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Improvement of Salt Stress Tolerance in Strawberry by Ethyl Methane Sulfonate Treatment

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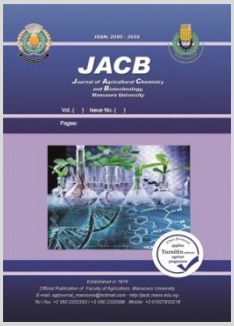

ABSTRACT

In Egypt, strawberry is one of the most important export vegetable crops and in addition,traded locally wide.Strawberry crop is sensitive to salinity of soil and irrigation water, as an increase in salinity over 700ppm has a negative effect on vegetative growth and yield. This study was conducted to obtain genetic variation and assess their tolerance to salt stress by ethyl methane sulfonate (EMS). EMS with three concentrations (0.0, 0.1 and 0.3 %)for (30, 60 and 90 min) applied to "Fortuna cv." runner tips. Strawberry seedlings were planted in the open field to assess vegetative growth and yield traits. Strains were evaluated under saline stress conditions (500 and 1000 ppm of Na Cl) after selecting the best.RAPD- PCR technique was used to detect the potentiality variability effects of EMS.The results showed that soaking in EMS treatment improved the vegetative growth and yield characteristics under salt stress conditions compared with the control such as plant height,number of leaves, chlorophyll content, number of fruits/plot and weight of fruits/plot. The results showed also high values for fruit quality parameters such as.,fats (%), proteins (%), ash (%), humidity (%),carbohydrates (%)and fibers (%)with EMS treatment compared to control plants. Using four RAPD primers, a total number of 15polymorphic bands were detected, out of 12 bands were resulted after EMS treatment. Eleven unique bands were observed, which eight from EMS treatment plants and other three bands amang the contral plants.This study revealed that EMS,as a chemical mutagen is considered is promising in chemical enhancing salt tolerance.

Keywords: ethyl methane sulfonate (EMS), salinity stress, vegetative growth, yield, chemical content.

INTRODUCTION

Strawberry belong to the family Rosaceae, genus *Fragaria* which consists of 20 species and approximately 600 varieties of strawberry (Mondal, 2010). According to (FAO, 2019) strawberry area harvested on Egypt were 11772 ha which produced 460245 tonnes, and Egypt exported 38543 tonnes. Mutation is considered as good a tool to study molecular nature and functions of genes. Ethyl methane sulfonate (EMS) used more commonly as a chemical mutagen on plants because of its high ability to induce mutations. EMS attaches its alkyl groups to the oxygen bonded to guanine through hydrogen bonds and produces 0-6 alkylguanine that pairs with thymine instead of cytosine and replaces A/T by G/C (Waungh *et al*., 2006).

Salinity is considered to be one of the most limiting environmental factors for plant growth and productivity in arid and semi-arid regions of the world (Turhan and Eris., 2004). Strawberry is categorized as one of the most salt-sensitive crops. Salinity causes leaf edge burn, necrosis, nutrient imbalance or specific ion toxicity, also reduction in fruit quality and yield as well as potential plant death on high salinity stress (Saied *et al*., 2005). The present study was planned to assess the genetic variations through chemical mutation using (EMS) on strawberry cv. Fortuna at morphological level, as well as molecular level in order to evaluate the tolerance to salt stress.

MATERIALS AND METHODS

This investigation was conducted at Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt, during the period from 2018 to 2020. This study aims to induce genetic variations on strawberry and evaluate the best clones under salinity stress.

Genetic materials:

Strawberry cultivar "Fortuna" runner tips was used as explants. Fortuna variety released by the University of Florida strawberry breeding program.

EMS treatment:

Explants were soaking in different EMS concentrations (0.0, 0.1 and 0.3 %) for different durations (30, 60 and 90 minutes). After the mutagen treatment, the plant material washed with water three times. Runners were planted in cups for one month then, seedlings were transplanted at the open field on September 2018. Runners were taken at the end of the experiment and then growing for evaluation of salt stress.

Salt stress evaluation:

Uniformed size plants from EMS treatments growing after selecting the best vegetative growth and yield traits from treatments (0.1% EMS for 60 and 90 min. in addition to control) in hydroponic system (deep water culture) were selected. For salt stress evaluation, two levels of salinity were used (500 and 1000 ppm) in addition to control (zero Na Cl).

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Data recorded:

Plants exposed to EMS treatments, after 60 days from growing at vegetative stage were used. Data were recorded for plant height (cm), number of leaves per plant, total number of fruits per plot (plot area m^2) and total weight of fruits (g) per plot 2.1 m^2). Some chemical analysis was measured in plants leaves for chlorophyll A (mg/g), chlorophyll B (mg/g) and total chlorophyll (mg/g) according to Arnon, D.I. (1949). In the same text, some other measurements were determined for fats (%), proteins (%), ash (%), humidity (%), carbohydrates (%) and fibers (%) by NIR Infrared Analyzer 1650 D, FOSS. On the other hand, plants exposed to saline treatments, after 60 days at vegetative stage , data were collected, such as plant height (cm), number of leaves per plant and root length (cm). Some chemical analyses were measured for sodium content (ppm), potassium content (ppm) and potassium /sodium ratio according to Chapman and Pratt (1978).

Molecular analysis study:

1- **DNA extraction:** The DNA was extracted from fresh leaf tissues and used as templates for four RAPD reactions to investigate the variation between strawberry samples in response to different Na Cl treatments and EMS.

2- **RAPD PCR technique**: RAPD markers were used to detect the possible genetic variation between treated and untreated samples in response to salt stress. Four oligonucleotide primers were used in this study (OPA-5: 5' AGG GGT CTT G 3' ; OPA-1: 5' CAG GCC CTT C 3' ; OPA-7: 5' GAA ACG GGT G 3' ; OPA-8: 5' GTG ACG TAG G 3').

3- **PCR reactions** were carried out in 25 µl volumes tube containing 2µl of 34 ng/µl-1 genomic DNA, 1 µl oligoprimer, DNA master mix (GoTaq@ G2 GreenMaster Mix 2X, Promega). The thermal cycler was programmed with an initial step of 5 min at 94 °C that was followed by 35 repeated cycles for 1 min at 94 °C, an annealing step of 1 min at 36 °C and an elongati on step of 2 min at 72 °C and finally, a 7 min extension at 72 °C. Ladder contains 1500 bp was used. Amplification products were separated on 1.5% agarose with EtBr stain, diluted with 100 ml of 10x TBE (Dongsheng Biotech). PCR products were visualized on UV light and photographed using a gel documentation system.

Experiment of design and Statistical analysis:

Treatments were arranged in Split Plot De, Main Plot(time), Subplot(concentrations) , signeach treatment applied with three replications, each replication in single row (3 m long x 0.7 m width) for the open field treatments, while in the deep water culture treatments, each replication contains nine plants. Obtained data were subjected to the analysis of variance according to Snedecor and Cochran (1980). The differences between various treatments means were tested by L.S.D.

RESULTS AND DISCUSSION

Vegetative growth parameters:

Data presented in Table (1) showed that there are significant differences between different soaking periods (30, 60 and 90 min.). Soaking on EMS for 30 min. achieved the highest plant height, chlorophyll A, chlorophyll B and total chlorophyll content f leaves (9.277 cm ,1.340, 0.452 and 1.792mg/g, respectively), while soaking in EMS for 60 min. achieved the highest number of leaves per plant with value of 11.222. Soaking in EMS for 90 min achieved the lowest plant height(6.444cm) , number of leaves per plant (10.000) and chlorophyllA(1.186 mg/g), chlorophyll B(0.350 mg/g) and total chlorophyll contents in leaves (1.536 mg/g). The Influence of different concentrations of EMS (0.0, 0.1 and 0.3%) also presented in Table (1) The results showed that there are significant differences among the concentrations of EMS. The control treatment achieved the highest plant height (9.333 cm), number of leaves per plant (11.555) , chlorophyll A(1.940 mg/g), chlorophyll B(0.567 mg/g) and total chlorophyll contents in leaves and (2.507 mg/g), while the concentration of 0.1% achieved the lowest number of leaves per plant 9.777. The concentration of 0.3% achieved the lowest plant height chlorophyll A, chlorophyll B and total chlorophyll contents in leaves (7. 333cm, 0.795, 0.227 and 1.023mg/g, respectively). The results are in conformity with many earlier researchers such as Jabeen and Mirza (2004) who observed the variability in plant height through EMS treatments in *Capsicum annuum*, Dhakshanamoorthy *et al.*, (2010) in *Jatropha curcas* and Giriraj *et al.*, (1990) and Jayakumar and Selvaraj (2003) in sunflower. The high dose treatment of EMS causing growth inhibition has been ascribed to the cell division or various damages in the entire genome. Similar result was previously reported in chickpea (Ya., 1996), Durum and bread wheat (Kalia *et al.*, 2001) and many other crop plants. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromeres or on proximal segments of chromosomes (Swaminathan., 1964). leaf color mutants might be due to the fact that changed characters are controlled by more than one gene and different genes interact to give this observed mutation (Hartl and Clark., 1997).Previous studies reported that chlorophyll development seems to be controlled by many genes that are located on different chromosomes (Larkin and Scowcroft, 1981; Wang *et al*., 2013).

Table 1. Effect of soaking period with chemical mutagen and concentrations of chemical mutagen on vegetative growth characteristics of strawberry plants after 60 days from growing at open field

Soaking period (minutes)	Plant height(cm)	Number of leaves/plant	Chlorophyll a (mg/g)	Chlorophyll b(mg/g)	Total Chlorophyll (mg/g)
30	9.277	11.055	1.340	0.452	1.792
60	9.222	11.222	1.253	0.379	1.632
90	6.444	10.000	1.186	0.350	1.536
L.S.D 5%	0.279	0.364	0.015	0.012	0.014
EMS (%)	Plant height (cm)	Number of leaves/plant	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
0	9.333	11.555	1.940	0.567	2.507
0.1	8.277	9.777	1.043	0.386	1.430
0.3	7.333	10.944	0.795	0.227	1.023
L.S.D 5%	0.363	0.326	0.017	0.025	0.017

Table (2) , showed the interaction between soaking periods and concentrations of EMS. The results showed that there are significant differences among different concentrations of EMS and soaking periods as well. The control treatment (zero concentration of EMS) gave the highest plant height chlorophyll A, chlorophyll B and total chlorophyll content in leaves (10.666 cm, 1.94, 0.567 and 2.507 mg/g, respectively) under the three periods, while the EMS concentration 0.3 % for 30 min, recorded maximum number of leaves per plant 12.00. Among different interactions, the EMS concentration 0.3 % for 90 min. achieved the lowest plant height (5.67cm), number of leaves per plant (9.17cm) and chlorophyll A, chlorophyll B and total chlorophyll content in leaves (0.685, 0.175 and 0.861 mg/g, respectively). The finding of the present investigation supports the results of Murti *et al*. (2013). Aruna .(2012) observed that the values of chlorophyll contents decreased in higher concentrations. Decreased chlorophyll content with increasing concentrations of chemical mutagen confirmed the results obtained by earlier workers (Rosen *et al*., 1961). Chaudhari *et al*. (2015) observed that when the seeds of *Psoralea corylifolia* IC were exposed to 15Mm, 30Mm, 45Mm and 60Mm of EMS, the chlorophyll content decreases with higher doses of EMS.

Table 2. Effect of interaction between soaking period with chemical mutagen and concentrations of chemical mutagen on vegetative growth characteristics of strawberry plants after 60 days from transplanting date in the open field

Soaking period (minutes)	EMS Cons. (%)	Plant height (cm)	Number of leaves/plant	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total Chlorophyll (mg/g)
30	0	10.666	11.666	1.940	0.567	2.507
	0.1	8.833	9.500	1.175	0.497	1.672
	0.3	8.333	12.000	0.905	0.291	1.197
60	0	10.666	11.666	1.940	0.567	2.507
	0.1	9.000	10.333	1.023	0.356	1.379
	0.3	8.000	11.666	0.796	0.215	1.011
90	0	10.666	11.666	1.940	0.567	2.507
	0.1	7.000	9.500	0.932	0.307	1.239
	0.3	5.666	9.166	0.685	0.175	0.861
L.S.D 5%		0.629	0.564	0.029	0.044	0.029

Yield parameters:

The fruit yield per plot was significantly affected by the different soaking periods of EMS (30, 60 and 90 min.) referring to the results presented in (Table 3). Soaking in EMS for 60 min. gave the highest total number and weight of fruits yield per plot (57.55 fruits/plot and 1047.99g/plot, respectively). While the lowest total number of fruits was obtained with soaking for 90 min. (49.44 fruits/plot). Also, soaking for 30 min recorded the lowest total weight per plot 875.64 g/plot. Similar trend was observed for the effect of 0.1% concentration comparing to control (0.0 %) and 0.3% concentration for the majority of yield components traits. Hence, it could be observed that from Table (3), that yield components for the estimated traits gave their maximum performance at the 0.1 % of EMS (84.00 fruit/plot) and (1689.43 g/plot). These values decreased in the case of control with values of 32.44 fruit/plot and 499.51 g/plot , respectively . Our results at the same line with Gandhi *et al*. (2014). The concentrations of EMS, duration of its treatment caused significant differences in total number and weight of fruit yield per plot.

Table 3. Effect of soaking period with chemical mutagen and concentrations of chemical mutagen on strawberry yield

Soaking period (minutes)	Total number of fruits/plot	Total weight of fruits/plot
30	54.33	875.64
60	57.55	1047.99
90	49.44	923.55
L.S.D 5%	0.18	10.31
EMS (%)	Total number of fruits/plot	Total weight of fruits/plot
0	32.44	499.05
0.1	84.00	1689.43
0.3	44.88	658.70
L.S.D 5%	0.78	13.56

Data in Table (4) illustrated that EMS concentration 0.1 % for 30 min. having maximum total number of fruit yield per plot(88.66 fruit/plot), while control treatment achieved the lowest total number of fruit 28.00 fruit/plot. On the other hand, EMS 0.1% for duration of 90 min gave maximum total weight per plot estimated by 1926.25g/plot, while the duration of 90 min. with concentration 0.3 % achieved the lowest total weight per plot estimated by 357.08g/plot. The results are in conformity with previous work which reported maximum number of fruits with 0.1 % EMS treatment in *capsicum* (Jabeen and Mirza.,2004). The higher doses have inhibitory effect and reduce the growth and yield in *Helianthus annuus L.* (Khursheed *et al*., 2009).

Table 4. Effect of interaction between soaking period with chemical mutagen and concentrations of chemical mutagen on strawberry yield

Soaking period (minutes)	EMS (%)	Total number of fruits/plot	Total weight of fruits/plot
30	0	28.00	430.51
	0.1	88.66	1379.55
	0.3	46.33	816.88
60	0	28.00	430.51
	0.1	81.00	1762.51
	0.3	54.00	802.14
90	0	28.00	430.51
	0.1	82.33	1926.25
	0.3	34.33	357.08
L.S.D 5%		1.36	23.48

Significant differences were observed among the three soaking times (30, 60 and 90 min) with EMS concentrations for fruit chemical composition traits in current study Table (5). Soaking in EMS for 30 min. achieved the highest fat%, moisture% and fibers% (0.846%, 7.998% and 13.311%, respectively). Meanwhile, the lowest

results were achieved with protein %, ash, and t-carbohydrate (10.392%, 3.592% and 64.145%, respectively). On the other hand, soaking in EMS for 90 min achieved the highest protein % and ash, 10.653% and 3.682%, respectively. Meanwhile, the lowest results were achieved with fat%, moisture% and fibers% with perentages of 0.794%, 7.301% and 13.055%, respectively. The highest t-carbohydrate values were obtained by soaking in EMS for 60 min 64.307%. Similar trend was observed for the effect of different EMS concentrations of control, 0.1% and 0.3% for the chemical content of the strawberry fruits. control treatment gave the highest value for fat%, moisture% and fibers% with perentages of (0.943%, 7.946% and 13.840%, respectively). Whereas the 0.3% EMS concentration gave the highest value of protein and ash, 10.954% and 3.866%, respectively. While achieved the lowest result, were achieved with fat%, moisture% and fibers% (0.684%, 7.012% and 12.570%, respectively). The highest t-carbohydrate value was obtained from 0.1% EMS concentration 64.496%.

Table 5. effect of soaking period with chemical mutagen and concentrations of chemical mutagen on chemical characteristics of strawberry fruits.

Soaking period (minutes)	FAT(%)	Moisture (%)	Protein content (%)	Ash (%)	Fiber (%)	T-Carbo.(%)
30	0.846	7.998	10.392	3.592	13.311	64.145
60	0.824	7.312	10.464	3.631	13.274	64.307
90	0.794	7.301	10.653	3.682	13.055	64.235
L.S.D 5%	0.011	0.021	0.018	0.015	0.020	0.014
EMS Cons. (%)	FAT (%)	Moisture (%)	Protein content (%)	Ash (%)	Fiber (%)	T-Carbo.(%)
0	0.943	7.946	10.073	3.336	13.840	63.930
0.1	0.837	7.653	10.482	3.702	13.231	64.496
0.3	0.684	7.012	10.954	3.866	12.570	64.262
L.S.D 5%	0.011	0.022	0.018	0.021	0.0170	0.0130

The data given in Table (6) revealed that the concentrations of EMS, duration of EMS treatment orientation resulted significant differences for the chemical content of strawberry fruits. Control treatment gave the highest value for fat% and fibers% 0.943% and 13.840%, respectively, while the lowest results were achieved for protein %, ash, and T- carbohydrate (10.073%, 3.336% and 63.930%, respectively). On the other hand, EMS 0.3% with 90 min. duration gave the maximum values of protein % and ash, 11.030% and 3.936%, respectively, while the lowest results were achieved with fat%, moisture% and fibers% (0.650%, 6.840% and 12.223%, respectively). EMS 0.1% for duration of 90 min gave maximum T-carbohydrate% values 64.753% and EMS 0.1% for duration of 30 min gave maximum moisture% values (8.723%). The improvement in fruit quality with lower doses of EMS may be due to the fact that EMS at a lower dose induces hormones which are responsible for the improvement of fruit quality. The mutagens at higher dose reduced fruit quality which might be due to the inhibitory effect of higher doses of ethyl methane sulphonate. The positive effects of EMS mutagenesis on biochemical composition. The positive effects of EMS mutagenesis on biochemical composition and nutritional quality of fruits in the present study are also reported by earlier researchers namely, (Bermego *et al*., 2012; and Kim *et al*., 2012 a &b) who had reported that gamma-irradiation mutagenesis had varied effects oninternal quality of fruits. The results of present study also indicated the varied effects of gamma irradiation on TSS, acidity and vitamin, without impairing nutritional quality. Similar results with gamma irradiation were also reported by (Goldenberg et al., 2014) in mandarin fruit. The results of the study are also supported by the findings of (Figueiredo et al., 2014) who observed better quality of papaya fruit with irradiation of gamma rays.

Table 6. Effect of interaction between soaking period with chemical mutagen and concentrations of chemical mutagen on chemical characteristics of strawberry fruits

Soaking period (minutes)	EMS (%)	FAT (%)	Moisture (%)	Protein content (%)	Ash (%)	Fiber (%)	T-Carbo (%)
	0	0.943	7.946	10.073	3.336	13.840	63.930
30	0.1	0.880	8.723	10.240	3.646	13.326	64.056
	0.3	0.716	7.326	10.863	3.793	12.766	64.450
	0	0.943	7.946	10.073	3.336	13.840	63.930
60	0.1	0.843	7.120	10.350	3.686	13.263	64.680
	0.3	0.686	6.870	10.970	3.870	12.720	64.313
	0	0.943	7.946	10.073	3.336	13.840	63.930
90	0.1	0.790	7.116	10.856	3.773	13.103	64.753
	0.3	0.650	6.840	11.030	3.936	12.223	64.023
L.S.D 5%		0.020	0.039	0.031	0.037	0.030	0.023

Salt Stress Evaluation

Vegetative growth parameters:

The data given in Table (7) revealed that there are significant differences among different soaking periods of EMS (0, 60 and 90 min.) on vegetative growth parameters of strawberry plants. Soaking in EMS for 90 min. achieved the highest number of leaves and root length/ plant (14.89 and 14.11cm, respectively) also soaking on EMS for 60 min. gave maximum plant height/ per plant (15.22cm). Control treatment achieved the lowest number of leaves**,** plant height and root length/ per plant (9.89, 12.44 and 8.00cm, respectively).

Results of Na Cl concentrations showed a significant difference among traits, control treatment achieved the highest number of leaves, plant height and root length/ per plant (18.11, 17.61 and 16.39cm, respectively). The Na Cl

concentration(1000 pmm)gave the lowest number of leaves, plant height and root length/ per plant (9.22, 11.61 and 8.11cm, respectively). The results are in line with the results of Alnayef (2012) who demonstrated that the leaves area and the number of leaves in strawberry cultivars negatively reduced in response to increasing salinity stress. Zhu (2001) noted that in a salt tolerant plant, the growth rate generally decreased owing to stomatal closure, limited CO_2 uptake and consequently inadequate photosynthesis and inhibition of cell division and expansion, but growth continued. Awang *et al*. (1993) found that addition of Na Cl into irrigation water decreased leaves number by 36%, leaf area by 48% and dry shoot weight by 47% compared with the control groups in strawberry plants.

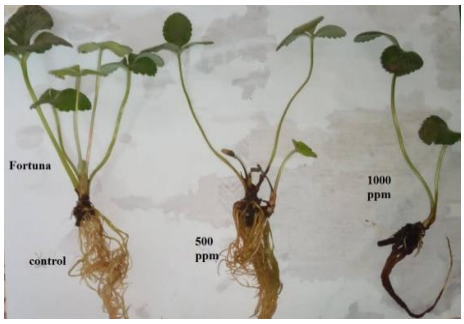
Table 7. Effect of soaking period with chemical mutagen and concentrations of NaCl on the vegetative growth characteristics of strawberry plants after 50 days from growing on deep water culture system

Soaking period (minutes)	No. of leaves	Plant height	Root length
0	9.89	12.44	8.00
60	14.11	15.22	11.67
90	14.89	13.83	14.11
L.S.D 5%	0.319	0.244	0.211
Na Cl Concentrations ppm	No. of leaves	Plant height	Root length
0	18.11	17.61	16.39
500	11.56	12.28	9.28
1000	9.22	11.61	8.11
L.S.D 5%	0.217	0.252	0.241

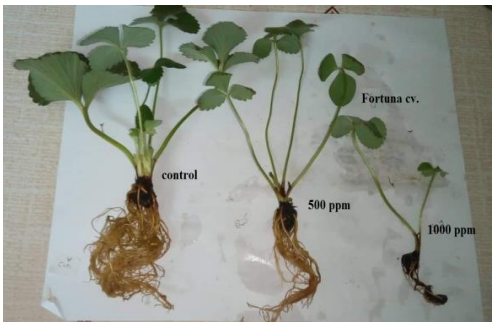
The obtained results from Table 8 showed that there was a significant difference among the interaction between Na Cl concentrations and soaking periods with EMS at concentration 0.1%. It could be observed that the great increase in number of leaves, plant height and root length/ per plant attributed to treatment by EMS for 90 min with the lack of any salinity stress (19.00, 18.00 and 18.33cm, respectively), while the Na Cl concentration(1000ppm) without applying EMS gave the lowest number of leaves, plant height and root length/ per plant (5.00 leaves, 9.50 cm and 4.67cm, respectively). Whereas, the treatment 90 min of soaking with 1000 ppm Na Cl gave the best response against high salinity stress for number of leaves and root length/ per plant that estimated by 12.00 and 11.50cm, respectively. On the other hand, the treatment 60 min of soaking with 1000 ppm Na Cl gave the best response against high salinity stress for plant height that estimated by 13.83cm. Our results are at the same trend with (Saba and Mirza., 2002). The chemical mutagen EMS has been used in crop breeding programs when different parts of plants are exposed to this mutagen, it disturbs the metabolic activities ultimately may resistance in plant (Salim *et al*., 2009). Generally, soaking with EMS treatment gave good performance ability for the strawberry plants under salt stress conditions compared with the control as shown in figure (1).

Table 8. Effect of interaction between soaking period with chemical mutagen and concentrations of Na Cl on the vegetative characteristics of strawberry plants after 50 days from growing on deep water culture system

Soaking period (minutes)	Na Cl concentrations (ppm)	No. of leaves	Plant height	Root length
0	0	18.33	17.83	14.33
	500	6.33	10.00	5.00
	1000	5.00	9.50	4.67
60	0	17.00	17.00	16.50
	500	14.67	14.83	10.33
	1000	10.67	13.83	8.17
90	0	19.00	18.00	18.33
	500	13.67	12.00	12.50
	1000	12.00	11.50	11.50
L.S.D 5%		0.375	0.436	0.418



(A) Na Cl stress without EMS treatment



(B) Soaking for 60 min. on EMS

(C) Soaking for 90 min. on EMS

Figure 1. Improvement in morphological parameters in Fortuna cultivar that mutagenized with EMS and subjected to Na Cl stress compared.

Potassium and Sodium Content

The Influence of different soaking periods (30, 60 and 90 min.) on potassium and sodium content of strawberry leaves showed in Table (9). Control treatment achieved the highest potassium content and the ratio of potassium /sodium (K / Na) that estimated by 24.53 ppm and 1.51, respectively. Whereas, soaking in EMS for 90 min. gave maximum sodium content that estimated by 23.80 ppm. On the other hand, control treatment achieved the lowest sodium content that estimated by 17.31ppm, while soaking in EMS for 60 min achieved the lowest potassium content that estimated by 21.47ppm. The ratio of potassium per sodium (K / Na) decreased with increasing different soaking periods.

The effects of salt applications on sodium (Na^+), potassium (K^+) show significant differences among salinity treatments, 500 and 1000 ppm of Na Cl. Control treatment achieved the lowest potassium and sodium content of strawberry leaves that estimated by 22.66ppm and 15.23ppm, respectively, while the same treatment gave the maximum ratio of K / Na that estimated by 1.60.

Generally, sodium content of strawberry leaves increased with increasing salinity level in the irrigation water. Na Cl concentration 1000 ppm gave the maximum sodium content that estimated by 26.78 ppm, while the same treatment gave the lowest ratio of (K / Na) that estimated by 0.90. Whereas, Na Cl concentration 500 ppm gave the maximum of the potassium content that estimated by 23.36 ppm. Therefore, plants with higher K/Na ratios under salinity conditions can tolerate salinity stress more effectively than plants with lower ratios (Ghadakchiasl., et al. 2017).

Table 9. Effect of soaking period with chemical mutagen and concentrations of Na Cl on potassium, sodium content and the ratio of potassium to sodium of strawberry leaves after 50 days from growing in hydroponic culture system

Soaking period (minutes)	K	Na	K/Na
0	24.53	17.31	1.51
60	21.47	20.56	1.22
90	23.01	23.80	0.98
L.S.D 5%	0.166	0.306	0.044
Na Cl concentrations (ppm)	K	Na	K/Na
0	22.66	15.23	1.60
500	23.36	19.67	1.21
100	22.99	26.78	0.90
L.S.D 5%	0.199	0.164	0.028

In Table (10), the presented results showed that there were significant differences among Na Cl different concentrations combined with soaking periods of EMS at concentration 0.1%. It could be observed that the great increase of the potassium content that estimated by 27.30 ppm, attributed to the treatment by 1000 ppm Na Cl concentration without applying EMS. Whereas, the treatment 60 min of soaking with 1000 ppm Na Cl gave the maximum sodium content that estimated by 32.12 ppm. The same treatment gave the lowest ratio of potassium per sodium (K / Na) that estimated by 0.64. On the other hand, control treatment achieved the lowest potassium and sodium contents of strawberry leaves that estimated by 20.48 and 10.54 ppm, respectively. The same treatment gave the maximum ratio of potassium per sodium (K / Na) that estimated by 1.95. Our results showed K^+ increment, although not always leaves of strawberry cultivar, there was no evidence of competition between Na+ and K+ in strawberry leaves. Similar results were reported by , Keutgen and Pawelzik., 2009, Sun *et al*., 2015, Adolf *et al*., 2013 and Jorge, *et al*.,2019).

Salinity increased the amount of Na concentration in the plant leaves when the salt applied to the aerial part of plant, Turhan and Atilla, (2004) and Kaya *et al.*, (2002) reported that Na concentration increased in leaves of two strawberry cultivars in the presence of Na Cl stress.

Table 10. Effect of interaction between soaking period and chemical mutagen and concentrations of Na Cl on potassium, sodium content and the ratio of potassium to sodium of strawberry leaves after 50 days from growing in hydroponic culture system

Soaking period (minutes)	Na Cl concentrations (ppm)	K	Na	K/Na
0	0	20.48	10.54	1.95
	500	25.81	19.67	1.31
	1000	27.30	21.74	1.26
60	0	22.36	13.23	1.70
	500	21.45	16.33	1.32
	1000	20.60	32.12	0.64
90	0	25.15	21.93	1.15
	500	22.81	23.00	0.99
	1000	21.06	26.48	0.80
L.S.D 5%		0.344	0.283	0.048

RAPD analysis:

RAPD markers have been used widely to detect the genomic DNA for identification and characterization of genetic diversity in different strawberry cultivars in response to abiotic stress conditions. It was apparent from Table (11) and figure (2) that there was certain variability between treated and untreated strawberry cultivars with EMS in respect to four primers (OPA-5; OPA-1; OPA-7; OPA-8).

The total numbers of bands obtained from DNA samples amplification using OPA-5 primer were 14 bands ranged from 808 to 154 bp. Three monomorphic band were detected at molecular size with 199.3, 239.6 and 387.3bp. In the same time, six bands were detected polymorphic without unique bands. The polymorphic bands were detected at molecular size with 495.4 bp, presented from control, control + Na Cl (1000ppm) and EMS (for 60min). Meanwhile, that polymorphic band was absent at the other concentrations. Also the other two polymorphic bands were detected at molecular size with 527.2 and 613.3bp presented at EMS (60min) + Na Cl(500 ppm) and EMS (60min) + Na Cl (1000ppm) but these two polymorphic bands were absent at the other concentrations including the control (untreated plants). On the other hand, the remain three polymorphic bands were detected at molecular size with 499.8, 694.7and 808.1 bp, respectively, and presented at EMS (90min) + Na Cl (500ppm) and EMS (90min) + Na Cl(1000ppm) but these three polymorphic bands were absent at the other concentrations including the untreated plants (control plants). There were new four unique bands were observed from treated plants compared with those untreated, and only one unique band was detected in untreated plants. Two unique bands were detected with EMS (90min) and apparent at molecular size with 309.1 and 471.0bp Meanwhile, the other two bands were present at the lower

concentration of EMS (60 min.) and with 1000 ppm Na Cl. The polymorphism percentage was 78.54% and the mean of bands frequency was 0.375.

In case of OPA-1 primer, the total numbers of detected bands were 11 bands ranged from 538.9 to 101.0 bp. Three monomorphic bands were detected and three were polymorphic without unique. One polymorphic band was detected at molecular size with 199.2 bp presented at all treatments except the control and control + Na Cl (1000 ppm). The other two polymorphic bands were detected at molecular size with 122.7and 147.4 bp, respectively, and presented at EMS (90min) + Na Cl (500 ppm) and EMS (90min) + Na Cl (1000 ppm). On the other hand, there were three unique bands which detected in treated plants, while two unique bands were observed in control plants. The unique bands that found in treated plants apparent at molecular size with 131.3, 164.1 and 538.9 bp. The polymorphism percentage was 72.73% and the mean of bands frequency was 0.443.

For OPA-7 primer, the total numbers of resulted bands were 7 bands ranged from 827 to 126 bp. Four monomorphic band were detected and two were polymorphic with unique. One polymorphic band was detected at molecular size with 524.3 bp presented at all treatment except control. The other polymorphic band was detected at molecular size with 681.5 bp presented at EMS (90min), EMS (90min) + Na Cl (500 ppm) and EMS (90min) + Na Cl (1000 ppm) but this polymorphic band was absent at the other concentrations. On the other hand, there were new bands apparent at treated plants with EMS (90min) compared with other treatments. That new bands apparent at molecular size with 827.8 bp. The polymorphism percentage was 42.86% and the mean of bands frequency was 0.768.

Table 11. Number of fragment bands and unique bands resulted from four RAPD primers for Fortuna that treated with EMS or treated with EMS + Na Cl compared to control.

Primer Name	Mono-morphic bands	Unique bands	Poly. Without unique bands	Poly. %	mean of band frequency	Mw (bp)	Treatments							
OPA-5	3	5	6	78.57%	37.50%	MW	FT1	FT2	FT3	FT4	FT5	FT6	FT7	FT8
14)808-154bp)						808.1	-	-	-	-	-	-	+	+
						694.7	-	-	-	-	-	-	+	+
						613.3	-	-	-	+	+	-	-	-
						527.2	-	-	-	+	+	-	-	-
						499.8	-	-	-	-	-	-	+	+
						495.4	+	+	+	-	-	-	-	-
						471.0	-	-	-	-	-	+	-	-
						387.3	+	+	+	+	+	+	+	+
						319.4	-	-	+	-	-	-	-	-
						309.1	-	-	-	-	-	+	-	-
						265.8	+	-	-	-	-	-	-	-
						239.6	+	+	+	+	+	+	+	+
						199.3	+	+	+	+	+	+	+	+
						154.4	-	+	-	-	-	-	-	-
OPA-1	3	5	3	72.73%	0.443	538.9	-	+	-	-	-	-	-	-
11(538-101bp)						391.5	+	+	+	+	+	+	+	+
						310.1	+	+	+	+	+	+	+	+
						239.2	+	+	+	+	+	+	+	+
						199.2	-	-	+	+	+	+	+	+
						164.1	-	-	+	-	-	-	-	-
						150.5	+	-	-	-	-	-	-	-
						147.4	-	-	-	-	-	-	+	+
						131.3	-	-	+	-	-	-	-	-
						122.7	-	-	-	-	-	-	+	+
						101.0	+	-	-	-	-	-	-	-
OPA-7	4	1	2	42.86%	0.768	827.8	-	-	-	-	-	+	-	-
7(827-126bp)						681.5	-	-	-	-	-	+	+	+
						524.3	-	+	+	+	+	+	+	+
						338.3	+	+	+	+	+	+	+	+
						254.7	+	+	+	+	+	+	+	+
						161.3	+	+	+	+	+	+	+	+
						126.8	+	+	+	+	+	+	+	+
OPA-8	5	0	4	44.44%	0.75	670.1	+	+	+	+	+	+	+	+
9(670-99bp)						575.8	+	+	+	+	+	-	-	-
						473.7	-	-	-	-	-	+	+	+
						381.8	-	-	-	-	-	+	+	+
						259.2	+	+	+	+	+	+	+	+
						213.2	+	+	+	+	+	+	+	+
						161.2	+	+	+	+	+	+	+	+
						131.1	+	+	+	+	+	+	+	+
						99.1	-	-	+	+	+	-	-	-

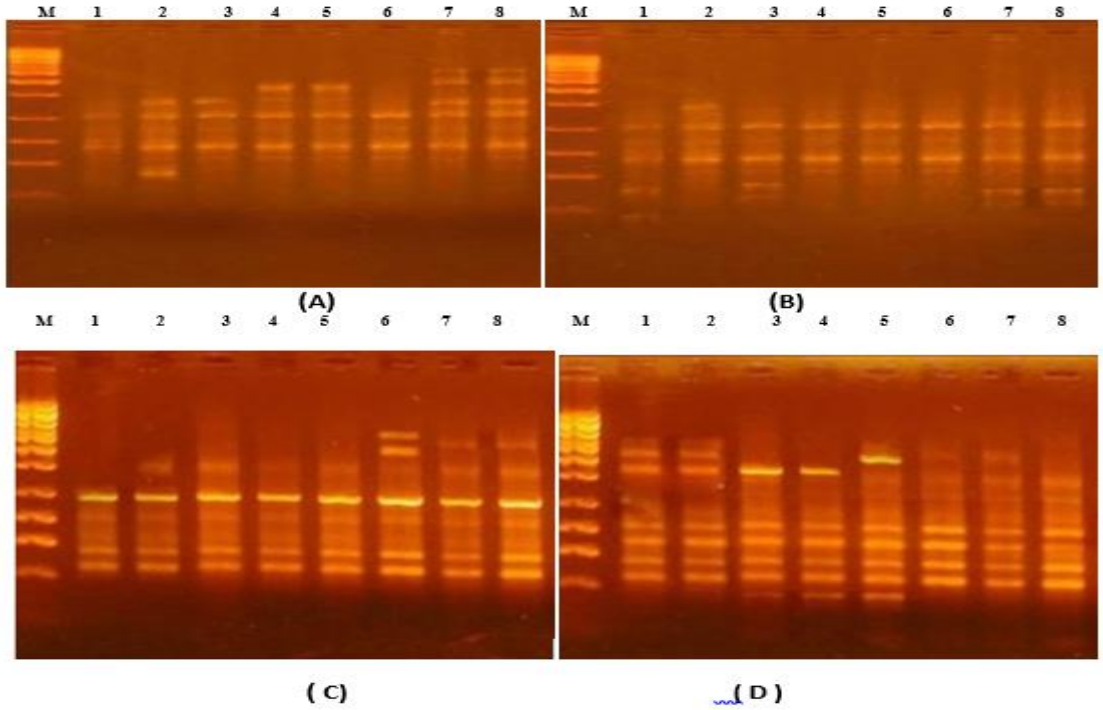


Figure 2. Agarose gel electrophoresis of PAPD profile for primers (A: OPA-5; B: OPA-1; C: OPA-7; D: OPA-8). M is DNA ladder (100 pb). The lanes numbered as following; 1: plants control, 2: plants treated with1000ppm NaCl, 3: plants treated with EMS 60 min. 4: plants treated with EMS 60min+ NaCl500ppm. 5: plants treated with EMS 60min+ NaCl1000ppm. 6: plants treated with EMS 90min .7: plants treated with EMS 90min+ NaCl500ppm. 8: plants treated with EMS 90min + NaCl1000ppm.

Finally, the total numbers of bands resulted from samples DNA amplification using OPA-8 primer were 9 bands ranged from 670.1 to 99.1 bp. Five monomorphic bands were detected and four were polymorphic with unique. One polymorphic band was detected at molecular size with 99.1 bp presented at EMS(60min), EMS(60min) + Na Cl (500 ppm) and EMS (60min) + Na Cl (1000 ppm), but the polymorphic band was absent at the other concentrations. Also, two polymorphic bands were detected at molecular size with 381.8 and 473.7 bp presented at EMS (90min), EMS (90min) + Na Cl (500 ppm) and EMS (90min) + Na Cl (1000 ppm), while that polymorphic bands were absent at the other concentrations. On the other hand, there was one polymorphic band was detected at molecular size with 575.8 bp presented at all treatment except EMS (90min), EMS (90min) + Na Cl (500 ppm) and EMS (90min) + Na Cl (1000 ppm). The polymorphism percentage was 44.44% and the mean of bands frequency was 0.75. Results showed Na Cl stress combined with EMS treatment which changed the genomic DNA of Fortuna genotype. Also, the results displayed different number of bands for some primers used individually in response to EMS or Na Cl treatments.

The results are in line with those of Coungiu *et al*. (2000) who tested 31 strawberry cultivars and found that 13 out of these cultivars had consistent bands differed from control. The results are also in agreement with the results of Gaafar and Saker (2006) who found that using RAPD-PCR for identification of different genomic strawberry cultivars to determine and estimate the genetic distances between cultivars and their genetic relationships since the results detected a high level of genetic variability among seven strawberry cultivars. Bhat *et al*. (2015) stated that leaf discs in strawberry treated with EMS led to positive change of phenotypic traits. RAPD was utilized effectively in strawberry to study genetic differences between cultivars (Graham *et al*., 1996). Abbas H.K. *et al*. (2018) revealed that EMS as a chemical mutagen is promising in enhancing Na Cl tolerance. EMS may modify G/C to A/T and induces mutations in some DNA sequences. This could be an evidence of the effect of EMS in creating mutation in plant cell and tolerant to salt stress as well.

CONCLUSION

From the obtained results of this study, it can be concluded that soaking of EMS treatment improved the vegetative growth and yield characteristics under salt stress conditions compared to the control such as plant height, number of leaves, chlorophyll contents, number of fruits/plot and weight of fruits/plot. High values for fruit quality parameters were found with EMS treatment compared to control plants. In this study , four RAPD primers were used and results revealed that a total number of 15 detected polymorphic bands, out of 12 bands were resulted after EMS treatment, 11 unique bands were observed, out of 6 with EMS treatment. So, EMS as a chemical mutagen is consider a promising in enhancing salt tolerance. Moreover, the results proved its usefulness for induction of genetic variability in this vegetable crops.

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تحسين تحمل الملوحة في نباتات الفراولة بإستخدام معاملات سلفونات ايثيل الميثان

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فى مصر تعتبر الفراولة من اهم محاصيل الفاكهة المصدرة ، كما يتم تداولها محليا على نطاق واسع. يعتبر محصول الفراولة حساس لملوحة التربة ومياه الرى حيث ان زيادة الملوحة عن ٧٠٠ جزء فى المليون لها تاثير سلبى على النمو الخضرى وكذلك على المحصول. أجريت هذه الدراسة بغرض الحصول على تباينات وراثية من صنف فورتونا لنبات الفراولة وتقييم مدى تحملها للاجهاد الملحى بواسطة سلفونات ايثيل الميثان. تم اضافة سلفونات ايثيل الميثان (EMS) بثلاثة تركيزات هى (٠,٠ ، ٠,١ و ٠,٣%) لثلاثة فترات زمنية مقدارها(٣٠ ،٦٠ و٩٠ دقيقة) على البراعم الطرفية (المدادات) لنباتات صنف فورتونا. ثم تم زراعة شتلات نباتات الفراولة المتحصل عليها فى الحقل المفتوح لتقدير صفات النمو الخضرى والمحصول. تم اختيار افضل السلالات لتقيمها تحت تركيزين من ظروف الاجهاد الملحى(٥٠٠ و ١٠٠٠جزء فى المليون من كلوريد الصوديوم). تم استخدام تقنية التسلسل المتعدد للحامض النووى (RAPD-PCR) لتحديد تاثير التباينات المتحصل عليها من استخدام سلفونات ايثيل الميثان. اوضحت النتائج ان النقع فى سلفونات ايثيل الميثان ادى الى تحسين النمو الخضرى وصفات المحصول تحت ظروف الاجهاد الملحى مقارنة بالنباتات الغير معاملة (المقارنة) لصفات ارتفاع النبات ، عددالاوراق ، محتوى الكلوروفيل وعدد ووزن الثمار/ وحدة تجريبية. اظهرت النتائج ايضا قيما مرتفعة لمعايير وصفات جودة الثمار مثل النسبة المئوية لكل من الدهون (%) ، البروتينات (%) ، الرماد (%)، الرطوبة (%) ، الكربوهيدرات (%) و الالياف (%). باستخدام اربعة معلمات جزيئية (RAPD) تم تحديد خمسة عشر حزمة متعددة الاشكال المظهرية، منها اثنتي عشرة حزمة ظهرت فى النباتات المعاملة بسلفونات ايثيل الميثان. تم تحديد احدى عشر حزمة فريدة منها ثمانية حزم فى النياتات المعاملة بسلفونات ايثيل الميثان. ولقد نتج عن هذة الدراسة انه يمكن اعتبار استخدام سلفونات ايثيل الميثان كمطفر كيميائيا واعدا فى تحسين وزيادة تحمل الملوحة لنباتات الفراولة. علاوة على ذلك، يمكن استخدام سلفونات ايثيل الميثان كأداة مفيدة وفعالة فى استحداث تباينات وراثية جديدة فى محصول الفراولة.