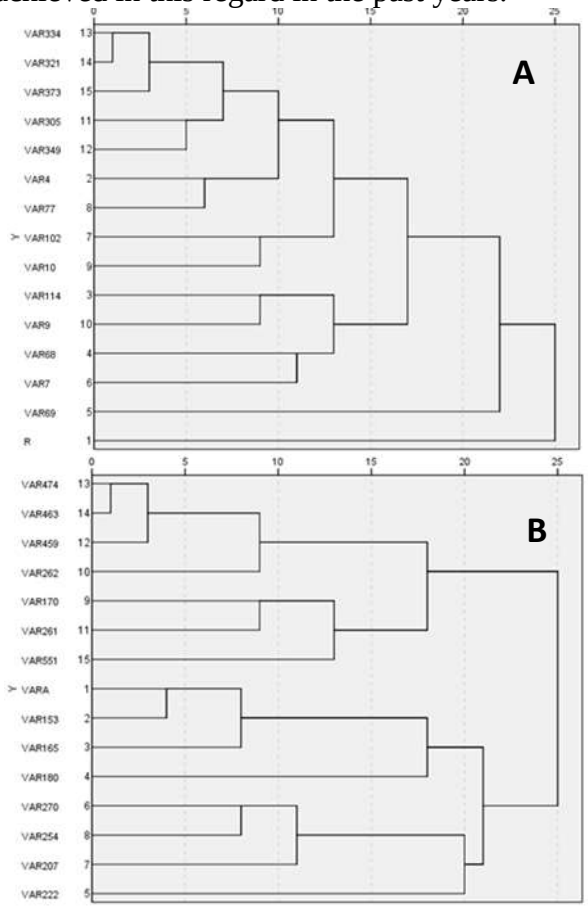

Figure 3. Dendrogram showing the genetic distances between induced mutagenic lines of potato cv. Rivera (R) and its derived mutants (A) and Arizona (A) and its derived mutants (B) based on the banding patterns produced by ISSR markers

Salt stress can be subdivided in to two main phenomena: the initial osmotic stress followed by accumulating Na^+ ions (15, 19). The suggested physiological mechanisms that deal with the early osmotic stress is by reducing the excessive loss of water with enhancing water absorption while the mechanism that reduces the effect of accumulating Na^+ ion involves the exclusion of Na^+ outside plant body through stomatal cavity or by the confinement of Na^+ in the cell vacuoles(5, 15). On the other hand, gene expression at the molecular level plays a vital role in enhancing salinity tolerance through sets of transcription factors families which are differentially expressed in response to uprising salinity stress (9). It was reported that, during salt stress, more than 5500 were up regulated in *Arabidopsis* (8). Mutation involves the alternation of gene sequences and changing the chromosomes structure and

number and can also shifts the transcription levels of some genes that are directly or indirectly contribute to the salinity tolerance scenario (21). The results noticed that produced mutagenic lines differentiated in its genetic distances and therefore some showed a promising salinity tolerance trait.

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