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INDUCTION OF GENETIC VARIATIONS USING SODIUM AZIDE IN SOME GENOTYPES DERIVED FROM RICE FOR SALINITY TOLERANCE *IN VITRO*.

Ahmed Ali Abdel Abbas Al Aboudi[1*], Abdulkadhim Jawad Musa[2] and Majid Sh.Hamdalla[3]

[1]College of Agricultural Engineering Sciences, University of Baghdad , Iraq
[2]Office of Agricultural Research, Ministry of Agriculture , Iraq
[3] Institute of Genetic Engineering and Biotechnology, University of Baghdad, Iraq
*Email: alia75455@gmail.com

Abstract

This study was conducted in the laboratories of the Division of Tissue Culture of the Research Department of Al-Najaf Al-Ashraf , Agricultural Research Office / Iraq. The study aimed to obtain salinity-tolerant genotypes by using sodium azide mutagenesis for some genotypes derived from rice under the influence of salt stress. CRD design was used with three replicates .The seeds of the genotypes (V1, V2, and V3) were mutated with sodium azide at two concentrations (1.5 and 2 mmol) in addition to non-mutation, with the addition of the non-mutagenic cultivar Anbar 33 for control. The seeds were cultured in a nutrient medium to obtain callus, and the callus was transferred to a nutrient medium with a salinity of 150 mmol to obtain a plantlets. After 6 weeks, the samples were taken and analyzed. The results showed the following: Mutagenesis with sodium azide increased potassium, decreased sodium, increased SOD enzyme activity and increased proline content, chlorophyll and carbohydrates. As for the genotypes, the K1 genotype in most traits excelled in increasing the effectiveness of antioxidant enzymes (SOD, POD, and CAT) and in increasing the proline and chlorophyll content compared to the V3 genotype and the Anber33 cultivar. While the V2 genotype, it did not produce plants below the salinity level of 150 mmol. We conclude from this that the V1 genotype is the best salinity-tolerant genotype, due to its excelled in most of the traits through which the plant can resist salinity. As for mutagenesis by increasing sodium, it can be used to increase the content of proline, chlorophyll, and carbohydrates, and to increase some antioxidant enzymes. The best combination for callus induction was 2 mg L^{-1} of 2,4-D and 0.1 mg L^{-1} of Kint, while the best combination for plant regeneration was 4 mg L^{-1} of BAP and 0.5 mg L^{-1} of NAA.

Keywords: sodium azide, rice, salinity tolerance.

Introduction:

Rice (*Oryza sativa* L.) is one of the important grain crops in the world, where it constitutes the main food for almost half of the world's population and comes second after wheat in nutritional and economic importance (Baishya et al., 2015) Iraq is at the forefront of the Arab and Asian countries in terms of the total area affected by salinity, where there are large areas of agricultural land that have become unsuitable for cultivation due to the accumulation of salts, which in turn led to a severe decline in the economic return from agricultural production .The central and

southern regions of Iraq are medium to highly saline, in addition to representing one of the obstacles to increasing agricultural production because of its direct negative effects of toxicity, osmosis, imbalance of nutrients and its indirect effects of physical and chemical properties of the soil .There are options to address the problem of salinity, the most important of which is coexistence with it by using genotypes that tolerate salinity by creating variations in the genotypes cultivated by mutagen.

or relying on tissue culture technology with / or without the use of mutagens and the ease of selection at the cellular level, which allows examining and separating cells that carry the desired traits from among thousands of cells in a short period of time. Limited space and the possibility of evaluating the selected traits of the resulting plantlets as well as the ease of dealing with single cells and studying the physiological basis of salinity tolerance with a greater opportunity for the emergence of genetic differences (Novak et al., 1991) Al-Saedi (2016) achieved in her study the enhancement of salinity tolerance in two genotypes of rice, Anbar 33 and Anbar Baghdad, by inducing genetic variations for them using the chemical mutagen ethyl methane sulfonate (EMS) and the physical mutagen (UV-B) with Employing different techniques of *in vitro* cultivation, the seeds of the two genotypes Anber 33 and Anber Baghdad were divided into two groups.The first group was exposed to different concentrations of EMS (0, 0.5, 1.0, 1.5, and 2.0%) for 3, 6, and 12 hours.As for the second group of seeds, they were exposed to different time periods (0, 20, 40 and 60 minutes) of ultraviolet (UV-B) radiation with a wavelength of 280-320 nm .Then callus was induced from mature seed embryos and then cultured in MS medium that was included with concentrations (0, 50, 100, 150, 180, and 200 mmol) of NaCl .The results of her study showed an increase in the percentage of germination, vegetative and root lengths, enzymatic antioxidant activity in mutant plantlets, and significant differences between the two genotypes in terms of percentage of callus production and callus fresh weight. Yousif et al. (2017) used Sodium Azide (SA) at two concentrations (0 and 1.5 mmol) for 4 hours as a mutagenic to induce genetic variations in two local cultivar of rice, Anber 33 and Anber Baghdad, with a drought tolerance test at the cellular level by including the Poly Ethylene Glycol nutrient medium. (PEG) (0, 0.5, 1.0, 1.5, and 2.0%) The effect of the chemical mutagenic sodium azide and Poly Ethylene Glycol on plant regeneration was tested by counting the number of plantlets regeneration from callus, and the accumulation of proline and carbohydrates in those plantlets was also tested .The results showed an increase in the accumulation of proline and a decrease in the accumulation of carbohydrates in both Varieties with an increase in the levels of polyethylene glycol and the use of a concentration of 1.5 mmol of the chemical mutagen sodium azide. In view of the prevalence of the problem of salinity and its spread 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 of the traits of salinity tolerance in rice using tissue culture technology ,Therefore, the aim of this study included obtaining the genotypes of rice that are tolerant to salinity by evaluating and screening the genotypes of rice (V1, V2, and V3) for salinity tolerance with the addition of the cultivar Anbar33 as a control and the introduction of the technology of mutagenesis with increased sodium *in vitro* in order to expand the base of genetic differences from which it is possible to select

from them for the trait of salinity tolerance or to expose the induced callus to a high salt level and to alter the plantlets from it, as well as to study the physiological indicators with the aim of adopting them as indicators in determining the tolerance of these genotypes to high levels of salinity.

MATERIALS AND METHODS

Factors used in the study: The study included the following factors: (1) the first factor: mutagenicity with sodium azide (0, 1.5, 2 mmol), (2) the second factor: genotypes (V1, V2, V3, and V4) .

Table 1. The genetic origin of the genotypes under study

genotype	genetic origin
V1	Al-Furat (♀)X Anber33 (♂)
V2	Anber33 (♀)X Al- Baraka(♂)
V3	Al-Furat (♀) X Al Ghadeer (♂)
V4	Local variety) (Anber33

The genotypes (V1, V2, and V3) above were produced through the application of the plant breeding and improvement program by the staff of the Al-Mishkhab rice research station for several previous seasons, up to the stage of genotype stability and submission for approval. A laboratory experiment was coundected in the Division of Tissue Culture (Research Department of Al-Najaf Al-Ashraf) affiliated to the Agricultural Research Office - Ministry of Agriculture ,Using the factorial experiment system according to the complete randomized design (CRD) and with three replicates, the first factor was the mutagenicity concentrations (0, 1.5, 2 mM) and the second factor was the genotypes (V1, V2, V3, anber33) and the salinity level of the food medium was 150 mmol.

Establishment of tissue cultures

It included three stages: the first stage was the callus induction stage for four genotypes of rice under study, the second stage was the plant regeneration stage of the induced callus, and the third stage was the rooting of the growing plantlets from the callus. It included the following action steps:

Sterilization of work tools

The work tools used in tissue culture were all sterilized from culture bottles of different sizes, tweezers, blade holders and Petri dishes by placing them in an oven at a temperature of 160 ° C for a period of 90 minutes.Then it was transferred to the Laminar Air Flow Cabinet to place the tweezers and blades in a glass vial containing ethyl alcohol at a concentration of 96%, and it was exposed to a flame during work to get rid of the alcohol to ensure the cultuer process in sterile conditions free of pollutants.

Preparation of the nutrient medium

Prepared MS (Murashige and Skooge, 1962) medium (made by Caisson) was used, to which sucrose was added in an amount of 30 g L^{-1}.The acidity of the pH was adjusted to 5.7 after adding

vitamins and growth regulators, according to the requirements of each stage, by adding a few drops of sodium hydroxide (NaOH) solution or hydrochloric acid (HCl), then complete the final volume with distilled water, then add agar (Agar-Agar) by 7 g. L^{-1},The medium was placed on a hot plate magnetic stirrer to dissolve the agar and homogenize the food medium. After that, the medium was sterilized using a steam sterilizer (Autoclave)at a temperature of 121 ˚ C and a pressure of 1.04 kg cm^{-2} for 20 minutes, after which the tubes were removed and left to cool and the medium to harden at a temperature of room temperature until used in culture

Preparing seeds for culture

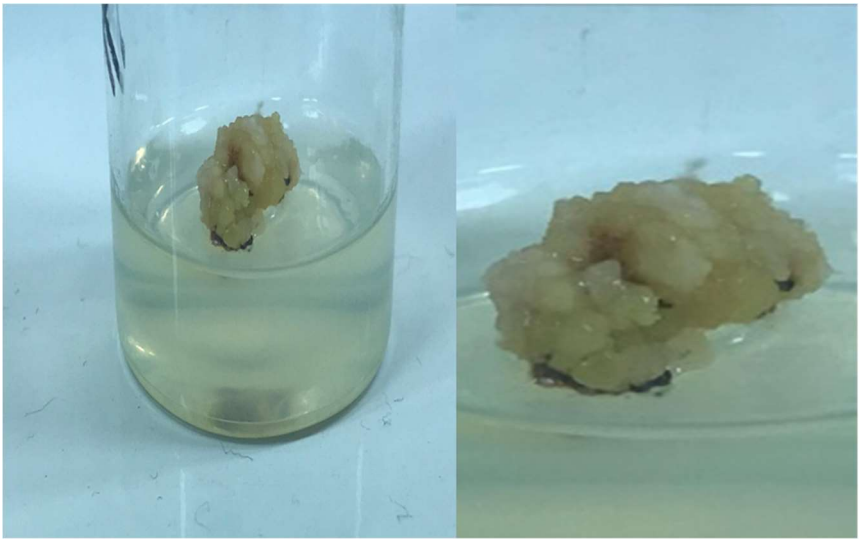
Ripe seeds of genotypes (V1, V2, and V3) were taken and washed with running water, then soaked in distilled water for 24 hours, and then treated with sodium azide solution at two concentrations (1.5 and 2 mmol) for 4 hours at a temperature of 28 °C and a pH of 3 by reducing the pH using acid.(Oraibi ,2013) Then the seeds were washed with tap water for half an hour, then the outer shell of the seeds was removed, then the seeds of the genotypes under study were sterilized by immersing them in 96% ethyl alcohol and then sodium hypochlorite solution (NaOCl) with a concentration of .2.5% (commercial minor type Fez, chlorine concentration of 6%) for 45 minutes. with constant stirring, After that, the seeds were washed three times with sterile distilled water for 3-5 minutes each time to get rid of traces of the sterile substance. The sterilization process was carried out inside the laminar air flow cabinet (Al-Fatlawi, 2020). The same steps were used with the cultivar Anber33, but without mutation.

Callus induction

The seeds of the four genotypes under study were sown after sterilization in the Laminar Air Flow Cabinet in the nutrient medium for callus induction composed of the previously described medium with the addition of 2 $mg.L^{-1}$ of 2,4-D and 0.1 $mg.L^{-1}$ of the KINT)After conducting an experiment showing the best concentration (Yousif, 2002), the culture vessels were incubated in the growth chamber for four weeks at a temperature of 25 ± 2 °C in the dark for the purpose of inducing callus.

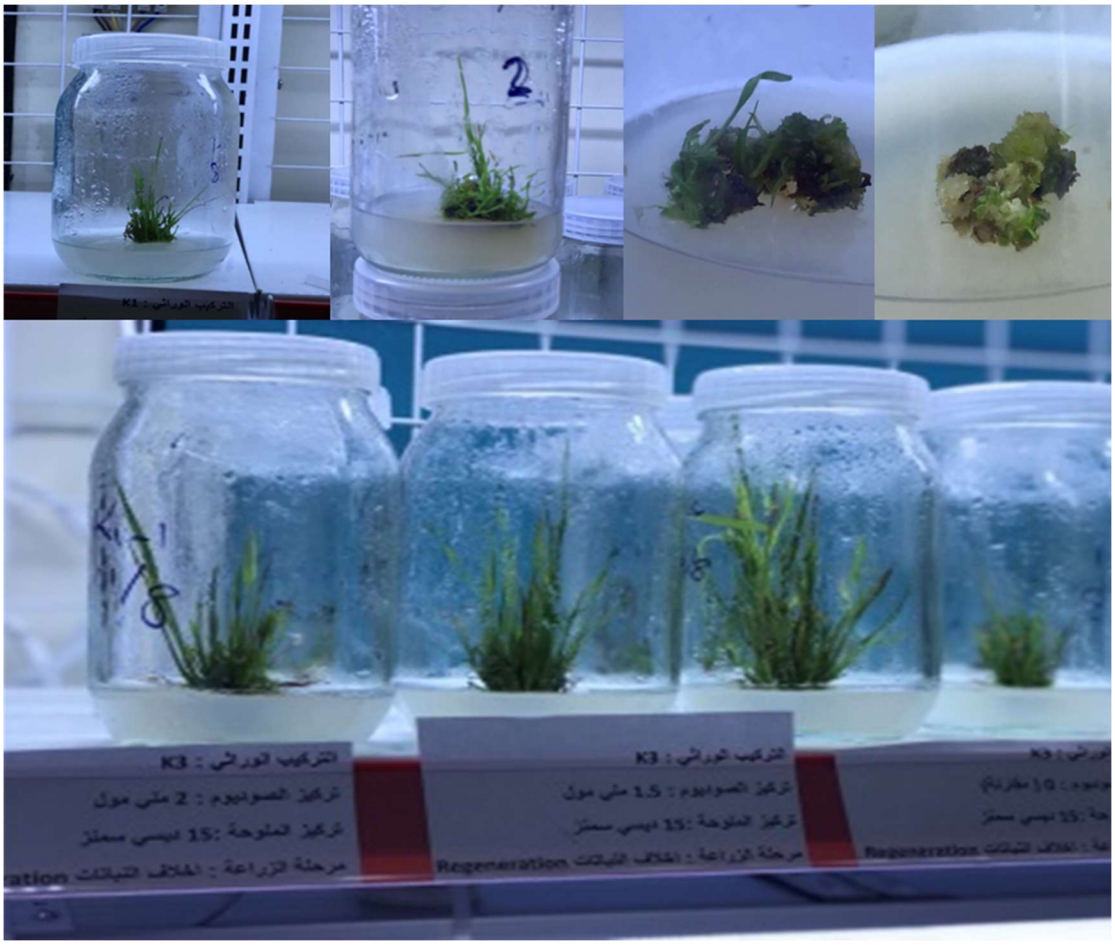
Callus culture on stress medium

The brine was prepared by purifying the local salt taken from the salts Al-Najaf Sea and dissolving it with distilled water at a concentration of 150 mmol (15 dS m^{-1}).Then a few drops of saline solution were added to the prepared nutrient medium for callus induction to sub-cultuer the growing callus in the saline nutrient medium, taking into account that the electrical conductivity of the medium (Electrical Conductivity) that was measured using the EC-Meter so that the level was obtained (15) $Ds.m^{-1}$ The induced callus was transferred, after cutting it into equal pieces by weight, into culture bottles containing 20 ml of saline medium, 25 vials for each genotype. Each vial was counted repeatedly. The culture vessels were incubated in the growth room for four weeks at a temperature of 25 ± 2 ºC in the dark to observe the surviving callus for the purpose of transferring it to the regeneration stage.



Plant regeneration

The plantlets were crossed from callus exposed to salt stress by transferring callus tolerant to salt stress of the genotypes under study (excluding the V2 genotype that failed to survive the callus) to the same components of the aforementioned nutrient medium except for the combination of growth regulators by adding 4 $mg.L^{-1}$ of BAP and 0.5 $mg.L^{-1}$ of NAAThe cultures were incubated in the growth chamber for six weeks at a temperature of 25 ± 2 ˚C and illumination for 16 hours $.Day^{-1}$.



rooting stage

Plantlets that were removed from the salt-tolerant callus were transferred to rooting medium supplemented with 1 mg.L^{-1} of IBA.They were incubated for four weeks in a growth chamber at a temperature of 25 ± 2 ˚C and illumination for 16 hours.Day^{-1}. After the end of the rooting period, the following properties were measured: antioxidant enzymes (SOD, POD, CAT), chlorophyll, proline, carbohydrates, potassium ions K+, sodium Na, and the ratio of potassium to sodium K+/Na.

Results and discussion

1- The percentage of potassium ions in the shoot content of the plantlets (K%)

Mutagenesis concentrations significantly affected the percentage of potassium (Table 2), as the mutagenic concentration of 1.5 mmol was significantly excelled on the treatment without mutagenicity by increasing potassium by 42.2%, and there was no significant difference between the two mutagenic concentrations (1.5 and 2 mmol) in the percentage of potassium.The cultivar Anber 33 (non-mutagen) was significantly different in potassium ion content from the mutagen V1 genotype according to the analysis of independent comparisons (Table 2),The cultivar Anbar 33 decreased from the genotype V1 mutagen at a concentration of 1.5 and 2 mmol, by 33.3% and 36.4%, respectively. While there was no significant difference between the cultivar Ambar 33 and the non-mutagenic V1 genotype, the cultivar Anbar 33 differed significantly with the non-mutagenic V3 genotype by 22.6% and by 39.2% and 30.5% from the mutagen V3 genotype at concentrations of 1.5 and 2 mmol, respectively.

Table (2) the effect of mutagenesis concentrations and genotypes and the interaction between them on the vegetative plant content of potassium ions % after six weeks of *in vitro* culture under the influence of salinity stress at the level of 150 mmol.

Sodium azide average	Genotypes		Sodium azide concentration (mM)
	K3	K1	
0.90	1.06	0.75	0
1.29	1.35	1.23	1.5
1.24	1.18	1.3	2
	1.19	1.09	Genotypes average
	0.82		Anber 33 cultivar
L.S.D.$_{(0.05)}$ Genotypes N.s = Mutagenesis 0.24 =interaction = N.sindependent comparisons0.17 =			

2- The percentage of sodium ions in the vegetative content of the plantlets (Na%)

The mutagenic concentrations differed significantly in the sodium ion content (Table 3), as the sodium ion content decreased with increasing mutagenic concentrations, the sodium concentration of 2 mmol was excelled on the treatment without mutagenicity and the mutagenic treatment with a concentration of 1.5 by 36% and 22.5%, respectively. The two genotypes (K1 and K3) had a significant effect on sodium ion content (Table 3), where the genotype K3 significantly excelled on the genotype k1 with a decrease of 57.2%.The cultivar Anber 33 (non-mutagenic) differed significantly in the content of sodium ions from the genotype V1 mutagenic and non-mutagenic, according to the analysis of independent comparisons (Table 3), Anber 33 decreased by 27.6% from the non-mutating V1 genotype and increased by 24.2% from the mutagenic V1 genotype at a concentration of 2 mmol, and there was no significant difference between Anber 33 and mutagen V1 at a concentration of 1.5 mmol.Also, the cultivar Anbar 33 differed significantly with the non-mutagenic genotype V3, where it increased by 89.2%. The cultivar Anbar 33 increased twice the content of sodium ions compared to the genotype V3 mutagen at concentrations of 1.5 and 2 mmol.

Table (3) the effect of mutagenesis concentrations and genotypes and the interaction between them on the vegetative content of plantlets sodium ions % after six weeks of *in vitro* culture under the influence of salinity stress at the level of 150 mmol.

Sodium azide average	Genotypes		Sodium azide concentration (mM(
	K3	K1	
2.3	1.3	3.4	0
1.79	1.06	2.53	1.5
1.50	1.02	1.98	2
	1.12	2.63	average Genotypes
	2.46		Anber 33 cultivar
L.S.D.$_{(0.05)}$ Genotypes **0.28** = Mutagenesis **0.34** =interaction = **0.49** independent comparisons0.43 =			

3- The percentage of potassium ions to sodium ions in the vegetative content of plantlets K/Na%

The mutagenesis concentrations significantly affected the ratio of potassium to sodium (Table 4), as the ratio of potassium to sodium increased with the increase in the mutagenic concentrations.The mutagenic concentration of 2 mmol was excelled by 69% to the treatment without mutagenicity and the mutagenic concentration of 1.5 mmol was excelled by 66% to the treatment without mutagenicity and there was no significant difference between the two mutagenic concentrations in the trait. The two genotypes (K1 and K3) differed in the ratio of potassium to sodium (Table 4), as the genotype K3 excelled on the genotype K1 with an increase of more than double the content of the genotype K1.The cultivar Anber 33 (non-mutagenic) differed significantly in the peracentage of potassium to sodium from the genotype V1 mutagen, according to the analysis of independent comparisons (Table 4), Anber 33 was lower than the mutagenic V1 genotype at a concentration of 1.5 and 2 mmol, by 31.2% and 48.4%, respectively, and there was no significant difference between the Anber 33 variety and the non-mutating V1 genotype. The cultivar Anbar 33 differed significantly with the non-mutagenic V3 genotype, where it decreased by 61.6%, and the Anbar 33 cultivar decreased by 24.2% and the genotype V3 mutagen decreased by 71.3% at concentrations of 1.5 and 2 mmol, respectively. The difference in the behavior of rice genotypes is evident in its content of sodium and potassium ions and the peracentage of K + / Na, which were treated with different concentrations of the chemical mutagen sodium azide and salt stress at a concentration of 150 mmol. This difference may be due to genetic reasons specific to each genotype. Mutation also works to reduce the toxicity of harmful ions such as sodium and increase

potassium ions, which are important for the vital functions of the plant cell, which have a close relationship with the tolerance of cells when exposed to salt stress, as well as the essential role of potassium in maintaining metabolic reactions within cells and its primary role in the process of osmotic modification and improving plant growth under The effect of environmental stressors (Ashraf et al., 2012).

Table (4) Effect of mutagenesis concentrations and genotypes and the interaction between them on shoot content of potassium to sodium percentage (K/Na)% after six weeks of *in vitro* culture under the influence of salinity stress at the level of 150 mmol.

Sodium azide average	Genotypes		Sodium azide concentration (mM(
	K3	K1	
0.53	0.86	0.21	0
0.88	1.28	0.48	1.5
0.9	1.15	0.64	2
	1.09	0.44	average Genotypes
	0.33		Anber 33 cultivar
L.S.D.$_{(0.05)}$Genotypes **0.15** = Mutagenesis **0.18** =interaction N.s = independent comparisons0.12 =			

4- The activity of the SOD enzyme in vegetative of plantlets (unit/g F.W.)

Mutagenic concentrations significantly affected the activity of the SOD enzyme (Table 5), as the mutagenic concentration 1.5 mmol was 30% higher than the non-mutagenic treatment, and there was no significant difference between the two concentrations of 1.5 and 2 mmol. This result is consistent with the findings of (Mohammed and Ibrahim, 2017). The two genotypes (K1 and K3) differed significantly in the activity of the SOD enzyme (Table 5). The K1 genotype was 32.4% superior to the K3 genotype, and this superiority is related to the nature of the genotype.The cultivar Anber 33 (non-mutagenic) differed significantly in the activity of the SOD enzyme from the genotype V1 mutagenic and non-mutagenic, according to the analysis of independent comparisons (Table 5), The cultivar Anbar 33 decreased from the non-mutagenic V1 genotype by 74% and by 77.2% and 75% from the mutagenic V1 genotype at a concentration of 1.5 and 2 mmol, respectively. The cultivar Anbar 33 differed significantly with the non-mutagenic V3 genotype by 60% and by 74% and 65.4% from the mutagenic V3 genotype with concentrations of 1.5 and 2 mmol, respectively.

Table (5) the effect of mutagenic concentrations and genotypes and the interaction between them on the activity of the enzyme SOD (unit/g F.W.) **for plantlets after six weeks of *in vitro* culture under the influence of salinity stress at a level of 150 mmol.**

Sodium azide average	Genotypes		Sodium azide concentration (mM(
	K3	K1	
4.06	3.2	4.93	0
5.28	4.94	5.63	1.5
4.42	3.71	5.13	2
	3.94	5.23	average Genotypes
	1.28		Anber 33 cultivar

L.S.D.$_{(0.05)}$Genotypes **0.41** = Mutagenesis **0.5** = interaction n.s = independent comparisons0.34 =

5- The activity of the POD enzyme in vegetative of plantlets (unit/g F.W.)

The two genotypes differed significantly in the activity of the POD enzyme (Table 6), as the genotype K1 was 30.8% excelled on the genotype K3.The cultivar Anber 33 (non-mutagenic) differed significantly in the activity of the POD enzyme from the genotype V1 mutagenic and non-mutagenic, according to the analysis of independent comparisons (Table 6), As the cultivar Anbar 33 decreased in POD activity from the V1 genotype by 23%, while there was no significant difference between the mutagenic and non-mutagenic V3 genotype.This indicates the excelled of the V1 genotype on Anbar 33 in this trait.

Table (6) the effect of mutagenic concentrations, genotypes and the interaction between them on the activity of POD (unit/g F.W.) **enzyme for plantlets after six weeks of *in vitro* culture under the influence of salinity stress at a level of 150 mmol.**

Sodium azide average	Genotypes		Sodium azide concentration (mM(
	K3	K1	
7.25	6.30	8.20	0
7.55	6.60	8.50	1.5
7.58	6.50	8.67	2

	6.46	**8.45**	**average Genotypes**
	6.4		**Anber 33 cultivar**
L.S.D.$_{(0.05)}$Genotypes **0.242** = Mutagenesis N.s =interaction = N.sindependent comparisons0.26 =			

6- CAT enzyme activity in vegetative of plantlets (katal/g.f.wt.)

The two genotypes had a significant effect on the activity of the CAT enzyme (Table 7), as the K1 genotype was 30.8% superior to the K3 genotype.The cultivar Amber 33 (non-mutagenic) differed significantly in the activity of the CAT enzyme from the genotype V1 mutagenic and non-mutagenic, according to the analysis of independent comparisons (Table 7), The cultivar Anbar 33 decreased from the non-mutagenic V1 genotype by 40.3% and by 45.7% and 48.4% from the mutagenic V1 genotype at a concentration of 1.5 and 2 mmol, respectively.The cultivar Anbar 33 differed significantly with the genotype V3 mutagen at a concentration of 2 mmol, as it decreased by 15.5%. This indicates the excelled of the mutagenic and non-mutagenic genotype V1 over the cultivar Anbar 33, as well as the excelled of the genotype V3 mutagen at a concentration of 2 mmol on the cultivar Anbar 33 in this trait.These results agree in their general framework with what was stated by Yousif et al. (2017), Mallik et al. (2011) and Daud et al. (2012) when exposing rice plantlets to mutagenesis and sodium chloride salt caused an increase in the activity of antioxidant enzymes such as peroxidase, catalase and superoxide dismutase. in rice plantlets *in vitro*, in that the SOD enzyme is the defensive line that scavenges the group of active free radicals ROS. The superoxide radical converts into hydrogen peroxide when the plantlets are exposed to salt stress, while the catalase converts the hydrogen peroxide into water.

Table (7) the effect of mutagenesis and genotype concentrations and the interaction between them on the CAT enzyme activity of plantlets (katal/g.f.wt.) after six weeks of *in vitro* culture under the influence of salinity stress at a level of 150 mmol.

Sodium azide average	**Genotypes**		**Sodium azide concentration (mM(**
	K3	**K1**	
1.53	**1.15**	**1.91**	**0**
1.72	**1.35**	**2.1**	**1.5**
1.68	**1.15**	**2.21**	**2**
	1.22	**2.07**	**average Genotypes**
	1.143		**Anber 33 cultivar**
L.S.D.$_{(0.05)}$Genotypes 0.16 = Mutagenesis n.s = interactionn.s = independent comparisons0.196 =			

Chlorophyll content of plantlets (µg gm $^{-1}$)

Mutagenesis concentrations significantly affected the chlorophyll content (Table 8), where the mutagenesis concentration of 2 mmol was significantly excelled on the non-mutagenic treatment and mutagenicity at a concentration of 1.5 mmol by 4.8%. This supports what was proven by Dewi et al.(2016) With sodium, increase the content of chlorophyll. The genotypes K1 and K3 differed significantly in chlorophyll content (Table 8), where the genotype k1 was 37.2% excelled on the genotype K3.The interaction between mutation concentrations and genotypes showed a significant difference in chlorophyll content (Table 8), The K1 mutagenic genotype at a concentration of 2 mmol excelled on the same non-mutagenic and mutagenic genotype at a concentration of 1.5 by 7.8% and 5.8%, respectively. In general, the genotype K1 mutagenic at a concentration of 2 mmol by 42.5% was excelled on the genotype K3 non-mutants, which did not differ significantly with the same genotype mutagenic at a concentration of 1.5 mmol. The cultivar Anber 33 (non-mutagenic) differed significantly in chlorophyll content from the genotype V1 mutagenic and non-mutagenic, according to the analysis of independent comparisons (Table 8), The cultivar Anber 33 decreased from the non-mutagenic V1 genotype by 26.2% and by 27.6% and 31.5% from the mutagenic V1 genotype at a concentration of 1.5 and 2 mmol, respectively.The cultivar also differed significantly with the genotype V3 mutagen at a concentration of 2 mmol, as it decreased by 3.4%.This indicates the excelled of the mutagenic and non-mutagenic genotype V1 on the cultivar Anbar 33, as well as the excelled of the genotype V3 mutagen at a concentration of 2 mmol on the cultivar Anbar 33 in this trait.

Table (8) Effect of mutation concentrations, genotypes and the interaction between them on the vegetative content of plantlets of total chlorophyll (µg.gm $^{-1}$) after six weeks of *in vitro* culture under the influence of salinity stress at the level of 150 mmol.

Sodium azide average	Genotypes		Sodium azide concentration (mM(
	K3	K1	
3.71	3.2	4.23	0
3.71	3.11	4.31	1.5
3.89	3.23	4.56	2
	3.18	4.36	average Genotypes
	3.12		Anber 33 cultivar
L.S.D.$_{(0.05)}$Genotypes **0.048** = Mutagenesis **0.059** = interaction **0.084** = independent comparisons0.087 =			

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

Mutagenesis concentrations had a significant effect on the effectiveness of proline content (Table 9), as the mutagenic concentration 2 mmol was 35% higher than the non-mutagenic treatment, and there was no significant difference between the two concentrations of 1.5 and 2 mmol. This supports what was stated by (Mensah et al. 2007) that proline content increased with increasing sodium azide concentration compared to the absence of mutagenicity. As the increase in the production of proline has a positive effect in reducing radiation damage because of its role in resisting free radicals resulting from radiation damage (Alexieva et al., 2001; Al-Enezi and Al-Khayri, 2012). Proline also works to reduce the damage caused by environmental stresses and to maintain the effectiveness of enzymes and balance the osmosis effort in plant cells.The two genotypes (K1 and K3) differed significantly in proline content (Table 9), where the genotype K1 was 22.7% excelled on the genotype K3.The cultivar Anber 33 (non-mutagenic) differed significantly in proline content from the genotype V1 mutagenic and non-mutagenic, according to the analysis of independent comparisons (Table 9), The cultivar Anbar 33 decreased from the non-mutagenic V1 genotype by 29.5% and by 44.8% and 48.3% from the mutagenic V1 genotype at a concentration of 1.5 and 2 mmol, respectively.The cultivar Anbar 33 differed significantly with the non-mutagenic V3 genotype by 11.1% and by 33.3% from the mutagenic V3 genotype at concentrations of 1.5 and 2 mmol.

Table (9) Effect of mutation concentrations, genotypes and the interaction between them on the vegetative content of plantlets of proline (µg.gm $^{-1}$) after six weeks of *in vitro* culture under the influence of salinity stress at the level of 150 mmol.

Sodium azide average	Genotypes		Sodium azide concentration (mM(
	K3	K1	
0.02052	0.01833	0.0227	0
0.02695	0.02473	0.02917	1.5
0.0279	0.02403	0.03177	2
	0.02237	0.02788	average Genotypes
	0.016		Anber 33 cultivar

L.S.D.$_{(0.05)}$Genotypes 0.0022 = Mutagenesis 0.0027 = interaction = N.Sindependent comparisons0.001 =

Percentage of carbohydrates in vegetative of plants %

Mutagenic concentrations had a significant effect on the carbohydrate content (Table 10), as the mutagenic concentration of 1.5 mmol was excelled by 8.8% over the non-mutagenic treatment and by 9.7% over the mutagenic treatment with a concentration of 2 mmol. This result is consistent with the findings of Bansod et al. (2019).As for the interaction between mutagenic concentrations and genotypes, it was significant (Table 10), as the K1 mutagenic genotype with a concentration of 1.5 and 2 mmol was significantly excelled on the same non-mutagenic genotype, and on the other hand, the K3 genotype with a concentration of 1.5 mmol was significantly excelled on the same genotype Non-mutagenic and mutagenic at a concentration of 2 mmol. The cultivar Anber 33 (non-mutagenic) differed significantly in carbohydrate content from the genotype V1 mutagenic and non-mutagenic, according to the analysis of independent comparisons (Table 10),The cultivar Anbar 33 decreased from the non-mutagenic V1 genotype by 12.8%, 16.3%, and 15.7% from the mutagenic V1 genotype, at a concentration of 1.5 and 2 mmol, respectively. The cultivar Anbar 33 also differed significantly with the non-mutagenic V3 genotype by 12.4% and 22.8%. And 7.7% for the genotype V3 mutagen at concentrations of 1.5 and 2 mmol, respectively.

Table (10) Effect of mutagenesis concentrations, genotypes and the interaction between them on the vegetative content of plantlets of total soluble carbohydrates % after six weeks of *in vitro* culture under the influence of salinity stress at the level of 150 mmol.

Sodium azide average	Genotypes		Sodium azide concentration (mM(
	K3	K1	
6.13	6.12	6.15	0
6.68	6.95	6.41	1.5
6.08	5.81	6.36	2
	6.29	6.30	average Genotypes
	5.36		Anber 33 cultivar
L.S.D.$_{(0.05)}$Genotypes **0.203** N.S = Mutagenesis = interaction = **0.288**independent comparisons0.156 =			

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