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INDUCTION OF GENETIC VARIATIONS IN TWO GENOTYPES OF RICE (*ORYZA SATIVA* L.) USING SODIUM AZIDE UNDER DIFFERENT SALINITY LEVELS.

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Abstract

The field experiment was conducted at the rice research station in Al- Mashkhab (Al-Najaf Governorate) during the 2021 season. Two rice genotypes (V1 and V3) were treated with two concentrations of sodium azide. The induced genotypes, in addition to the amber cultivar, were irrigated with different concentrations of NaCl, which included control (river water), 100 and 150 mmol NaCl. The objectives of the study were (1) to induce genetic variations for salt tolerance, (2) to evaluate the effect of sodium chloride on some agricultural traits and grain yield for the studied genotypes and (3) quantification of gene expression of the OsCIPK15 gene. Irrigation with different salinity concentrations negatively affected most of the studied traits compared to river water. Sodium azide generated genetic differences in some traits, as it reduced DTF, plant height, number of spikes, number of grains/spike, yield of grains/plant, while it had no significant effect on the weight of 1000 grain . Treatment with sodium azide also improved the salinity tolerance of V1 and V3 genotypes. With the increase of salinity levels of irrigation water, the gene expression of both the sodium azide-treated and untreated (V1 & V3) genotypes increased, while the gene expression of the amber cultivar decreased. Sodium azide-treated genotypes outperformed their non-treated counterparts in the rate of gene expression, which indicates sodium azide induction of the OsCIPK15 gene responsible for transduction of the signalling cascade in response to salinity stress.

Keywords: rice, salinity, sodium izide, gene expression.

Introduction

Rice (*Oryza sativa* L.) is one of the most important grain crops in the world, as it constitutes the main food for nearly half of the world's population and comes in second place after wheat in terms of nutritional and economic importance (Baishya et al., 2015) . Its cultivation spread in 114 out of 193 countries in the world, and Asia alone produces and consumes rice with 90% of global rice production . The global production is 742.54 million tons, with a cultivated area estimated at 160.06 million hectares, with an average yield of 4.64 tons. ha^{-1} (FAO, 2020), while the local production in Iraq for the year 2020 is about 464,200 tons, with a cultivated area estimated at about 102 hectares, and an average yield of 4.56 tons. ha^{1} (Statistics of the Ministry of Agriculture, 2020). The local production of rice in Iraq has not reached self-sufficiency due to the decline in the cultivation of the crop as a result of the drop in river water levels and the high salinity of irrigation water and agricultural lands. As well as the weak government support , low economic return for

the productivity of agricultural lands and dependence on importing foodstuffs. (Lami, 2021).The problem of salinity is one of the most important problems obstacle agriculture all over the world, especially in the arid and semi-arid areas, as about 20% of the cultivated lands in the world are affected by salinity, and about 30% of the area of rice cultivation in the world is affected by salinity (Singh 2021). 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 lands that have become unsuitable for cultivation due to the accumulation of salts, which in turn led to a severe decrease in the economic yield from agricultural production. The central and southern regions of Iraq are medium to highly saline, and the most important soluble salts in the soil are sodium chloride, magnesium, calcium, sodium and magnesium sulfate . In addition, salinity has direct negative effects of toxicity, osmosis and imbalance of nutrients and its indirect effects of physical and chemical properties of the soil. There are options to address the problem of salinity, including the reclamation of lands affected by salinity or coexistence with it using salinity-tolerant genotypes by induction variations in the cultivated genotypes through mutagens. Given the spread of the problem of salinity and its prevalence in large lands of Iraq and the urgent need for salinity-tolerant genetic genotypes , as well as the scarcity of studies and research in the domain of developing genetic variations for salinity tolerance in. The aim of this study included developing genotypes of salt-tolerant rice by evaluating and screening two genotypes of rice (V1 and V3) in addition to the amber cultivar , for salinity tolerance and using sodium azide to induce genetic variations

Materials and methods

The study included the following factors: (1) The first factor: salinity levels (river water, 100 mM, 150 mM), (2) The second factor: mutagen sodium azide (0, 1.5 mM, 2 mM), (3) Genotypes (V1, V3, Amber cultivar).

Table1. The pedigree of the genotypes under study

Genotype	Genetic origin
V1	**Amber (♂) X Al-Furat (♀)**
V3	**Al Ghadeer (♂) X Al-Furat (♀)**
Amber	**Certified local genotype**

The above genotypes (V1 and V2) were produced through a programm of plant breeding at the rice research station in Al-Mishkhab and for several previous seasons, up to the stage of genetic stability. The rice genotypes 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, as the PH was adjusted by phosphoric acid (Oraibi, 2013). Then the seeds were washed with tap water for half an hour. A field experiment was carried out at the Rice Research Station in Al-Mashkhab (Najaf Research Department) of 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 alluvial clay soil using a split-split plot system, according to a randomized complete block design (RCBD), with three replications. The salt concentrations (0, 100, 150 mM) occupied the main

plots and the sodium azide concentrations (0, 1.5 and 2 mM) occupied the sub plots , while the genotypes (V1, V3, Amber) occupied the sub- sub plots. The field was irrigated with water with different concentrations of sodium chloride between the day of irrigation and the day of drying, and the irrigation operations continued in this manner until the plants reached the stage of physiological maturity. The area of the experimental unit was 3 x 2 m .The plants were planted with lines, and the distance between one line and another and between plants was 25 cm. Weeds have been removed manually and as needed. The experimental land was fertilized by adding DAP fertilizer (N:P 18:18) with an amount of 400 kg.ha^{-1} mixed with the soil before cultivation. As for urea fertilizer (46% N), it was added at an amount of 280 kg.ha^{-1} in two equal batches, the first batch 10 days after cultivation, and the second batch a month after the first batch (Hassan, 2011). The traits of 50% of DTF (day to flowering), plant height (cm), number of fertile branches (branches carrying panicle .m^{-2}), number of grains per panicle , and weight of a 1000 grains (g) and grain yield (ton.ha^{-1}) The moisture of the grain yield was adjusted to 14% according to Hamdallah (2004) were studied, in addition to estimate the gene expression ratio of the OsCIPK15 gene according to Livak (2001) calibraor 2^(-ΔΔcT) method. The data were statistically analyzed using the analysis Genestat software to detect the differences between the studied treatments. least significant difference (LSD) at the level of significance of 0.05 has been used to compare the means (Al-Rawi and Khalaf Allah, 2000).

Results and discussion

Number of days from planting to 50% flowering

Salinity levels differed significantly in its effect on the number of days to reach 50% flowering (Table 2), as it took the treatment of using river water the least time to reach 50% flowering at an average of 7% and 9% for the salinity level (100 and 150 mM) respectively . The treatment of salinity of 100 mM also differed from the treatment of 150 mM, significantly reducing the number of days to flowering by 3.6% .The reason for the delay in flowering under the influence of salt stress may be attributed to the physiological reduction in the readiness of water, where salinity stress limits the absorption of water by plants, subsequently, retard seedlings growth and weaks them leading in delay the vegetative and flowering phases. It is also clear from Table (2) that mutagen concentrations had a significant effect in reducing the number of days to reach flowering in 50% of the plants, where mutagenesis at a concentration of 2 mM and 1.5 mM was the cause of early flowering compared to non-mutant plants, at a rate of 8.5% and 5.1%, respectively, compared to treatment without mutation. The mutagen concentration of 2 mM decrased significantly the number of days to reach 50% flowering by 2.7% as compare to 1.5 mM treatment. Some researchers mentioned that different mutagen concentrations may be a reason for early flowering of rice genotypes (omoregie and Ikhajiagbe, 2020). No significant differences in the number of days to reach 50% flowering among the studied genotypes, nor in two way and three way interactions. The cultivar Amber (non-mutant) differed significantly in the number of days from planting to flowering 50% from the rest of the mutated and non-mutated genotypes (V1M1,V1M2, V1M3, V3M1,V3M2, V3M3) at salinity level of 150 mM and river water treatment, while there was no significant difference with the salinity level of 100 mM according to the analysis of single orthogonals analysis (Table 2). The Amber cultivar took the longest period to reach 50%

flowering, which were 107 and 112 days for river water and 150 mM salinity treatments sequentially compared to the rest of genotype.

Table (2) Effect of salinity levels, mutagen concentrations and genotypes on the number of days from planting to flowering 50%

VMS		S1	S2	S3	Average
V1	M1	103	107	113	107.6
	M2	98	104	107	103
	M3	94	102	105	100.3
V3	M1	105	110	114	109.6
	M2	97	105	109	103.6
	M3	95	102	105	100.6
Lsd		N.S			
salinity average		98.6	105	108	
Amber cultivar		107	109	112	
Single orthogonals analysis		3.4**	N.S	3.0**	

VM	M1	M2	M3	Score Lsd	
V1	107.6	103	100.3	*	significant
V3	109.6	103.6	100.6	**	high significant
Lsd	N.S			n.s	significant

VS	S1	S2	S3	V
V1	98.3	104.3	108.3	103.6
V3	99	105.67	109.3	104.6
Lsd	N.S			N.S

MS	S1	S2	S3	M
M1	104	108.5	113.5	108.6
M2	97.5	104.5	108	103.3
M3	94.5	102	105	100.5
Lsd	N.S			1.86**

S	S1	S2	S3	Lsd

	98.6	**105**	**108.8**	**1.2 ****

S= salinity levels, M = mutagen concentrations, V = genotypes

Plant height

Irrigation treatments with saline water negatively affected the plant height (Table 3), as the plant height decreased at the 100 and 150 mM treatment by 17.8% and 24% respectively compared to the river water, and the plant height at the salinity level of 150 mM decreased by 7.5% from the salinity level of 100 mM,The decrease in plant height with an increase in salinity levels may be attributed to the very slow growth as a result of the decrease in the increase in the size of cells and the decrease in vital activities and damage to the chloroplasts with the short length of the internodes, which leads to a decrease in the height of the plant. These results are consistent with that of Soltabateva et al., 2021. The plant height characteristic differed significantly according to the different mutagenic concentrations (Table 3), the mutation caused by a concentration of 2 mM reduced plant height by 2.8% than the non-mutant plants, and no significant difference was recorded between the mutagenicity at a concentration of 1.5 mM or not. Shortening of mutated plants height at a concentration of 2 mM may be caused by to inhibit the activity of enzymes that catalyze the biosynthesis of gibberellins and that play a role in stem elongation (Dewi, 2016). Khan (2009) mentioned that sodium azide is a strong mutagenic and strongly inhibited the growth of plant parts with increasing concentration. These results agree with what was stated by Raina et al., 2022) which proved that mutagenicity with sodium azide leads to a shortening of plant height.The two genotypes V1 and V3 differed from each other in plant height (Table 3), whereby genotype V1 was 23.57% superior to genotype V3 in giving the highest plant height. The plant height characteristic varies between crops, some are under qualitative genetics and others are under quantitative genetics .This result is consistent with what was found by Al-Anawy (2015) and Al-Aboudi,(2016) and khuit et al,(2022) , who showed in their study on local genotypes of rice that the height of the plant varies according to the genes associated with this trait. Two way and three way interactions had no significant effect on the trait .The amber cultivar (non-mutagenic) differed significantly in plant height from the rest of the mutagenic and non-mutagenic genotypes (V1M1,V1M2, V1M3, V3M1,V3M2, V3M3) for all studied salinity levels according to the analysis of single orthogonals analysis (Table 3), and the cultivar Amber gave greater Average plant height reached 139.33, 107 and 98.33 cm with increased salinity levels compared to the rest of the studied genotypes. It is clear that the salt stress had an effect on the plant height for all the studied genotype .However, the decrease in the height of the amber cultivar was greater when the salt stress levels increased compared to the studied genotypes, despite the excelled of the amber cultivar in the average of this trait. This indicates that the Amber cultivar does not possess sufficient mechanisms to reduce the harmful effect of salt stress.

Table (3) Effect of salinity levels, mutagenic concentrations and genotypes on plant height (cm)

VMS		S1	S2	S3	Average
V1	M1	103.47	85.10	80.77	89.77
	M2	99.33	85.87	83.03	89.41
	M3	97	83.10	76.83	85.64
V3	M1	87	65.33	60.33	70.88
	M2	85.53	70.97	62.57	73.02
	M3	84.73	67	59.47	70.4
Lsd		N.S			
salinity average		92.84	76.23	70.50	
Amber cultivar		139.33	107	98.33	
Single orthogonals analysis		3.77 **	5.66 **	6.67**	

VM	M1	M2	M3	Score Lsd	
V1	89.78	89.41	85.64	*	significant
V3	70.89	73.02	70.40	**	high significant
Lsd	N.S			n.s	significant

VS	S1	S2	S3	V
V1	99.93	84.69	80.21	88.28
V3	85.76	67.77	60.79	71.44
Lsd	N.S			1.69**

MS	S1	S2	S3	M
M1	95.23	75.22	70.55	80.33
M2	92.43	78.42	72.80	81.22
M3	90.87	75.05	68.15	78.02
Lsd	N.S			2.01*

S	S1	S2	S3	Lsd
	92.84	76.23	70.50	4.35**

s = salinity levels, M = mutagen concentrations, V = genotypes

Number of panicle (panicle .m^{-2}):

Salinity levels differed significantly in their effect on the number of panicles (Table 4), as irrigation with river water gave the largest number of panicle per unit area, while the average of the trait decreased in the two treatments 100 and 150 mM by 14.4% and 26.3%, respectively, than the treatment of irrigation with river water. Also, the difference was significant between treatment 150 mM and treatment 100 mM, which decreased by 13.8%. The reason for the decrease in the number of panicles with an increase in salinity levels may be due to the prior effect of salts on the growth of cells and their ability to divide due to the lack of metabolites needed for growth, which leads to abort the primordium and reduction in the number of the number of total branches (The data do not shown), which is consider one of the most stages of rice growth sensitivity to salinity. (Rad et al., 2012), this result agrees with the findings of Shereen et al. (2020), who confirmed a decrease in the number of panicles with an increase in salt concentrations. It is also clear from Table (4) that mutagen concentrations had a significant and negative effect on the number of panicles per unit area which decreased in mutated plants for both concentrations (1.5 and 2 mmol) by 6.3% and 3.9%, respectively, than the treatment of non-mutated plants, and the mutagen concentration 1.5 differed significantly from the concentration of 2 mM, with an increase in the number of panicles by 2.7%.The genotypes V1 and V3 differed in the number of panicles per unit area, as the V3 genotype was 9.8% excelled on the V1 genotype. The superiority of the V3 genotype may be due to its excelled in the number of total branches (The data do not shown) . The phenomenon of branching is inversely related to the height of the plant, as the shorter the height (Table 3), the lower the apical dominance, which helps in the emergence of lateral buds, which later become branches (Assuero and tognetti (2010) ، This agrees with the results of (Al-Hasanie et al ، 2017) in the variation of cultivars and genotypes of rice in branching ability and the number of panicles per square meter. The interaction was significant between mutagenic concentrations and salinity levels used in the study (Table 4), as the plants irrigated with river water had a significant difference between mutagenic and non-mutant plants, where the number of panicles in mutagenic plants decreased with both concentrations compared to non-mutant plants. While there was no significant difference between mutated and non-mutant plants under the influence of salinity levels (100 and 150 mM). In general, the highest panicle number per unit area was for the treatment without mutagenesis and irrigated with river water (M1S1), which excelled on the mutagenesis treatment at a concentration of 2 mM, which was irrigated with water of a salinity level of 150 mM, Although the response of the two genotypes was similar during the used salinity levels, a significant interaction was present due to the difference in the size of the response (Table 4). The highest average number of panicle per unit area for genotype V3 when irrigated with fresh water (S1) compared to the genotype V1 irrigated with salinity level 150 mM, which decreased by 36.8%, which gave the lowest rate for the trait. It is noticed that the characteristic rate of genotype V1 did not differ during the levels S1 and S2, while the average trait of genotype V3 differed during all levels of salinity studied. Despite the excelled of the genotype V3 on the genotype V1 when irrigated with river water, but the genotype V1 was excelled when irrigated with water with

a salt concentration of 100 mM . The interaction was significant between the genotypes and mutagen concentrations (Table 4), where the mutagenic treatment with a concentration of 2 mM for genotype V3 was excelled by giving the largest number of panicles, (18%) as compare with genotype V1 at a concentration of 1.5 mmol. The effect of sodium azide concentration (2 mM) was negatively on the number of panicles of the genotype V1 and positively on the genotype V3, while the traits did not differ between the non-mutagenic and mutagenic treatment at a concentration of 1.5 mM. The three war interaction was significant , where Table (4) indicated that the non-mutant V1 genotype was significantly excelled on the mutated plants under all studied salinity levels by increasing the number of panicles per unit area, in contrast to the genotype V3 whose mutagenic plants excelled in both concentrations on non-mutant plants under salt stress levels. In general, genotype V3 mutagenic at a concentration of 2 mM and irrigated with river water recorded the highest number of panicles, excelled on genotype V1 and mutagenic at a concentration of 1.5 mM and irrigated with salinity water of 150 mM, which decreased by 72%. The plants of the genotype V3 mutated at both concentrations 1.5 and 2 mM and irrigated with a salinity level of 100 mM outperformed the non-mutated plants by giving them the highest number of panicles, where non-mutant plants decreased by 11.8% and 7.4% respectively, while the mutated V3 plants with a concentration of 1.5 mM excelled on the non-mutated plants by 11% under the salinity level of 150 mM, this result agrees with what was mentioned by omoregie and ikhajiagbe (2020), that the number of panicles increased in the mutated plants with sodium azide under salt stress as compare with the non-mutagenic plants of some genotypes. The cultivar Amber (non-mutated) differed significantly in the trait from the rest of the mutated and non-mutated genotypes (V1M1,V1M2, V1M3, V3M1,V3M2, V3M3) for all studied salinity levels according to the analysis of single orthogonals analysis (Table 4). The cultivar Amber gave the lowest average number of panicles per unit area of 225, 119 and 84 panicles .m^{-2} for salinity treatments S1, S2 and S3 respectively compared to the rest of genotype. This indicates the excelled of the genotypes V1 and V3 on the Ambar cultivar in this trait.

Table (4) Effect of salinity levels, mutagen concentrations, genotypes and the interaction between them on the number of panicle (panicle .m^{-2}).

VMS		S1	S2	S3	Average
V1	M1	285.33	275	212.67	257.6
	M2	239	242.67	195	225.5
	M3	246.33	245	200	230.4
V3	M1	341	224	212	259
	M2	284.67	254	236	258.2
	M3	336.33	242	221	266.4
Lsd		11.94**			
salinity average		288.78	247.11	212.78	

Amber cultivar		**225**	**119**	**84**	
Single orthogonals analysis		**12.79****	**11.36****	**12.73****	
VM	**M1**	**M2**	**M3**	**Score Lsd**	
V1	**257.67**	**225.56**	**230.44**	*	**significant**
V3	**259**	**258.22**	**266.44**	**	**high significant**
Lsd	**6.38****			**n.s**	**significant**
VS	**S1**	**S2**	**S3**	**V**	
V1	**256.89**	**254.22**	**202.56**	**237.89**	
V3	**320.67**	**240**	**223**	**261.22**	
Lsd	**8.55****			**3.95****	
MS	**S1**	**S2**	**S3**	**M**	
M1	**313.17**	**249.5**	**212.33**	**258.33**	
M2	**261.83**	**248.33**	**215.5**	**241.89**	
M3	**291.33**	**243.5**	**210.5**	**248.44**	
Lsd	**9.26****			**4.59****	
S	**S1**	**S2**	**S3**	**Lsd**	
	288.78	**247.11**	**212.78**	**8.38****	

The number of grains in panicle (grain. panicle $^{-1}$):

The salinity levels differed significantly in the trait of the number of grains in panicle (Table 5), where the number of grains decreased with the increase in salinity concentration (100 and 150 mM) by 42.1% and 53.8%, respectively, for river water. While the level of salinity 100 mM excelled on the level of salinity 150 mM, with a relative increase of 25.3% . The decrease in the number of grains with increasing salinity concentration may be due to the effect of salt stress on the number and vitality of pollen grains. As salinity causes a decrease in pollen grains activity leading to the failure of fertilization, or to a weakening of the ability of stigmas to receive pollen, or both (Abdla et al., 2001). This result is consistent with Aref (2013). Sodium concentrations affected significantly in the number of grains in panicle (Table 5), as the number of grains at a concentration of 2mM decreased by 16.2% than the treatment without mutagenicity, while there was no significant difference between the mutagenic concentrations at a concentration of 1.5 mM from the non-mutagenic treatment. The two genotypes V1 and V3 differed from each other in the

number of grains in panicle (Table 5), as the V1 genotype was 77.9% excelled on the V3 genotype, The increase in the number of grains of the V1 genotype may be due to its superiority in the flag leaf area, the length of the panicle and the number of its branches (data do not show). Perhaps the number of fewer panicles in the V1 genotype (Table 4) may have contributed to the decrease in competition for the products of photosynthesis, which contributed to an increase in the number of grains. There was no significant difference for the interactions between the studied factors except for the interaction between genotypes and salinity, which was significant. As (Table 5) shows a decrease in the number of grains in genotype V1 with an increase in salinity levels from 100 mM and 150 mM by 39% and 54% compared to irrigation with river water sequentially, and in genotype V3 by 47% and 53% % compared to irrigation with water river respectively for the same treatments. However, the levels of salinity 100 mM and 150 mM did not differ significantly from each other for genotype V3, but they differed with the treatment of irrigation with river water for the same genotype. In general, genotype V1 irrigated with river water gave the highest number of grains, while genotype V3 irrigated with salinity water 150 mM decreased by 72.8% of V1S1. The number of grains per panicle decreased for cultivar Amber across the salinity levels which gave 102, 68 and 42 grains for treatments salinity 0, 100 ad 150 mM respectively.

Table (5) Effect of salinity levels, mutagen concentrations, genotypes and the interaction between them on the number of grains

VMS		S1	S2	S3	Average
V1	M1	180	110	88	126
	M2	178	105	85	122.6
	M3	162	100	65	109
V3	M1	119	59	44	74
	M2	103	53.3	49	68.4
	M3	80	47.7	48	58.5
Lsd		N.S			
salinity average		137	79.2	63.2	
Amber cultivar		102	68.3	40	
Single orthogonals analysis		17.83**	N.S	15.71**	
VM	M1	M2	M3	Score Lsd	
V1	126	122.7	109	*	significant
V3	74	68.4	58.6	**	high significant
Lsd	N.S			n.s	significant

VS	S1	S2	S3	V
V1	173.3	105	79.3	119.2
V3	100.7	53.3	47	67
Lsd	13.24**			5.82**
MS	S1	S2	S3	M
M1	149.5	84.5	66	100
M2	140.5	79.2	67	95.6
M3	121	73.8	56.5	83.8
Lsd	N.S			6.12**
S	S1	S2	S3	Lsd
	137	79.2	63.2	13.13**

Weight of 1000 grains (gm):

The salinity levels differed significantly in the weight of 1000 grains (Table 6), where the weight of 1000 grains decreased with an increase in salinity levels of 100 and 150 mM by 19.13% and 24%, respectively, compared to river water, and there was no significant difference between the salinity level of 100 and 150 mM, The decrease in the weight of the grain under the influence of salt stress may be due to its negative effect on the leaf area and its content of chlorophyll , as well as the effect of salt stress in reducing the amount of carbohydrates manufactured by the plant and its direct impact on the weight of the grains. All these factors and others lead to a decrease in the process of photosynthesis, which reduces the amount of material accumulated and transported to the grain (Hasegawa et al., 2000). This result agreed with Nahar (2018) who showed a decrease in grain weight with an increase in salinity concentration. The genotypes V1 and V3 differed significantly in the weight of 1000 grains (Table 6), where the genotype V1 significantly excelled on the genotype V3 with a relative increase of 8.5%,The reason for the excelled of the genotype V1 may be due to the increase in the height of its plants (Table 3) and its efficiency in transferring the represented materials from the source to the sink, which lead to increase the weight of the dry matter stored in the stems, that subsequently contributes to the filling of the grains . As the sources of grain filling are what is transferred from the products of photosynthesis to green tissues throughout the period of filling, as well as the dry matter temporarily stored in the stems, especially before the flowering stage (Shiyam et al., 2014) and (Akhgari) and others, 2013) This result agrees with (Hadi and salem، 2016). As for the interaction between salinity levels and genotypes, it was significant (Table 6), where the average weight of 1000 grains for the two genotypes decreased with an increase in salinity concentrations. However, the interference arose from the difference in the amount of response. There was no significant difference between salinity levels (100 and 150 mM) for genotype V3, which differed from the treatment of irrigation with river water. In general,

genotype V1 irrigated with river water gave the highest weight per 1000 grains, while genotype V3 irrigated with 150 mM salinity water decreased by 28.7%. The concentrations of mutagen and other interactions did not have any significant difference for the trait. The cultivar Amber (non-mutagenic) differed significantly in the trait of weight of 1000 grains from the rest of the mutated and non-mutated genotypes of some genotypes across the studied salinity levels according to the analysis of single orthogonal analysis (Table 6), The Amber cultivar gave the lowest average weight of 1000 grains which was 20.57, 16.47 and 15.77 gm for salinity treatments S1, S2 and S3 respectively compared to the rest of the genotypes. The percentages of decline were close among all the studied genotype as a result of irrigation with water of different salinity, and this indicates the similarity of the mechanisms of salinity tolerance of the 1000 grain weight.

Table (6) Effect of salinity levels, mutagen concentrations, genotypes and the interaction between them on the weight of one thousand grains (gm)

VMS		S1	S2	S3	Average
V1	M1	23.66	19.31	17.49	20.15
	M2	21.5	16.82	15.98	18.10
	M3	23.78	19.13	17.86	20.25
V3	M1	21.86	17.87	17.52	19.08
	M2	23.45	19.54	17.3	20.09
	M3	21.55	17.11	16.99	18.55
Lsd		N.S			
salinity average		22.63	18.3	17.19	
Amber cultivar		20.57	16.47	15.77	
Single orthogonals analysis		0.84**	1.48**	1.48**	

VM	M1	M2	M3	Score Lsd	
V1	20.15	20.26	20.10	*	significant
V3	18.1	19.08	18.55	**	high significant
Lsd	N.S			n.s	significant

VS	S1	S2	S3	V
V1	23.63	19.33	17.55	20.17
V3	21.64	17.27	16.83	18.58
Lsd	1.22*			0.43**

MS	S1	S2	S3	M
M1	22.58	18.07	16.73	19.13
M2	22.82	18.5	17.69	19.67
M3	22.5	18.32	17.14	19.32
Lsd	N.S			N.S
S	S1	S2	S3	Lsd
	22.63	18.3	17.19	1.24**

Grain yield (ton.ha $^{-1}$)

Salinity levels differed significantly in their effect on grain yield (Table 7), as river water gave the largest grain yield and decreased it by 59.2% and 74.4% for the two treatments 100 and 150 mM respectively, and the grain yield for the 150 mM treatment significantly decreased from the salinity level of 100 mM by 37.4%,The reason for the decrease in grain yield may be due to the effects of salt stress in all growth traits and its effect on reducing the yield components, which ultimately led to a reduction in grain yield, This result is consistent with the findings of (Shereen et al. 2020) and (Aref, 2013), who confirmed that increasing salinity levels reduces grain yield. It is also clear from Table (7) that the mutagenic concentrations (1.5 and 2 mM) significantly and negatively affected the grain yield, as the grain yield decreased with the increase of the mutagenic concentration by 10.7% and 18.7%, respectively, than the non-mutated plants. The yield of plants mutated at a concentration of 100 mM decreased by 8.9% than that of plants mutated at a concentration of 1.5 mM, as Vinithasri et al. (2020) mentioned there is a linear relationship between an increase in sodium concentration and an increase in the number of sterile branches and sterility of pollen grains depending on the dose .The low concentrations of the mutants proved to be more effective and efficient than the medium and high concentrations. This was supported by Ambavane et al. (2015) when he proved that the low concentration of sodium azide was more efficient than the medium and high. The two genotypes V1 and V3 differed from each other in the grain yield (Table 7), where the genotype V1 was superior by giving the highest yield compared to the genotype V3 which decreased by 38.8%. The excelled of the genotype V1 is due to its superiority in the yield components, except for the number of panicles per unit area (Table 4). However, the genotype V1 was excelled in the number of grains (Table 5) and the weight of a 1000 grains (Table 6).We note that the percentages of decrease for the two genotypes V1 and V3 are approximately equal when irrigated with a concentration of 150 µmol salinity compared to irrigating with river water. This indicates the similar performance of the two genotypes in tolerance of salinity despite the difference in the yield when irrigated with river water in favour of the genotype V1.There was a significant interaction between mutagenic concentrations and salinity levels used in the study (Table 7). The grain yield decreased with increasing salinity levels and mutagenic concentrations. Unmutated plants irrigated with river water gave the highest grain yield, while mutated plants at a

concentration of 2mM and irrigated with salinity level of 150 mM decreased by 78.9%, which gave the lowest yield. Mutation levels of 1.5 and 2 mM did not differ within each salinity level. On the other hand, the treatments of non-mutation did not differ from mutagenicity at a concentration of 1.5 mM for the two salt levels 100 and 150 mM. Although the response of the two genotypes was similar during the used salinity levels, significant interaction was present due to the difference in the size of the response (Table 7). The grain yield of the genotype V1 reached 9.6 tons.ha^{-1} when irrigated with river water, but it decreased by 52% when irrigated with a salinity level of 100 µmol and reached 4.6 tons.ha^{-1} and to 76.3% when irrigated with a salinity level of 150, reaching 2.5 tons.ha^{-1} ,As for the genotype V3, the highest grain yield was 6.6 tons.ha^{-1} when irrigated with river water, and it decreased to 69% when irrigated with a salinity level of 100mM and reached 2.04 tons.ha^{-1} and to 76% at a salinity level of 150 µmol with an average of 1.6 tons.ha^{-1}.)In general, the highest yield of genotype V1 irrigated with river water was lower than that of genotype V3 irrigated with salinity level of 150 mM by 83.4%.The interactions between mutagen concentrations and genotypes and the three way interaction did not reach significance for the trait. The cultivar Amber (non-mutagenic) differed significantly in grain yield from the rest of the mutated and non-mutated genotypes (V1M1,V1M2, V1M3, V3M1,V3M2, V3M3) for all studied salinity levels according to the analysis of single orthogonals analysis (Table 7),The Amber cultivar gave the lowest grain yield (4.34 tons.ha^{-1}), (1.1 tons.ha^{-1}) and (0.63 tons.ha^{-1}) for salinity treatments S1, S2 and S3 respectively compared to the rest of the genotypes. It is noted that the percentages of yield drop for the Amber cultivar for treatments 100 and 150 mM were higher as compare to the irrigated with river water. This indicates the low ability of the cultivar Amber to withstand salt stress. Also, when comparing the grain yield of the cultivar Amber with the genotypes V1 and V3 when irrigated with river water, it was clear that the genotypes V1 and V3 were excelled on the genotype Amber in this trait.

Table (7) Effect of salinity levels, mutagen concentrations, genotypes and the interaction between them on the grain yield (tons.ha^{-1}**)**

VMS		S1	S2	S3	Average
V1	M1	10.49	5.30	2.90	6.23
	M2	9.62	4.50	2.56	5.56
	M3	8.95	4.10	2.30	5.11
V3	M1	8.28	2.02	1.22	3.84
	M2	6.07	2.26	1.96	3.43
	M3	5.72	1.85	1.64	3.07
Lsd		N.S			
salinity average		8.19	3.34	2.09	
Amber cultivar		4.34	1.1	0.63	

Single orthogonals analysis		**1.34****	**0.87****	**0.49****	
VM	**M1**	**M2**	**M3**	**Score Lsd**	
V1	**6.23**	**5.56**	**5.11**	*	**significant**
V3	**3.84**	**3.43**	**3.07**	**	**high significant**
Lsd	**N.S**			**n.s**	**significant**
VS	**S1**	**S2**	**S3**	**V**	
V1	**9.68**	**4.63**	**2.58**	**5.63**	
V3	**6.69**	**2.04**	**1.60**	**3.44**	
Lsd	**0.58****			**0.37****	
MS	**S1**	**S2**	**S3**	**M**	
M1	**9.38**	**3.66**	**2.06**	**5.03**	
M2	**7.84**	**3.38**	**2.26**	**4.49**	
M3	**7.35**	**2.97**	**1.97**	**4.09**	
Lsd	**0.55****			**0.29****	
S	**S1**	**S2**	**S3**	**Lsd**	
	8.19	**3.34**	**2.09**	**0.47****	

Gene expression:

Identification of the genes involved in stress tolerance, their patterns and understanding their functions in stress adaptation provides a basis for engineering stress tolerance. Understanding the level of the expression pattern of OsCIPK15 during salinity stress and the physiological effect on rice lines may help breeders to select and breed salt-tolerant cultivars for cultivation in affected areas. The results in Table (8) showed the values of gene expression (folding) of the OsCIPK15 gene for seven studied genotype, namely, genotype V1 and genotype V3 without mutagenicity and mutagenicity at a concentration of 1.5 and 2 mM, in addition to the non-mutagenic Amber cultivar under the studied salinity levels (irrigation with river water and salinity of 100 and 150 mM). As for the V1 genotype , the gene expression increased significantly with the increase in the levels of mutagenesis. As for the V3 genotype, its gene expression increased for the mutated genotype compared to the non-mutated genotype, but no difference was observed between the two mutagen concentrations for the V3 genotype. The saline concentrations used affected the difference in gene expression, but this difference was not symmetrically, where the highest gene expression occurred for the concentration 150 mM in the genotype V1, while the highest gene

expression occurred at the 100 mM salt level for the genotype V3 for all levels of mutagenicity, as well as for the non-mutated genotype V1. These results may indicate the positive effect of the mutation in genotype V1, which led to a greater response of gene expression to this genotype at higher salt stress. As for the mutagenic effect on the V3 genotype, it improved the performance of the gene and led to an increase in the gene expression of the mutated genotype compared to the non-mutated V3 genotype, but the response was higher at the average salt level (100 mM). Excessive stress induced the gene expression of OsCIPK15 gene, thus affecting the physiological homeostasis of the plant. The difference in the response of the two genotypes may be related to the nature of the genotype and the relationship of the studied gene with other genes that code for the same enzyme. OsCIPK15 is a silent gene, but it is stimulated by increased salt content and then transcribes proteins in response to biological stresses (Kamanga, 2016). As for the Amber cultivar , which was not treated with mutagens in this study, its gene expression decreased with increasing salinity levels, and this indicates that the Amber cultivar may not have this gene or that it is silently present, and this is confirmed by the results of the gene expression analysis, where the non-mutated V1 and V3 genotype showed higher levels of gene expression at salinity levels compared to when irrigated with river water, while the cultivar Amber, its gene expression was decreased when using high salinity levels. When comparing the values of gene expression for the two genotypes without mutation and after mutation, we note that the values of gene expression increased at high salinity levels of the mutagenic genotype, and this indicates the positive effect of sodium azide in inducing mutation of the studied gene at least. As for the Amber cultivar, the opposite occurred, and this indicates that the Amber cultivar does not possess salinity-tolerant genes. It should be noted that sodium azide, which was associated with an increase in gene expression, does not necessarily leads to an increase in yield. This is due to (1) that the mutation may have improved the tolerance to salinity but not the yield or one of its components; (2) That the yield, as is known, a quantitative trait depends on the action of large numbers of genes, The two genotypes V1 and V3, which showed high gene expression under salt stress levels, can be used in breeding programs Abiotic stress is a quantitative trait that is governed by a large number of genes (Jake et al., 2010). Benitez et al. (2013), demonstrated that different loci in the genome act in concert to mitigate abiotic stresses including salinity. This means that the physiological and morphological modification of the plant is the product of several genetic loci in the plant genome that work concurrently to adapt under stress conditions. Accordingly, the identification and detection of the number of genes responsible for tolerance to abiotic stresses in local cultivars is the first step in the development of these cultivars for salt tolerance.

Table (8) values of gene expression for the studied genotype

Genotypes	Trt. (Nacl)mM	Ct (GAPDH)	Ct(OsCIPK15)	ΔCt	ΔΔCt	Folding
K1-0	0	24.9	35.21	10.31	0	1
	100	25.21	33.14	7.93	-2.38	5.2053
	150	24.36	32.69	8.33	-1.98	3.9449

K1-1.5	0	18.99	27.1	8.11	0	1
	100	16.55	21.22	4.67	-3.44	10.852
	150	23.51	27.81	4.3	-3.81	14.025
K1-2	0	17.55	25.44	7.89	0	1
	100	18.65	23	4.35	-3.54	11.63
	150	21.53	25.5	3.97	-3.92	15.13
K3-0	0	24.9	35.21	10.31	0	1
	100	22.54	30.52	7.98	-2.33	5.028
	150	24.33	33.66	9.33	-0.98	1.972
K3-1.5	0	18.01	27.11	9.1	0	1
	100	20.24	25.56	5.32	-3.78	13.737
	150	27.62	33.36	5.74	-3.36	10.267
K3-2	0	16.33	25	8.67	0	1
	100	22.36	27.33	4.97	-3.7	12.996
	150	24.84	30.12	5.28	-3.39	10.483
Amber	0	20.32	30.22	9.9	0	1
	100	23.24	33.25	10.01	0.11	0.926
	150	25.66	36.31	10.65	0.75	0.594

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