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EFFECT OF ETHYL METHANE SULPHONATE ON IMPROVING THE PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF TWO STRAWBERRY CULTIVARS

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Abstract

A study was conducted in the laboratory of plant tissue culture in the College of Agriculture/ University of Anbar to determine the effect of four concentrations of ethyl methane sulfonate (EMS) 0, 1, 2 and 3% on the biochemical characteristics of two cultivars of Albion and Rubjem under three levels of water stress using four levels of 0, 3, 6 and 9% of polyethylene glycol (PEG-6000). A factorial experiment was carried out using ten replicates according to CRD design. Albion cultivar plants treated with 9.0% PEG and 3.0% EMS concentration recorded the highest average APX enzyme activity of 0.5033 mmol min^{-1} mg^{-1} and CAT enzyme activity of 21.067 mmol min^{-1}. The treatment of Albion cultivar plants with 9.0% PEG and 1.0% EMS concentration recorded the highest average for each of the iron contents, which reached 53.80 mg g^{-1} DW. Albion cultivar also achieved the highest average for GPX activity, which reached 0.160 mmol min^{-1} mg^{-1} under the level of 9.0% PEG and 2.0% EMS concentration. While the same cultivar Rubigim recorded the lowest significant reduction in H_2O_2 content at the two concentrations of 3.0% PEG and 3.0% EMS, which reached 0.540 µg g^{-1} FW. While the treatment with 3.0% EMS and 3.0% PEG

concentrations for the same cultivar showed the highest average for the content of the amino acid proline, which reached 2.90 µg g^{-1}.

Keywords: Fragaria ananassa Duch, EMS, PEG, Antioxidant Enzyes.

تأثير Ethyl Methane Sulphonate في تحسين الخصائص الفسيولوجية والكيميائية الحيوية لصنفين من الشليك

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الخلاصة

أجريت دراسة في مختبر زراعة الانسجة النباتية في كلية الزراعة/ جامعة الانبار لمعرفة تاثير أربعة تراكيز من مركب اثيل ميثان سلفونيت (EMS) 0 و1 و2 و3% في الصفات الكيموحيوية لصنفين من الشليك البيون وروبجيم تحت ثلاث مستويات من الاجهاد المائي باستخدام اربعه مستويات 0 و3 و6 و9% من البولي اثيلين كلايكول (PEG– 6000). نفذت تجربة عاملية باستخدام عشرة مكررات وفق تصميم CRD. سجلت نبيتات صنف البيون المُعاملة بالمستوى 9.0% من PEG والتركيز 3.0% من EMS أعلى متوسط لفعالية انزيم APX بلغ 0.5033 ملي مول دقيقة$^{-1}$ ملغم$^{-1}$ وفعالية انزيم CAT بلغ 21.067 ملي مول دقيقة$^{-1}$. اما معاملة نبيتات الصنف البيون بتركيز 9.0% من PEG والتركيز 1.0% من EMS فقد سجل اعلى متوسط لكل من محتوى الحديد بلغ 53.80 ملغم غم$^{-1}$ وزن جاف. كما حقق الصنف البيون اعلى متوسط لفعالية انزيم GPX بلغ 0.160 ملي مول دقيقة$^{-1}$ ملغم$^{-1}$ تحت المستوى 9.0% من PEG والتركيز 2.0% من EMS. بينما سجل ذات الصنف روبيجيم عند التركيزين 3.0% من PEG و 3.0% من EMS أدنى تقليص معنوي لمحتوى H_2O_2 بلغ 0.540 مايكرو غم غم$^{-1}$ وزن طري. في حين أظهرت المعاملة بالتركيز 3.0% لمركب EMS و3.0% PEG لنفس الصنف اعلى متوسط من محتوى الحامض الاميني البرولين بلغ 2.90 مايكروغرام غم$^{-1}$.

كلمات مفتاحية: الشليك، EMS، PEG، مضادات الأكسدة الانزيمية.

Introduction

Fragaria ananassa Duch is an important fruit crop known for its nutritional and medical benefits for the human body, as it is characterized by its high content of carotenoids, phenols, flavonoids, anthocyanins, tannins and vitamins. All of these chemical components have important biological activities by being antioxidant,

antibacterial, anti-inflammatory (30), anticancer and antimicrobial (11 and 15) and is used in the treatment of diabetes, heart diseases, atherosclerosis and blood vessels (13) and helps in activating memory and delaying the onset of aging (6 and 8).

There is still a large gap in the level of local production and consumption of the strawberry plant, and this is due to many reasons, including, but not limited to, the lack of use of appropriate varieties and the lack of use of modern scientific methods in agriculture, in addition to the problems resulting from irrigation and salinization of lands suitable for agriculture. All of these factors are likely to affect the level of production.

Drought stress is one of the most dangerous and common problems that affect plants among other abiotic stress, especially in dry and semi-dry areas, including Iraq, due to climate change. The strawberry plant is classified as a drought-sensitive plant due to its fibrous and superficial roots (10). Resorting to biotechnology in general and tissue culture in particular has become inevitable, to keep pace with the challenge of growing abiotic stresses, most notably drought stress, in order to improve the performance of plant species in the desired direction. Plant tissue culture has several positive characteristics that distinguish it from traditional methods of preserving and propagating plant species. It is based on the ability of many of these species to produce a complete plant from a cell or plant part. It thus contributes to facilitating and accelerating programs for obtaining stress-tolerant plants with an understanding of the mechanisms of tolerance and the accompanying biochemical changes that have positive effects on plant physiology, causing a change in plant growth and enhancing the tolerance of different plant species. Chemical mutation is one of the modern methods used to create desired genetic changes, which can increase plant tolerance to various stress. Many chemical compounds are used to induce mutations in vitro, taking into account caution when using them because they are carcinogenic materials, which made resorting to cell cultures safer than field spraying, in addition to the fact that the latter requires increasing the concentration of the active substance and thus increasing the accumulation of these materials in the tissues and increasing their risk (25). Ethyl methane sulphonate (EMS) is one of the chemical agents that cause mutations at the point level, and mutations are changes in the DNA sequence and are divided into genetic mutations and chromosomal mutations and are a source of genetic variations (23). EMS is considered a chemical mutagen and is the least harmful alkyl agent to (Deoxyribonucleic acid) DNA. Mutation was resorted to to obtain new varieties and obtain plants with good specifications and high productivity. This compound was used to develop a limited trait or multiple traits. Chemical mutagen was used with tissue culture as a means to induce many genetic variations to improve plant traits. The chemical mutagen EMS is considered the most important point mutagen that leads to genetic variations (28). Therefore, the current study aimed to determine the effect of EMS compound in two rows of strawberry under different levels of drought stress outside the living body by studying some physiological and biochemical.

Materials and Methods

The study was conducted at the College of Agriculture/ University of Anbar in the Tissue Culture Laboratory of the Department of Horticulture and Landscape

Engineering for 2022-2023. Two strawberry varieties, Albion and Ruby gem, were used. A factorial experiment was carried out using a completely randomized design (CRD) with ten replications, and the means were compared with the least difference test. Significant LSD and at the probability level of 0.05 using the statistical program Statistical Analysis System (27); the metal and glass tools, distilled water, and food media used in the experiment were sterilized according to the method described by (4); the standard MS medium that was discovered by (20) was used, where 30 g L^{-1} was sucrose. Then, the plant growth regulators were added according to each stage of the experiment's requirements, adding 0.5 mg L^{-1} of the growth regulator BA, to which 0.1 mg L^{-1} of the growth regulator IBA in the embryonic stage. While concentrations of 1.0 mg L^{-1} of the growth regulator BA were added, 0.1 mg L^{-1} of the growth regulator IBA was added during the multiplication stage. The pH was then adjusted to 5.7±1.

Estimation of H_2O_2 content (µg g^{-1} FW): The method described by AOAC (1) was used to determine the content of H_2O_2 in plant samples.

Estimation of MDA content (µmol gm^{-1} FW): Malondialdehyde (MDA) content was estimated according to the method described by Carmak and Horst (9), according to the equation described by Gao (12).

Estimation of CAT activity (mmol $min^{-1}mg^{-1}$): The method described by Luhova et al., (16) was used to determine of CAT activity in plant samples.

Estimation of APX activity: The APX activity was estimated according to the method proven by Anjum et al., (5).

Estimation of GPX activity: The GPX activity was measured according to the method described Maehly and Chance (17).

Estimation of iron content (mg g^{-1} DW): This is carried out according to the method proven, and the iron element is estimated using an absorption atomic device.

Extraction and estimation of proline (µg g^{-1} FW): The method described by Bates et al., (7) was used to extraction and estimation of proline in plant samples.

Results and Discussion

H_2O_2 content: It is noted from Table 1 that there is a significant effect of all study factors and their interactions on the (H_2O_2) content of the Albion and Ruby gem varieties, where the Albion cultivar excelled with the highest significant value for this characteristic amounting to 0.72 µg g^{-1} FW, while the Ruby gem cultivar recorded the least significant difference of 0.69 µg g^{-1} FW and increasing the water stress led to an increase in the hydrogen peroxide content, as the concentration of 3% achieved the highest significant value for the trait amounting to 0.78 µg g^{-1} FW, while the comparison treatment recorded the lowest significant value for the characteristic amounting to 0.65 µg g^{-1} FW, as for adding concentrations of the chemical mutagen EMS, it achieved significant differences in the trait, as the concentration exceeded 2%, recording the highest significant difference of 0.78 µg g^{-1} FW, while the 3% concentration gave the lowest significant difference in hydrogen peroxide content, amounting to 0.57 µg g^{-1} FW. Regarding the bilateral interactions between the cultivar and water stress, the Albion cultivar with a 3% concentration of water stress recorded the highest significant value for the trait, amounting to 0.80 µg g^{-1} FW, while the lowest

significant value was for the Ruby gem cultivar, and the comparison treatment for water stress amounted to 0.64 µg g^{-1} FW. As for the bilateral interaction between the cultivar and the mutagen, the treatment with a concentration of 2% of the mutagen for the Albion cultivar achieved the highest significant value for the trait, which amounted to 0.80 µg g^{-1} FW, while the lowest significant value was for the Ruby gem cultivar at a concentration of 3% of the mutagen, as it reached 0.56 µg g^{-1} FW As for the binary interaction between the mutagen and water stress, there were significant differences between the study factors as well, as the comparison treatment of the mutagen and a 3% concentration of the water stress recorded the highest significant value of 0.92 µg g^{-1} FW. In comparison, the treatment with a 1% concentration of the mutagen and the unexposed group was achieved. The water stress had the lowest significant value, which amounted to 0.54 µg g^{-1} FW. Also, the triple intervention achieved significant differences for the trait, reaching the highest value when the comparison treatment of the mutagen and the 3% concentration of water stress for the Albion cultivar amounted to 0.94 µg g^{-1} FW. The lowest value at a concentration of 1% for the mutagen not subject to water stress was for the Ruby gem cultivar, as it reached 0.69 µg g^{-1} FW.

Table 1: Effect of cultivar, PEG and EMS and their interactions in H_2O_2 content (µg g^{-1} FW) in vitro.

Cultivar V	EMS	PEG (%)				EMS×V	Mean V
		0	3	6	9		
Albion	0	0.80	0.94	0.73	0.63	0.77	0.72
	1	0.56	0.90	0.64	0.75	0.71	
	2	0.72	0.78	0.83	0.90	0.80	
	3	0.60	0.59	0.59	0.60	0.59	
Ruby gem	0	0.78	0.90	0.69	0.73	0.77	0.69
	1	0.53	0.88	0.62	0.70	0.68	
	2	0.69	0.70	0.80	0.87	0.76	
	3	0.57	0.54	0.55	0.58	0.56	
LSD 0.05		0.038				0.076	0.009
V×PEG		%0	%3	%6	%9	LSD 0.05=0.096	
Albion		0.67	0.80	0.69	0.72		
Ruby gem		0.64	0.75	0.66	0.72		
EMS×PEG		0%	3%	6%	9%	Mean EMS	
%0		0.79	0.92	0.71	0.68	0.77	
%1		0.54	0.89	0.63	0.72	0.69	
%2		0.70	0.74	0.81	0.88	0.78	
%3		0.58	0.56	0.57	0.59	0.57	
0.05 LSD		0.038				LSD0.05= 0.01	
Mean PEG		0.65	0.78	0.68	0.72		
LSD 0.05		0.013					

MDA content: The results of Table 2 show that all study factors and their interactions significantly affect the average MDA content of the two strawberry varieties under different concentrations of the mutagen and levels of drought stress. The table shows that there are significant differences between the cultivar, as the Albion cultivar achieved the highest significant difference for the trait, amounting to 18.69 µmol g^{-1} FW, while the least significant difference was for the Ruby gem cultivar, which amounted to 18.51 µmol g^{-1} FW. Also, there were significant

differences between the levels of water stress, as the treatment with the 3% concentration recorded the highest significant difference for the trait, amounting to 18.72 µmol g^{-1} FW. In contrast, the comparison treatment recorded the least significant difference for this trait, amounting to 18.42 µmol g^{-1} FW. Also, treating with the EMS gave significant differences for the trait, as the comparison treatment recorded the highest significant difference for the trait amounting to 18.80 µmol g^{-1} weight Fresh, while the least significant difference for the trait at the concentration of 3% for the mutagen was 18.33 µmol g^{-1} FW. The binary interaction also significantly affected the trait, as the Albion cultivar at the concentration of 9% recorded the highest significant difference for the trait, amounting to 18.96 µmol g^{-1} FW. At the same time, the least significant difference for the trait in the comparison treatment for the Albion also amounted to 18.42 µmol g^{-1} FW. As for the binary interaction between the cultivar and the mutagen, there were no significant differences between the concentrations for the highest significant difference, while the treatment recorded a concentration of 3% for the mutagen. For the cultivar Ruby gem, the lowest significant difference was 17.93 µmol g^{-1} FW. At the same time, the binary interaction between the mutagen and water stress achieved significant differences for the trait, where the highest significant difference was when the control treatment of the mutagen and 3% concentration of water stress was 19.08 µmol g^{-1} FW, while treatment with a concentration of 3% for the mutagen and 9% for the water stress achieved the lowest significant difference amounting to 18.09 µmol g^{-1} FW. The triple interaction between the study factors also showed a significant effect on the trait, as the concentration of 3% for the mutagen and 9% for the water stress for the Albion cultivar gave the highest significant difference.

Table 2: Effect of cultivar, PEG and EMS and their interactions in MDA content (µmol g^{-1} FW) in vitro.

Cultivar V	EMS	PEG (%)				EMS×V	Mean V
		0	3	6	9		
Albion	0	19.00	19.09	18.66	18.50	18.81	18.69
	1	17.98	18.93	18.53	18.67	18.52	
	2	18.50	18.60	18.89	18.90	18.72	
	3	18.20	18.83	18.10	19.77	18.72	
Ruby gem	0	19.13	19.08	18.74	18.20	18.78	18.51
	1	18.05	18.93	18.64	18.44	18.51	
	2	18.45	18.90	18.90	19.00	18.81	
	3	18.11	17.45	18.08	18.09	17.93	
LSD 0.05		0.37				0.34	0.09
V×PEG		%0	%3	%6	%9	LSD 0.05= 0.38	
Albion		18.42	18.86	18.54	18.96		
Ruby gem		18.43	18.59	18.59	18.43		
EMS×PEG		%0	%3	%6	%9	Mean EMS	
%0		19.06	19.08	18.70	18.35	18.80	
%1		18.01	18.93	18.58	18.55	18.52	
%2		18.47	18.75	18.89	18.95	18.76	
%3		18.15	18.14	18.09	18.93	18.33	
0.05 LSD		0.42				LSD0.05= 0.13	
Mean PEG		18.42	18.72	18.57	18.69		
LSD 0.05		0.13					

It reached 19.77 µmol g^{-1} FW, while the least significant difference for this trait was at the concentration of 3% for mutagen and water stress of the Ruby gem cultivar, which amounted to 17.45 µmol g^{-1} FW.

CAT activity: Table 3 shows the presence of a significant effect of the study factors and their interactions on the catalase activity for the studied varieties, where the Albion cultivar was distinguished by giving the highest significant difference for the trait, amounting to 19.13 mmol min^{-1} mg^{-1}, while the Ruby gem cultivar gave the lowest significant difference for the trait, amounting to 18.65 mmol min^{-1} mg^{-1}, As for the levels of dehydration, they showed significant differences for the trait, as the 9% concentration gave the highest significant difference amounting to 19.44 mmol min^{-1} mg^{-1}, while the comparison treatment gave the least significant difference for the trait amounting to 18.48 mmol min^{-1} mg^{-1}, Also, treatment with different concentrations of the EMS recorded significant differences for the trait, as the 3% concentration gave the highest significant difference amounting to 20.32 mmol min^{-1} mg^{-1}, while the least significant difference was in the comparison treatment, where it was recorded 17.07 mmol min^{-1} mg^{-1}. As for the bilateral interaction between cultivar and water stress, significant differences were also recorded for the trait, as it gave the highest significant difference for the trait at a concentration of 9% for the water stress of Albion cultivar, which gave 19.77 mmol min^{-1} mg^{-1}, While the least significant difference in comparison treatment for the same cultivar was 18.17 mmol min^{-1} mg^{-1}. Also, binary interaction between cultivar and mutagen gave significant differences for the trait, as the highest significant difference for the trait at 3% concentration of mutagen for Albion cultivar was 20.42 mmol min^{-1} $mg^{-1,}$ and the least significant difference amounted to 16.75 mmol min^{-1} mg^{-1} when treating comparison for Ruby gem cultivar. As for the bilateral interaction between mutagen and water stress, the trait had significant differences, as the highest average for enzyme activity was reached. Catalase at a concentration of 3% for mutagen and a concentration of 9% for water stress reached 20.75 mmol min^{-1} mg^{-1}. The lowest average for trait was in comparison treatment of mutagen and water stress, which gave 16.68 mmol min^{-1} mg^{-1}. As for triple interaction between the study, factors showed a significant difference for the trait, as the highest average of catalase enzyme activity was recorded at a concentration of 3% for mutagen and 9% for the water stress of Albion cultivar, amounting to 21.05 mmol min^{-1} mg^{-1}. However, the least significant difference for the triple interaction was in the comparison treatment for the mutagen and the stress. The water content of the Ruby gem cultivar reached 16.06 mmol min^{-1} mg^{-1}.

Table 3: Effect of cultivar, PEG and EMS and their interactions in CAT activity (mmol min^{-1} mg^{-1}) in vitro.

Cultivar V	EMS	PEG (%)				EMS×V	Mean V
		0	3	6	9		
Albion	0	17.30	17.35	17.40	17.56	17.40	19.13
	1	18.00	18.10	19.00	20.20	18.82	
	2	19.60	19.78	19.90	20.29	19.89	
	3	20.11	20.20	20.35	21.05	20.42	
Ruby gem	0	16.06	16.82	17.00	17.14	16.75	18.65
	1	17.80	17.94	18.46	18.94	18.28	
	2	18.94	19.04	19.46	19,89	19.33	
	3	20.04	20.12	20.30	20.45	20.22	
LSD 0.05		0.13				0.37	0.03
V×PEG		%0	%3	%6	%9	LSD 0.05= 1.06	
Albion		18.17	18.85	19.16	19.77		
Ruby gem		18.21	18.48	18.80	19.10		
EMS×PEG		%0	%3	%6	%9	Mean EMS	
%0		16.68	17.08	17.20	17.35	17.07	
%1		17.90	18.02	18.73	19.57	18.55	
%2		19.27	19.41	19.68	20.09	19.61	
%3		20.07	20.16	20.32	20.75	20.32	
0.05 LSD		0.387				LSD0.05= 0.04	
Mean PEG		18.48	18.67	18.98	19.44		
LSD 0.05		0.04					

APX content: Table 4 shows the significant effects of the studied factors and their interactions on the activity of the APX enzyme for the two strawberry varieties under the influence of different concentrations of EMS and four different concentrations of PEG. The table shows significant differences between the two varieties, where the Albion cultivar gave the highest significant value for the trait. It reached 0.39 mmol min^{-1} mg^{-1}, while the lowest significant value for the trait was for the Ruby gem cultivar, which was 0.35 mmol min^{-1} mg^{-1}; as for drought levels, significant differences were also achieved for the trait, as the highest significant difference was at 9% concentration of drought stress, which reached 0.39 mmol min^{-1} mg^{-1}, while the lowest value for drought stress was in the comparison treatment, which also reached 0.34 mmol min^{-1} mg^{-1}. With mutagen, significant differences were achieved for the trait, as the highest significant value was recorded at the 3% concentration of the mutagen, which was 0.40 mmol min^{-1} mg^{-1}, while the lowest value was recorded in the comparison treatment, which was 0.35 mmol min^{-1} mg^{-1}; as for bilateral interaction between the cultivar and the water stress, there were significant differences for the trait, as the treatment of 9% for the water stress of the Albion cultivar recorded the highest value. Significance amounted to 0.43 mmol min^{-1} mg^{-1}, while the comparison treatment for water stress for the Ruby gem cultivar recorded the lowest significant difference, amounting to 0.3 mmol min^{-1} mg^{-1}; also, binary interaction between the cultivar and the mutagen recorded significant differences for a trait as well, as the highest significant difference for the trait at a concentration of 3% for the mutagen for the Albion cultivar was 0.42 mmol min^{-1} mg^{-1}, while the lowest significant difference for

trait was at comparison treatment for the mutagen for the Ruby gem cultivar reached 0.32 mmol min^{-1} mg^{-1}.

In contrast, the binary interaction between the mutagen and the water stress had a significant effect, as the treatment with 3% of the mutagen and a concentration of 9% of the water stress recorded the highest significant value of 0.46 mmol min^{-1} mg^{-1}. In contrast, the remaining concentrations did not differ in recording the lowest significant value. As for the interactions. The triplicate gave significant differences for the trait, and the treatment with a concentration of 3% and a concentration of 9% for water stress for the Albion cultivar recorded the highest significant value of 0.50 mmol min^{-1} mg^{-1}, while the lowest value was for the comparison treatment of the mutagen and a concentration of 9% for water stress, which amounted to 0.29 mmol min^{-1} mg^{-1}.

Table 4: Effect of cultivar, PEG and EMS and their interactions in APX activity (mmol min^{-1} mg^{-1}) in vitro.

Cultivar V	EMS	PEG (%)				EMS×V	Mean V
		0	3	6	9		
Albion	0	0.35	0.39	0.37	0.41	0.38	
	1	0.38	0.40	0.44	0.39	0.40	0.39
	2	0.36	0.38	0.39	0.41	0.38	
	3	0.36	0.35	0.47	0.50	0.42	
Ruby gem	0	0.33	0.36	0.32	0.29	0.32	
	1	0.30	0.41	0.33	0.36	0.35	0.35
	2	0.34	0.37	0.38	0.38	0.36	
	3	0.35	0.37	0.41	0.42	0.39	
LSD 0.05		0.06				0.03	0.01
V×PEG		%0	%3	%6	%9	LSD 0.05= 0.03	
Albion		0.36	0.38	0.41	0.43		
Ruby gem		0.33	0.38	0.36	0.36		
EMS×PEG		%0	%3	%6	%9	Mean EMS	
%0		0.34	0.38	0.34	0.35	0.35	
%1		0.34	0.40	0.38	0.38	0.37	
%2		0.35	0.37	0.38	0.39	0.37	
%3		0.35	0.36	0.44	0.46	0.40	
0.05 LSD		0.05				LSD0.05= 0.02	
Mean PEG		0.34	0.38	0.38	0.39		
LSD 0.05		0.02					

GPX activity: The results of Table 5 indicate that all study factors and their interactions have a significant effect on the GPX activity for the Albion and Ruby gem varieties under concentrations of water stress in vitro. The table shows that the Albion cultivar excelled with the highest significant average for the trait, reaching 0.08 mmol min^{-1} mg^{-1}, while the lowest significant average was for the Ruby gem cultivar, where It reached 0.07 mmol min^{-1} mg^{-1}. Also, the levels of drought showed significant differences for the trait, as the highest significant difference was at the 9% concentration, which amounted to 0.09. In contrast, the remaining concentrations did not differ significantly, as the comparison treatment and the 6% concentration treatment gave the lowest significant difference, amounting to 0.07 mmol min^{-1} mg^{-1}. As for the levels of mutagenesis, there were significant differences. Also, the treatment with the concentration of 3% showed the highest significant difference, amounting to

0.11 mmol min^{-1} mg^{-1}, while the comparison treatment showed the lowest significant difference for the trait, amounting to 0.05 mmol min^{-1} mg^{-1}. As for the binary interactions, it gave significant differences for the trait as well, as the interaction between the cultivar and water stress recorded the highest significant difference at the concentration of 9% for the Albion cultivar, amounting to 0.11 mmol min^{-1} mg^{-1}, while The concentration of 6% for the cultivar Ruby gem recorded the lowest significant difference of 0.06 mmol min^{-1} mg^{-1}, while the binary interaction between the cultivar and the mutagen gave significant differences for the trait as well, as the concentration of 3% for the cultivar Albion achieved the highest significant difference for the trait of 0.13 mmol min^{-1} mg^{-1}.

In contrast, the comparison treatment for the cultivar Ruby gem also gave the lowest significant difference. The combination between the mutagen and the drought stress gave significant differences, as the 3% concentration treatment for the mutagen and the water stress gave the highest significant average for the trait, which amounted to 0.12 mmol min^{-1} mg^{-1}. The lowest significant average for the trait was when the comparison treatment of the mutagen with a 3% concentration for the water stress reached 0.03. Significant differences were achieved concerning the triple interactions of the study factors, also for the trait, where the treatment with a concentration of 3% for the mutagen and 9% for the water stress of the Albion cultivar gave the highest significant difference for the trait amounting to 0.15 mmol min^{-1} mg^{-1}, while the lowest significant difference for the trait when the comparison treatment of the mutagen and a concentration of 3% of the water stress for the Ruby gem cultivar reached 0.03 mmol min^{-1} mg^{-1}.

Table 5: Effect of cultivar, PEG and EMS and their interactions in GPX activity (mmol min^{-1} mg^{-1}) in vitro.

Cultivar V	EMS	PEG (%)				EMS×V	Mean V
		0	3	6	9		
Albion	0	0.06	0.04	0.05	0.09	0.06	
	1	0.08	0.09	0.10	0.11	0.09	0.08
	2	0.05	0.08	0.04	0.09	0.06	
	3	0.10	0.13	0.16	0.15	0.13	
Ruby gem	0	0.05	0.03	0.04	0.08	0.05	
	1	0.08	0.09	0.10	0.11	0.09	0.07
	2	0.06	0.09	0.05	0.08	0.07	
	3	0.10	0.11	0.07	0.08	0.09	
LSD 0.05		0.020				0.017	0.005
V×PEG		%0	%3	%6	%9	LSD 0.05= 0.025	
Albion		0.07	0.08	0.08	0.11		
Ruby gem		0.07	0.08	0.06	0.08		
EMS×PEG		%0	%3	%6	%9	Mean EMS	
%0		0.05	0.03	0.04	0.08	0.05	
%1		0.08	0.09	0.10	0.11	0.09	
%2		0.05	0.08	0.04	0.08	0.06	
%3		0.10	0.12	0.11	0.11	0.11	
0.05 LSD		0.023				LSD0.05= 0.007	
Mean PEG		0.07	0.08	0.07	0.09		
LSD 0.05		0.007					

Iron content: The results of the statistical analysis in Table 6 show that the addition of the EMS under the influence of water stress levels in vitro led to the emergence of significant differences between treatments, as the Albion cultivar recorded the highest significant difference in the amount of iron, reaching 31.17mg g^{-1} DW, while Albion cultivar recorded the highest significant difference in the amount of iron, amounting to 31.17 mg g^{-1} DW. Ruby gem cultivar had the least significant difference, amounting to 28.21 mg g^{-1} DW. The addition of PEG led to the emergence of significant differences, as the highest value for the amount of iron in plants not exposed to drought stress amounted to 39.56 mg g^{-1} DW, while the lowest value was when treated with the highest concentration of water stress at a concentration of 9%, as it reached 19.66 mg g^{-1} DW. Also, the addition of EMS had an effect in causing significant differences in the trait, as the highest value for the amount of iron was at a concentration of 3%. Of mutagen, it amounted to 46.34 mg g^{-1} DW, while the lowest value was at 1% concentration, amounting to 22.48 mg g^{-1} DW. Binary interactions between study factors also influenced significant differences in traits, as the interaction between the cultivar and water stress had an effect. Significantly in the amount of iron, as this interaction gave the highest significant difference in the comparison treatment for the Albion cultivar, amounting to 41.16 mg g^{-1} DW, while the least significant difference was at the concentration of 9% for the Ruby gem cultivar, amounting to 18.52 mg g^{-1} DW. As for the double interaction between the cultivar and the mutagen, there were also significant differences in traits. Its highest value was at a concentration of 3% for the Albion cultivar, amounting to 47.70 mg g^{-1} DW. The lowest value was when comparison was treated for the Ruby gem cultivar. Interaction between mutagen and water stress also recorded a significant effect between treatments, as treatment with 3% of mutagen and water stress recorded the highest percentage, amounting to 51.28 mg g^{-1} DW.

In contrast, treatment not exposed to mutagen, with a 9% concentration of water stress, recorded the lowest percentage, amounting to 9.49 mg g^{-1} DW. As for the interaction between the three study factors, there were significant differences between treatments, as treatment with a 3% concentration of mutagen and water stress recorded the highest significant value for the trait, amounting to 53.80 mg g^{-1} DW. In contrast, the lowest significant value was for a 9% concentration of water stress and not exposed to mutagenicity with EMS, amounting to 8.12. mg g^{-1} DW.

Table 6: Effect of cultivar, PEG and EMS and their interactions in iron content (mg g^{-1} DW) in vitro.

Cultivar V	EMS	PEG (%)				EMS×V	Mean V
		0	3	6	9		
Albion	0	32.01	13.00	40.05	10.86	23.98	31.17
	1	48.00	13.90	15.71	17.22	23.70	
	2	34.05	30.00	37.01	16.20	29.31	
	3	50.60	53.80	47.50	38.90	47.70	
Ruby gem	0	30.03	10.15	35.90	8.12	21.05	28.21
	1	45.07	10.99	13.87	15.09	21.25	
	2	28.09	24.30	35.00	14.90	25.57	
	3	48.66	48.76	46.53	35.99	44.98	
LSD 0.05		1.31				8.82	0.32
V×PEG		%0	%3	%6	%9	LSD 0.05= 10.26	
Albion		41.16	27.67	35.06	20.79		
Ruby gem		37.96	23.55	32.82	18.52		
EMS×PEG		%0	%3	%6	%9	Mean EMS	
%0		31.02	11.57	37.97	9.49	22.51	
%1		46.53	12.44	14.79	16.15	22.48	
%2		31.07	27.15	36.00	15.55	27.44	
%3		49.63	51.28	47.01	37.44	46.34	
0.05 LSD		2.23				LSD 0.05= 0.46	
Mean PEG		39.56	25.61	33.94	19.66		
LSD 0.05		0.46					

Proline content: The results of table 7 indicated that there were significant differences when adding a chemical mutagen under water stress and the extent of its effect on the amount of amino acid proline in the Albion and Ruby gem varieties, where the Ruby gem cultivar gave the highest significant difference for trait, amounting to 1.88µg g^{-1} FW, while lowest difference was It was significant for trait for Albion cultivar, where it was recorded at 1.84 µg g^{-1} FW. Water stress levels also gave a significant difference for the trait, as 3% concentration gave the highest significant difference, 2.12 µg g^{-1} FW. The least significant difference was that at the concentration of 6%, it reached 1.67 µg g^{-1} FW. The table also shows that the addition of chemical mutagen (EMS) recorded significant differences for trait, as the concentration of 3% gave the highest significant difference, which amounted to 2.02 µg g^{-1} FW, while the least significant difference was In the comparison treatment, which gave 1.54 µg g^{-1} FW, bilateral interaction between cultivar and water stress recorded significant differences, as 3% concentration of the Ruby gem cultivar gave highest significant difference, amounting to 2.21 µg g^{-1} FW, while least significant difference for trait was 1.67 µg g^{-1} FW, at a concentration of 6% for the same cultivar. As for the interaction between two factors of study, cultivar and chemical mutagen, it gave significant differences for a trait as well, and its highest value at a concentration of 3% for cultivar Ruby gem amounted to 2.24 µg g^{-1} FW, While the lowest value was in comparison treatment for the same cultivar, as it reached 1.52 µg g^{-1} FW. The interaction between mutagen and water stress gave significant differences for the trait, as the highest average reached 2.61 µg g^{-1} FW, with a concentration of 2% for mutagen and 3% for stress. Water and the lowest mean for trait was at a 2% treatment for

mutagen and not exposed to water stress. As for triple interactions of study factors, there were significant differences, and treatment with a 3% concentration of mutagen and water stress for the Ruby gem cultivar recorded the highest significant value of 2.90 µg g^{-1} FW, while the lowest value was in control treatment of mutagen and water stress for Ruby gem cultivar as well, where it was recorded as 1.49 µg g^{-1} FW.

Table 7: Effect of cultivar, PEG and EMS and their interactions in proline content (µg g^{-1} FW) in vitro.

Cultivar V	EMS	PEG (%)				V×EMS	Mean V
		0	3	6	9		
Albion	0	1.55	1.53	1.57	1.59	1.56	
	1	1.91	1.96	2.00	2.50	2.09	1.88
	2	1.52	2.67	1.53	1.87	1.90	
	3	1.82	1.99	1.62	1.59	1.80	
Ruby gem	0	1.49	1.55	1.53	1.50	1.52	
	1	1.89	1.85	1.94	2.00	1.92	1.84
	2	1.50	2.55	1.53	1.87	1.86	
	3	2.50	2.90	1.70	1.86	2.24	
LSD 0.05		0.04				0.25	0.01
V×PEG		%0	%3	%6	%9	LSD 0.05= 0.27	
Albion		1.70	2.04	1.68	1.93		
Ruby gem		1.84	2.21	1.67	1.80		
EMS×PEG		%0	%3	%6	%9	Mean EMS	
%0		1.52	1.54	1.55	1.54	1.54	
%1		1.90	1.90	1.97	2.25	2.00	
%2		1.51	2.61	1.53	1.87	1.88	
%3		2.16	2.44	1.66	1.82	2.02	
0.05 LSD		0.20				LSD0.05= 0.01	
Mean PEG		1.77	2.12	1.67	1.87		
LSD 0.05		0.01					

The results of Tables 1, 2, 3, 4, 5, 6, and 7 show that the use of chemical mutagen (EMS) has improved the biochemical and physiological properties of two strawberry varieties under the influence of levels of drought stress, as strawberries are a crop of utmost importance in Everywhere (29), but like others, it is affected by environmental stresses, especially water stresses (19 and 22). PEG causes water stress in plants (21 and 24). However, the use of mutagens led to improved plant properties and resistance to water stress (14 and 18) because the chemical mutagen (EMS) is ethyl methane sulfonate, which is most commonly used on Strawberries are one of the chemical mutagens that work to improve the characteristics of the plant and increase its resistance to drought (3), use of mutagens is to create genetic variations that are the backbone of selection and genetic improvement. Accordingly, the main goal of using mutagens is to create a genetic variation that is supposed not to exist or has a very low frequency. In general, mutagenesis and genetic improvement programs are for developing one or a minimal number of desirable traits. Increasing the frequency of mutations and thus increasing the chances of finding and selecting them. The most important characteristics that can be improved through mutation breeding programs are resistance to infection with pathogens or insects, resistance to the effects of some pesticides, tolerance to salinity, drought, and heat, improving the qualitative and

nutritional characteristics of the plant type, and post-harvest, and increasing productivity by improving one or more of the characteristics of the crop components. Improving some phenotypic or physiological traits, or creating them, and creating new traits by linking modern life technologies with mutation breeding programs and bringing them to the integration of technical packages to produce and create plants or traits that nature has not and will not provide, and creating an inherited effect by creating genetic variations requires changing one of the nucleotide bases of the DNA, which is called a genetic mutation, or creating a change in the structure of the chromosome, whether it is loss, addition, duplication, duplication, or transfer of part of the chromosome, which is called a chromosomal mutation. This can be achieved by the method of acute treatment that leads to the creation of valuable mutations with a specific frequency (2), as tissue culture technology has helped to accelerate breeding programs in general and mutagenesis and screening programs for heterozygous mutations in particular (26). Selection and propagation are carried out by continuing cultivation in In Vitro, which has helped improve strawberry traits and increase its resistance to drought stress.

Conclusions

We conclude from this study that using mutagenesis technology on two strawberry varieties under the influence of water stress in vitro led to improved and increased quality characteristics.

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