Effect of sodium azide on some physiological traits of genotypes of rice (*Oryza sativa* L.) under different salinity levels

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Abstract

A field experiment was conducted at rice research station in Al-Mishkhab (Al-Najaf provainc) during (2021) season. The split-split-plot system was used with a randomized complete block design (RCBD),As the first factor was represented by salinity levels (river water, 100 mmol, 150 mmol) and the second factor was mutagenesis by sodium increase in different concentrations (1.5 mmol and 2 mmol with treatment without mutagen) and the third factor was the genotypes (V1 and V3) and the Ambar 33 cultivar for control at the end of the season, samples were taken and analyzed, and the results appeared as follows: Increasing salt levels led to an increase in proline content and an increase in the activity of antioxidant enzymes (SOD, POD, and CAT). Mutation with sodium azide increased proline content, increased POD and SOD enzyme activity, and improved tolerance to salt stress. As for the genotypes, the V1 genotype was excelled in most physiological traits compared to the V3 genotype and Ambar 33.

introduction

Rice is a staple food crop for more than 50% of the world's population, and soil salinity greatly limits its production as it is a salt-sensitive plant. Therefore, the study of the physiological characteristics of rice under the influence of salt stress is necessary. Climate change causes changes in the surrounding environment and soil salinization appears significantly, and the rise in temperatures in arid and semi-arid regions has led to increased evaporation, which, when associated with ineffective irrigation systems used in developing countries, has caused surface soil salinization to varying degrees (Munns, 2005). The salinity problem is one of the most important problems facing agriculture all over the world, especially in the arid and semi-arid regions, as about 20% of the cultivated land in the world is affected by salinity, and nearly 30% of the cultivated area Rice in the world is affected by salinity (Singh, 2021). Salinity is an important physical factor affecting rice production, and salinity can cause severe damage at any stage of rice growth and development, leading to yield loss. Plants have developed several biochemical and molecular mechanisms to deal with the toxic effects of salinity, including the regulation of genes that have a role in the uptake, transport or fragmentation of Na+ or K+, therefore, The study of different strategies to make rice plants more tolerant and improve their productivity under the influence of salinity represents an important challenge for researchers in order to deal with the decline in food production due to soil salinization (Porcel et al., 2016).Plants have also developed complex antioxidant enzymes against ROS, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in different cell (Batels and Sunkar, 2005), The enzyme catalase (CTA) converts hydrogen peroxide H202 into water and molecular oxygen, while the enzyme Superoxide dismutase (SOD) works to suppress the harmful effect of negative superoxide ions (0^{-2}) (Radhakrishman, 2009).There are options to address the problem of salinity, including reclamation of

land affected by salinity or coexistence with it using genotypes that tolerate salinity by creating variations in genotypes cultivated by mutagen. In view of the prevalence of the problem of salinity and its prevalence in large lands of Iraq and the urgent need for genotypes that are tolerant to salinity as well as the scarcity of studies and research in the field of creating genetic variations for the trait of salinity tolerance in rice, so the aim of this study included obtaining genotypes of rice that are tolerant to salinity by evaluating And screening of the genotypes of rice (V1, V2 and V3) for salinity tolerance with the addition of the local Ambar 33 cultivar for control.

Materials and Methods

Factors used in the study: The study included the following factors: (1) the first factor: salinity levels (river water, 100 mmol, 150 mmol), (2) the second factor: mutagenesis with sodium azide (0, 1.5 mmol, 2 mmol)), (3) Genotypes (V1, V3, Amber 33).

Table1. The genetic origin of the genotypes under study

genotype	genetic origin
V1	Amber (♂) X Al-Furat (♀)
V3	Al Ghadeer (♂) X Al-Furat (♀)
amber	Certified local brand

The genotypes (V1 and V3) above were extracted by plant breeders at the rice research station in Al-Mishkhab for several previous seasons, to reach the stage of genotype stability and submission for approval .The rice genotype seeds were soaked in distilled water for 24 hours.Then they were treated with sodium azide solution at two concentrations (1.5 and 2 mmol) for 4 hours at a temperature of 28 C and PH = 3, where the PH was reduced by phosphoric acid (Oraibi, 2013), then the seeds were washed with tap water for half an hour. A field experiment was conducted at the rice research station in Al-Mishkhab (Al-Najaf Research Department) affiliated to the Agricultural Research Department - Ministry of Agriculture, during the 2021 agricultural season, located at latitude 31 north and longitude 44 east, at an altitude of 70 m above sea level in clayey loamy soil. Using the completely randomized block design (RCBD) according to split- split-plot design arrangement and with three replications, where the salt concentrations (S1: 0 , S2: 100 , S3:150 mmol) (main plots) and sodium azide concentrations (M1: 0 , M2: 1.5 and M3: 2 mmol) were occupied Sub plots and genotypes (V1, V3, Amber 33) in sub-subplots). The field was irrigated with water with different concentrations of sodium chloride salt between the day of irrigation and the day of drying. Irrigation operations continued in this methods until the plants reached the stage of physiological maturity. The area of the experimental unit was 2 x 3 m, the plants were planted in lines, and the distance between one line and another, and between one plant and another, was 25 cm. As for the weeds, they were weeding by hand, as needed. The experimental land was fertilized by adding (compound fertilizer N.p 18:18) in an amount of 400 $kg.ha^{-1}$ mixed with the soil before planting, while urea fertilizer (46% N) was added in an amount of 280 $kg.ha^{-1}$ and in two equal batches, the first batch after 10 days of seedling in the field and The second one, a month after the first batch (Hassan, 2011).The physiological trait of proline, chlorophyll, potassium ions, sodium ions, and the ratio between them, and antioxidant enzymes (SOD, POD, and CAT) were studied. The experiment was conducted using the split-plot system with a randomized complete block design (RCBD), and the ready-made statistical analysis system (Genstat12th) was used under the Windows computer operating system to perform statistical analyses. between the averages of treatment, As well as the use of

independent comparisons analysis to compare between amber 33 (non-mutagenic) and the two genotypes (mutagenic with sodium azide concentrations of 0, 1.5 and 2 mmol) during the salinity levels used in the study. the level of probability LSD 5%

Results and discussion:

Determination of the percentage of potassium in the leaves (%)

Salinity levels differed in the percentage of potassium ions in the leaves (Table 2), where the percentage of potassium ions was inversely proportional to the increase in salinity levels, where it decreased at the treatment of 100 and 150 mmol by 26.1% and 39.5%, respectively, compared to river water.It also decreased at the salinity level of 150 mmol by 18.2% from the level of salinity 100 mmol. The decrease in the percentage of potassium ions in the leaves with the increase in salinity levels is due to the increase in the percentage of the Na+ ion, which is the potassium ion +K (Garcia morales et al., 2012). This result is consistent with what was mentioned by (Rasel et al., 2021).Also, the concentrations of mutagenesis differed significantly in the percentage of potassium ions in the leaves (Table 2), as the mutagenicity caused by a concentration of 2 mmol reduced potassium by 7.1% compared to non-mutagenic plants, and no significant difference was recorded between mutagenic plants with a concentration of 1.5 and 2 mmol and between plants with a concentration of 1.5 and 2 mmol Mutagenic plants at a concentration of 1.5 mmol and non-mutagenic plants.The two genotypes V1 and V3 differed from each other in the percentage of potassium ions (Table 2), where the genotype V1 was 15.4% excelled in the genotype V3 in giving the highest percentage of potassium in the leaves, which reflects a higher tolerance to salinity compared to the genotype V3. These results were consistent with what happened. According to Emon et al. (2015) in rice, who showed that the genotypes differed in the proportion of potassium ions in vegetative growth.The cultivar Ambar 33 (non-mutagenic) differed significantly in potassium percentage with the genotype V1 mutagenic and non-mutagenic when irrigated with river water (Table 2), As the percentage of potassium was higher for the V1 genotype, and there was no significant difference between the cultivar Ambar 33 and the mutagenic and non-mutagenic V3 genotype. The salinity level was 100 mmol.The non-mutagenic and mutagenic genotype V1 at a concentration of 1.5 mmol on the cultivar Ambar 33 increased potassium, and the cultivar Anbar 33 did not differ significantly with all genotypes in the salinity level of 150 mmol.

Determination of Sodium Percentage in Leaves (%)

The salinity levels affected significantly the percentage of sodium in the leaves (Table 3), where the percentage of sodium +aN was directly proportional to the increase in the levels of salinity. The level of salinity of 100 mmol did not differ significantly with the level of salinity of 150 mmol. The increase in the percentage of sodium in conjunction with the increase in salinity levels is due to its intense competition for the potassium ion on protein carriers in the absorption sites inside the cell and its hindrance to its absorption from the soil solution (Murat et al., 2007) supports this The result with the findings of (Jini and Joseph, 2017).The concentrations of mutagenesis also differed significantly in the percentage of sodium in the leaves (Table 3), where the cause of mutagenesis at a concentration of 2 mmol increased the percentage of sodium by rates of 10.7% and 15% for mutagenic plants with a concentration of 1.5 mmol and non-mutated plants, respectively, and the percentage of plants increased mutagens at a concentration of 1.5 mmol, with a rate of 3.9% compared to non-mutagenic plants. Table (3) shows that there is an overlap between salinity levels and mutagenic concentrations, as the mutagen plants did not differ from non-mutagenic plants when irrigated with river water, but the

difference occurred when irrigated with a salinity level of 100 mmol. Sodium level decreased in the leaves of mutant plants at a concentration of 1.5 mmol by 23% compared to mutagen plants at a concentration of 2 mmol, and there was no significant difference between mutagen plants at a concentration of 1.5 mmol and non-mutated plants. Salinity 150 mmol. Amber 33 (non-mutagenic) did not differ significantly in sodium percentage from the rest of the mutagenic and non-mutagenic genotypes (V1M1, V1M2, V1M3, V3M1, V3M2, V3M3) for all studied salinity levels according to the independent comparisons analysis (Table 3).

Table (2) The effect of salinity levels, mutagenesis concentrations, genotypes and the interaction between them on the percentage of potassium ions (%) in leaves.

VMS		S1	S2	S3	Average
V1	M1	0.974	0.784	0.635	0.797
	M2	0.943	0.693	0.521	0.719
	M3	0.914	0.637	0.507	0.686
V3	M1	0.833	0.563	0.471	0.622
	M2	0.811	0.615	0.532	0.652
	M3	0.786	0.597	0.514	0.632
L.S.D 5%		n.s			
Amber 33 cultivar		0.733	0.539	0.451	
independent comparisons L.S.D5%		*0.1804	*0.1307	n.s	
VM	M1	M2	M3	Score of L.S.D 5%	
V1	0.798	0.719	0.686	*	Significant
V3	0.622	0.653	0.632	**	high significant
L.S.D	n.s			n.s	non significant
VS	S1	S2	S3	V	
V1	0.944	0.705	0.555	0.734	
V3	0.810	0.592	0.506	0.636	
L.S.D5%	n.s			**0.0509	
MS	S1	S2	S3	M	
M1	0.903	0.674	0.553	0.710	
M2	0.877	0.654	0.527	0.686	
M3	0.850	0.617	0.510	0.659	
L.S.D5%	n.s			*0.0392	
S	S1	S2	S3	L.S.D5%	
	0.877	0.648	0.530	**0.0575	

Table (3) Effect of salinity levels, mutagenesis concentrations, genotypes and the interaction between them on the percentage of sodium ions (%) in leaves.

VMS		S1	S2	S3	Average
V1	M1	0.860	1.122	1.280	1.087
	M2	0.891	1.168	1.538	1.199
	M3	0.913	1.592	1.539	1.348
V3	M1	0.916	1.210	1.367	1.164
	M2	0.955	1.147	1.320	1.140
	M3	0.958	1.417	1.359	1.244
L.S.D 5%		n.s			
Amber 33 cultivar		0.853	1.320	1.515	
independent comparisons L.S.D5%		n.s	n.s	n.s	
VM	M1	M2	M3	Score of L.S.D 5%	
V1	1.087	1.199	1.348	*	Significant
V3	1.164	1.140	1.245	**	high significant
L.S.D5%	n.s			N.s	Non significant
VS	S1	S2	S3	V	
V1	0.888	1.294	1.452	1.211	
V3	0.943	1.258	1.348	1.183	
L.S.D5%	n.s			n.s	
MS	S1	S2	S3	M	
M1	0.888	1.166	1.323	1.126	
M2	0.923	1.158	1.429	1.170	
M3	0.936	1.505	1.449	1.296	
L.S.D	**0.2286			**0.0610	
S	S1	S2	S3	L.S.D5%	
	0.916	1.276	1.400	**0.2334	

Determination of the percentage of potassium to sodium in leaves (%)

Salinity levels significantly affected the potassium-to-sodium ratio in the leaves (Table 4), where salinity levels were inversely proportional to the potassium-to-sodium ratio. the river, There was no significant difference between the salinity level 100 mmol and 150 mmol. The reason may be due to the fact that the increase in the concentration of sodium in the plant cells changes the nutritional balance in the plant, which leads to an obstruction of potassium absorption and then a decrease in its ratio, which leads to a decrease in growth and production rates, and this effect is competitive.Therefore, the presence of an amount of potassium under saline conditions is important for plant growth and continuity. (Khorshidi and Hassanpanah, 2009) showed that the presence of a high percentage of potassium in plant tissues indicates that the plant is salt-tolerant, and then the potassium-to-sodium ratio is a measure of the extent of this endurance, which was confirmed by Turan et al. (2009) and Burki Al-(2017). The cultivar Amber 33 (non-mutagenic) did not differ significantly in the ratio of potassium to sodium from the rest of the mutagenic and non-mutagenic genotypes (V1M1, V1M2, V1M3, V3M1, V3M2, V3M3) for all studied salinity levels according to the independent comparisons analysis (Table 4).

Determination of SOD superoxide dismutase activity (unit. mg^{-1} protein)

The levels of salinity differed significantly in the activity of the SOD enzyme (Table 5), as the activity of the enzyme increased with increasing levels of salinity.The salinity level of 150 mmol also differed by increasing the activity of the SOD enzyme over the salinity level of 100 mmol by 50%. The SOD enzyme is the first line of defense against reactive oxygen species generated during stress, including salt stress, It can play a role in removing harmful free radicals, but at the same time other enzymes are necessary to remove hydrogen peroxide generated by SOD and these enzymes are CAT and POD (Landi et al., 2012 and Zeafyadeh et al., 2009) This result agrees with what Yaghubi concluded. et al (2014). It is also clear from Table (5) that the interaction between salinity levels and mutagenic concentrations was significant, where the activity of the SOD enzyme decreased in the mutagen plants when irrigated with fresh water, while the activity of the enzyme increased for the mutagenic plants when watering at salinity levels of 100 and 150 mmol, where the mutagenic treatment excelled At a concentration of 1.5 mmol when irrigating at a salinity level of 150, the same mutagenesis treatment was obtained when irrigating with river water by up to 200%.Amber 33 (non-mutagenic) was significantly different in the activity of the SOD enzyme from the rest of the mutagenic and non-mutagenic genotypes (V1M1, V1M2, V1M3, V3M1, V3M2, V3M3) and for a salinity level of 150 mmol, while there was no significant difference at a salinity level of 100 mmol and River water treatment according to the analysis of independent comparisons (Table 5), the variety Anbar 33 recorded the least effective salinity treatments of 150 mmol compared to the rest of the formulations.

Table (4) Effect of salinity levels, mutagenesis concentrations, genotypes and the interaction between them on the ratio of potassium to sodium (%) in leaves.

VMS		S1	S2	S3	Average
V1	M1	1.139	0.698	0.499	0.778
	M2	1.123	0.594	0.336	0.684
	M3	1.010	0.402	0.334	0.582
V3	M1	0.943	0.463	0.346	0.584
	M2	0.876	0.536	0.401	0.604
	M3	0.837	0.415	0.555	0.602
L.S.D 5%		n.s			
Amber 33 cultivar		0.854	0.408	0.300	
independent comparisons L.S.D		n.s	n.s	n.s	
VM	M1	M2	M3	Score of L.S.D 5%	
V1	0.779	0.684	0.582	*	Significant
V3	0.584	0.604	0.602	**	high significant
L.S.D5%	n.s			N.s	Non significant
VS	S1	S2	S3	V	
V1	1.091	0.565	0.389	0.682	
V3	0.885	0.471	0.434	0.597	
L.S.D5%	n.s			n.s	
MS	S1	S2	S3	M	
M1	1.041	0.580	0.423	0.681	
M2	0.999	0.565	0.368	0.644	
M3	0.924	0.409	0.444	0.592	
L.S.D5%	n.s			n.s	
S	S1	S2	S3	L.S.D5%	
	0.988	0.518	0.412	**0.2194	

Table (5) The effect of salinity levels, mutagenesis concentrations, genotypes and the interaction between them on the activity of the SOD enzyme (unit. mg^{-1} protein)

VMS		S1	S2	S3	Average
V1	M1	3.55	5.91	8.15	5.87
	M2	3.26	6.67	9.42	6.45
	M3	3.16	6.13	9.32	6.20
V3	M1	4.79	4.37	7.16	5.44
	M2	2.54	5.64	8.57	5.58
	M3	2.15	4.15	6.95	4.41
L.S.D 5%		n.s			
Amber 33 cultivar		2.56	3.01	5.23	
independent comparisons L.S.D5%		n.s	n.s	**2.164	
VM	M1	M2	M3	Score of L.S.D 5%	
V1	5.87	6.45	6.20	*	Significant
V3	5.44	5.58	4.42	**	high significant
L.S.D	n.s			n.s	non significant
VS	S1	S2	S3	V	
V1	3.32	6.24	8.96	6.17	
V3	3.16	4.72	7.56	5.15	
L.S.D5%	n.s			n.s	
MS	S1	S2	S3	M	
M1	4.17	5.14	7.65	5.65	
M2	2.90	6.15	8.99	6.02	
M3	2.65	5.14	8.13	5.31	
L.S.D5%	**1.48			n.s	
S	S1	S2	S3	L.S.D5%	
	3.24	5.48	8.26	**1.454	

Determination of the activity of the peroxidase enzyme (POD peroxidase) (absorption unit.gm^{-1} fresh weight)

Salinity levels differed significantly in the activity of the POD enzyme (Table 6), where the activity of the enzyme increased in the salinity level 150 mmol by 159% for river water, and there was no significant difference between the level of salinity 100 mmol and river water and between the level of salinity 100 mmol and the level of salinity 150 mmol

.Several studies indicate that when plants are exposed to a certain stress, the activity of antioxidant enzymes increases, and that the increase in SOD and POD enzymes is always associated with an increase in plant tolerance to environmental stresses. This result is consistent with what was mentioned by Singh et al. (2022), who confirmed that the activity of antioxidant enzymes increased with the increase in salinity levels.It is also clear from Table (6) that mutagenic concentrations significantly affected the activity of the POD enzyme, as mutagenic plants with both concentrations (1.5 and 2 mmol) were significantly superior to non-mutagenic plants.The mutagenic concentration of 1.5 mmol was excelled on 31.5% over non-mutagenic plants, and mutagenic plants with a concentration of 2 mmol were superior to non-mutagenic plants by 22.4%, while there was no significant difference between the two mutagenic concentrations (1.5 and 2 mmol).Amber 33 (non-mutagenic) was significantly different in the activity of the POD enzyme than the rest of the mutagenic and non-mutating genotypes (V1M1, V1M2, V1M3, V3M1, V3M2, V3M3) and for a salinity level of 150 mmol, while there was no significant difference at a salinity level of 100 mmol and River water treatment according to the analysis of independent comparisons (Table 6), the Ambar 33 cultivar recorded the least effective salinity treatments of 150 mmol compared to the rest of the formulations.

Estimation of the activity of the enzyme CAT (CATalase) (absorbent unit.gm^{-1} fresh weight)

The levels of salinity were significantly different in the activity of the CAT enzyme (Table 7), as the activity of the enzyme increased at the level of salinity 150 mmol by 97% over the level of salinity 100 mmol and river water.While there was no significant difference between the salinity level of 100 mmol and the river water. This result agrees with what Singh et al. (2022) found that antioxidant enzymes increase with increasing levels of salinity, including the CAT enzyme.It is also clear from Table (7) that the genotypes significantly affected the effectiveness of the CAT enzyme, as the V1 genotype was excelled on the V3 genotype, with an increase in the enzyme activity by 48.2%. This supports the findings of Abdelaziz et al. (2018) that the genotypes differ in the activity of the CAT enzyme, where the salinity-tolerant structures have a high activity of the CAT enzyme.The cultivar Amber 33 (non-mutagenic) was significantly different in the activity of the CAT enzyme than the rest of the genotypes mutagenic and non-mutagenic (V1M1, V1M2, V1M3, V3M1, V3M2, V3M3) and for a salinity level of 100 mmol. While there was no significant difference at the level of salinity of 150 mmol and the treatment of river water according to the analysis of independent comparisons (Table 7), the variety Anbar 33 recorded less effectiveness for the treatments of 100 mmol of salinity compared to the rest of the formulations.

Table (6) Effect of salinity levels, mutagenesis concentrations, genotypes and the interaction between them on POD enzyme activity (absorption unit.g^{-1} fresh weight)

VMS		S1	S2	S3	Average
V1	M1	1.77	3.40	5.28	3.48
	M2	2.47	4.63	6.17	4.42
	M3	2.23	4.31	5.73	4.09
V3	M1	1.29	3.70	4.35	3.11
	M2	2.70	4.39	5.70	4.26
	M3	2.20	4.17	5.63	4
L.S.D5%		n.s			
Amber 33 cultivar		2.53	2.50	3.51	
independent comparisons L.S.D5%		n.s	n.s	*2.455	
VM	M1	M2	M3	Score of L.S.D 5%	
V1	3.48	4.43	4.09	*	Significant
V3	3.11	4.26	4.00	**	high significant
L.S.D	n.s			n.s	non significant
VS	S1	S2	S3	V	
V1	2.16	4.11	5.72	4.00	
V3	2.06	4.09	5.22	3.79	
L.S.D5%	n.s			n.s	
MS	S1	S2	S3	M	
M1	1.53	3.55	4.81	3.30	
M2	2.59	4.51	5.93	4.34	
M3	2.22	4.24	5.68	4.04	
L.S.D5%	n.s			**0.669	
S	S1	S2	S3	L.S.D5%	
	2.11	4.10	5.47	*2.029	

Table (7) Effect of salinity levels, mutagenesis concentrations, genotypes and the interaction between them on CAT enzyme activity (absorption unit.gm^{-1} fresh weight)

VMS		S1	S2	S3	Average
V1	M1	2.13	2.55	4.47	3.05
	M2	1.29	2.70	4.30	2.76
	M3	2.80	2.20	4.70	3.23
V3	M1	1.14	1.70	3.20	2.01
	M2	1.37	1.57	3.50	2.14
	M3	1.12	1.20	3.43	1.91
L.S.D5%		n.s			
Amber 33 cultivar		1.17	1.29	2.73	
independent comparisons L.S.D5%		n.s	0.859*	n.s	
VM	M1	M2	M3	Score of L.S.D 5%	
V1	3.05	2.76	3.23	*	Significant
V3	2.01	2.15	1.92	**	high significant
L.S.D5%	n.s			n.s	non significant
VS	S1	S2	S3	V	
V1	2.07	2.48	4.49	3.01	
V3	1.21	1.49	3.38	2.03	
L.S.D	n.s			0.441**	
MS	S1	S2	S3	M	
M1	1.63	2.12	3.83	2.53	
M2	1.33	2.13	3.90	2.46	
M3	1.96	1.70	4.07	2.58	
L.S.D5%	n.s			n.s	
S	S1	S2	S3	L.S.D5%	
	1.64	1.99	3.93	**1.012	

Measurement of chlorophyll in leaves (Spad)

The salinity levels differed significantly in their effect on the chlorophyll content (Table 8), as the river water gave the highest content of chlorophyll, while the mean of the trait decreased in the two treatments of 100 and 150 mmol by 33.4% and 47.2%, respectively, for river water.Also, the difference was significant between the 150 mmol treatment and the 100 mmol treatment, which decreased

by 20.8%. The reason for the breakdown of chlorophyll in plants exposed to salt stress is the production of some chlorophyll-degrading enzymes, such as the chlorophyllase enzyme, which is produced when the plant is exposed to abiotic stresses, and salinity causes the destruction of a protein. Plastids and chlorophyll reduction (Fisarakis et al., 2001).Some studies also indicate that the high sodium and chloride ions in the cytoplasm of leaf cells directly affect photosynthetic enzymes, light reactions and photosynthetic pigments (Ghanem et al., 2008, Karper et al., 2012, and Nahar, 2018).High concentrations of salinity have a negative impact on the process of photosynthesis, through its effect on the composition of chloroplasts, where the membranes of these organelles shrink with distortion of the chlorophyll-bearing plates, and this is due to the restriction of the absorption of the elements necessary to build the chlorophyll molecule (Al-Wahaibi, 2009).It is also clear from Table (8) that the mutagenic concentrations affected significantly the chlorophyll content in the leaves, where the chlorophyll content decreased in the mutagenic plants at both concentrations (1.5 and 2 mmol) by 11% and 18.9%, respectively, when compared to the treatment of non-mutagenic plants, while it did not. There was no significant difference between the two concentrations of mutagenicity (1.5 and 2 mmol).The reason for the low chlorophyll content in the mutagenic plants may be due to the time of measuring the chlorophyll content in the field, as the readings were taken at the time of flowering of all experimental units. Whereas, the mutagenic plants began flowering early, while the non-mutagenic plants were late, and the plants that flowered early reached advanced stages and began to tend to yellowing, and this is a natural result of low chlorophyll content.The genotypes V1 and V3 differed from each other in the content of chlorophyll in the leaves (Table 8), where the genotype V1 excelled on the genotype V3 by 69.3%. It has the efficiency of its vegetative covering to intercept light and represent substances, including an increase in chlorophyll pigment, and this was confirmed by Noor Al-Jannah (2021).The interaction was significant between the concentrations of the mutagen and the levels of salinity used in the study (Table 8), where the irrigation treatment with river water had a significant difference between mutagen and non-mutagen plants, where the chlorophyll content decreased in mutagen plants with both concentrations compared to non-mutant plants. Also, there was a significant difference between the mutated and non-mutated plants under the influence of salinity levels (100 and 150 mmol) with a decrease in the chlorophyll content of the mutated plants compared to the non-mutated plants. At a concentration of 2 mmol, which was irrigated with water with a salinity level of 150 mmol (M3S3), with a rate of 146%.Despite the similarity of the response of the two genotypes through the salinity levels used, the significant overlap was present due to the difference in the size of the response (Table 8). The highest mean of chlorophyll content for genotype V1 when irrigated with fresh water (S1) compared to genotype V3 irrigated at salinity level 150 mmol, which decreased by 69.5%, which gave the lowest rate for the trait. The interaction was significant between genotypes and mutagenic concentrations (Table 8), as the V1 genotype differed during mutagenesis concentrations and reached the highest average chlorophyll content for non-mutagenic plants and decreased by 16.1% and 24.2% for mutated plants with both concentrations of 1.5 and 2 mmol, respectively. While the V3 genotype did not differ during mutagenesis concentrations.The cultivar Anbar 33 (non-mutagenic) differed significantly in chlorophyll content with some mutagenic and non-mutagenic genotypes watered with river water according to the analysis of independent comparisons (Table 8), as the genotype V1 non-mutagenic was significantly excelled in chlorophyll content by 24.2% over the cultivar Ambar 33 and there was no difference Significant between the mutagenic V1 genotype at both concentrations (1.5 and 2 mmol) with the cultivar Ambar 33, While the variety Ambar

33 was excelled the genotype V3 mutagen and non-mutagen in chlorophyll content, but when irrigated at the salinity level of 100 and 150 mmol, there was no significant difference between the cultivar Ambar 33 and the genotype V1 mutagen and non-mutagen, but the significant difference occurred with the genotype V3 mutagen and non- The mutagen increased the chlorophyll content in favor of Anbar 33.

Table (8) Effect of salinity levels, mutagenic concentrations, genotypes and the interaction between them on chlorophyll content(spad)

VMS		S1	S2	S3	Average
V1	M1	42.67	27.00	21.00	30.22
	M2	33.33	22.00	20.67	25.33
	M3	30.33	22.33	16.00	22.88
V3	M1	24.00	14.00	10.00	16
	M2	21.00	15.00	11.33	15.77
	M3	19.33	13.33	11.00	14.55
L.S.D5%		n.s			
Amber 33 cultivar		34.33	25.33	18.00	
independent comparisons L.S.D5%		**5.583	**4.864	*3.221	
VM	M1	M2	M3	Score of L.S.D 5%	
V1	30.22	25.33	22.89	*	Significant
V3	16.00	15.78	14.56	**	high significant
L.S.D	**2.615			n.s	non significant
VS	S1	S2	S3	V	
V1	35.44	23.78	19.22	26.15	
V3	21.44	14.11	10.78	15.44	
L.S.D5%	**2.865			**1.543	
MS	S1	S2	S3	M	
M1	33.33	20.50	24.83	23.11	
M2	27.17	18.50	17.83	20.56	
M3	24.83	16.00	13.50	18.72	
L.S.D5%	* 3.417			**1.979	
S	S1	S2	S3	L.S.D5%	
	28.44	18.94	15.00	**2.66	

Proline content of leaves (µg.gm $^{-1}$)

Table (9) shows that salinity levels significantly affected the increase in proline content, where it increased at the treatment of 100 and 150 mmol by 49.7% and 89.5%, respectively, compared to river water. 100 mmol. The increase in the leaf content of proline with an increase in salinity levels is attributed to its role as a protection against environmental stresses, increasing plant tolerance to it and stimulating the production of ATP, which is part of the plant's response mechanisms to salt stress (szabados and savore, 2010).As well as its role in reducing the negative effects of salt by reducing the osmotic pressure and then maintaining the integrity of the plasma membrane and its functions (Nounjan et al., 2012). This result is consistent with a number of studies that confirmed an increase in proline level with an increase in equal salinity, including what was confirmed by Zhao et al. (2007).The concentrations of mutagens differed significantly in the content of proline (Table 9), where the cause of mutagenesis at a concentration of 1.5 and 2 mmol was an increase in the content of proline by rates of 23.3% and 27.4% for non-mutagenic plants, and no significant difference was recorded between mutagenic plants at a concentration of 1.5 and 2 mmol..The two genotypes V1 and V3 differed from each other in proline content (Table 9), as the V1 genotype was 23.2% superior to the V3 genotype in giving the highest proline content.The cultivar Amber 33 (non-mutagenic) was significantly different in proline content from the rest of the mutagenic and non-mutagenic genotypes (V1M1, V1M2, V1M3, V3M1, V3M2, V3M3) for all studied salinity levels according to independent comparisons analysis (Table 9). The cultivar Anbar 33 gave the lowest rate of proline content (0.0297, 0.0367, and 0.0527 µg gm-1) for salinization treatments S1, S2, and S3, respectively, compared to the rest of the compositions, and this indicates the genotypes V1 and V3 excelled on the cultivar Ambar 33 in this trait.

Table (9) Effect of salinity levels, mutagenesis concentrations, genotypes and the interaction between them on proline content (µg.g^{-1})

VMS		S1	S2	S3	Average
V1	M1	0.0410	0.0620	0.0910	0.0646
	M2	0.0530	0.0860	0.0973	0.0787
	M3	0.0597	0.0923	0.0990	0.0836
V3	M1	0.0320	0.0530	0.0727	0.0525
	M2	0.0420	0.0660	0.0893	0.0657
	M3	0.0533	0.0610	0.0830	0.0657
L.S.D 5%		n.s			
Amber 33 cultivar		0.0297	0.0367	0.0527	
independent comparisons L.S.D5%		*0.01859	**0.03201	**0.0328	
VM	M1	M2	M3	Score of L.S.D 5%	

V1	**0.0647**	**0.0788**	**0.0837**	*	**Significant**
V3	**0.0526**	**0.0658**	**0.0658**	**	**high significant**
L.S.D5%	**n.s**			**n.s**	**non significant**
VS	**S1**	**S2**	**S3**	**V**	
V1	**0.0512**	**0.0801**	**0.0958**	**0.0757**	
V3	**0.0424**	**0.0600**	**0.0817**	**0.0614**	
L.S.D5%	**n.s**			****0.00779**	
MS	**S1**	**S2**	**S3**	**M**	
M1	**0.0365**	**0.0575**	**0.0818**	**0.0586**	
M2	**0.0475**	**0.0760**	**0.0933**	**0.0723**	
M3	**0.0565**	**0.0767**	**0.0910**	**0.0747**	
L.S.D5%	**n.s**			***0.01305**	
S	**S1**	**S2**	**S3**	**L.S.D**5%	
	0.0468	**0.0701**	**0.0887**	****0.01795**	

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