Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

USING PLANT TISSUE CULTURE TECHNIQUE TO EVALUATE FOUR GENOTYPES OF MAIZE TO SALINITY TOLERANCE

M. I. Salih *[1], A. S. Abed [2], E. N. Ismail [3] S. F. H. Kassad [4]
*[1,4] Instit Genetic Engin.Biotech., University of Baghdad, Baghdad, Iraq
*[2,3] Biotec Res Cent, College of Sciences, Al-Nahrain University, Baghdad, Iraq

ABSTRACT

Salinity is an important abiotic factor that negatively impacts maize cultivation, especially in areas with saline soils. The present study evaluates the influence of salinity on the growth and physiological reactions of seven maize genotypes. Highly tolerant cultivars were excluded, while callus cultures *were* produced from moderately salt-tolerant cultivars and salt-sensitive genotypes on MS medium fortified with 3 mg/L 2,4-D and 0.5 mg/L Kin. The finding showed that high levels of salinity significantly decreased the callus weight. The moderate cultivars (Alfajr and Almaha) demonstrated a high salt tolerance index (STI) compared with sensitive genotypes (T1 and T6). The ionic analysis indicated that genotypes T1 and T6 revealed a higher concentration of the ions Na^+ and Cl^-. On the contrary, the cultivars Alfajr and Almaha accumulated a high concentration of the cations K^+ and Ca^{2+}, along with a greater carbohydrates and proline content that probably led to their developed salt tolerance. Additionally, the molecular analysis based on the RAPD test, classified the tolerant cultivars and sensitive genotypes into two separate groups, confirming the presence of genetic differences related to salinity tolerance. The results highlight the role of ion regulation, osmotic adaptation, and genetic factors to mitigate the impact of salinity on maize. The study provides helpful insights that could assist breeding procedures for developing salt-tolerant maize genotypes that are necessary for maintaining maize agriculture in areas affected by high salt stress.

Keywords: Maize, Salinity tolerance, RAPD PCR, callus cultures.

Received: 16/12/2024, Accepted: 23/3/2025, Published: 30/5/2026

INTRODUCTION

The presence of high salinity levels represents an important risk to the overall stability of the food supply worldwide, affecting the efficiency of crop growth and the long-term viability of agriculture (Mukhopadhyay *et al* 2021). The increasing salinity levels in the soil, mostly caused by inappropriate irrigation techniques, rising sea levels due to climate change, and the degradation of soil, have become urgent issues for agricultural cultivation on a global scale (Stavi *et al* 2021). Maize (*Zea mays* L.) is an important crop in worldwide food production, serving as a primary source of nutrition for millions of people and playing a critical role in animal feed and numerous industries (Gheith *et al* 2022). However, the increasing prevalence of salt stress in maize cultivation areas has raised concerns about its potential impact on crop output and quality (Li *et al* 2023). Inducing osmotic disorder, ion toxicity, and oxidative damage are some of the ways that salinity stress hurts plants (Farooq *et al* 2015), this leads to slower growth, lower yields, and poor nutritional quality (Ibrahim *et al* 2022). Many physiological functions in maize crop cooperate to reduce the negatively impacts of

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

salinity stress, including: modulation of stomatal behavior, reducing cytoplasmic toxicity by split extra Na+ into vacuoles, and increasing Cl- transportor activities (Cao *et al* 2023). The accumulation of some osmotic substances like starch and proline also increased in salt-grown maize; to adapt to decrease water potential (Farooq *et al* 2015). To address these difficulties, it is crucial to cultivate varieties of maize that can flourish in saline environments and sustain productivity in salt-affected soils (Zhou *et al* 2022). Traditionally, the assessment of tolerance to salt in crops has depended on trials in the field and greenhouse investigations, which are frequently time-consuming, resource-intensive, and susceptible to environmental fluctuations (Sun *et al* 2018). To assist these constraints and expedite the progress of salt tolerance studies, innovative methods employing plant tissue culture and molecular techniques have gained traction. The controlled environment of plant tissue culture makes it possible to study how plants´ bodies and chemicals respond to salt stress (Taratima *et al* 2022). This permits for a better understanding of the mechanisms that contribute to salt tolerance in maize. Molecular analysis methods, like Random Amplified Polymorphic DNA (RAPD), is also being used to test for salt tolerance (Mazumder *et al* 2020). In addition to physiological tests, molecular studies help us understand salt-responsive DNA markers and genetic variants that are connected to salt stress tolerance (Kim *et al* 2023). By employing a multidisciplinary approach, a thorough analysis of salt tolerance pathways in maize can be achieved, leading to valuable insights for developing specific methods to enhance crop quality (Ibrahim *et al* 2022). The study aimed to employ tissue culture techniques and molecular analysis to comprehend the response of maize to salt stress and to identify potential genotypes for use in breeding programs to enhance salt tolerance. Moreover, this study contributes to the development of sustainable maize production in response to increasing salinity difficulties. It provides valuable insights that can guide future efforts to improve crops and reduce the negative impact of saline stress on agricultural systems.

MATERIALS AND METHODS

Plant materials and surface sterilization After screening seven maize genotypes for salinity tolerance in the Department of Agricultural Research of Iraqi Agriculture Ministry, four genotypes were selected to evaluate the impact of salt stress on each genotype, including two moderately salt-tolerant cultivars, Alfajir and Almaha, and two sensitive salt-tolerant genotypes, T1 and T6. The investigation and *in vitro* study were carried out at the Biotechnology Research Center/ Al Nahrain University. The surface sterilization was applied under a laminar air flow cabinet, by soaking the washed seeds in 70% ethanol for 1 minute, followed by 2% sodium hypochlorite for 15 minutes (Al-Alwani *et al* 2023). The rewashing with sterilized distilled water was applied to remove the disinfectant solution. The sterilized seeds were kept for 24 h in the refrigerator at 7 ºC to facilitate the removal of mature embryos and use them as explants.

Establishment of callus cultures: Callus cultures were induced by inoculating mature embryos on Murashige and Skoog medium (MS) (Murashige *et al* 1962) fortified with 3.0 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 mg/L Kinetin (Kin), 30 g/L sucrose, and 7 g/L Agar. The pH was adjusted to 5.7 using 1N of NaOH or HCl. Steam sterilization was carried out by an autoclave for 20 minutes at 121 ºC (a pressure of 1.04 kg/cm^2) (Singh *et al* 2011). The cultured explants were kept in darkness at 25 ± 1 °C in the growth chamber (Jassim *et al* 2021). After 30 days, the initiated callus cultures from four maize genotypes were sub cultured every four weeks for three intervals to perform the maintenance stage.

Impact of salinity stress: Three levels of salt stress were applied by adding NaCl to the MS culture medium to obtain 2, 8, and 16 dS/m of electrical conductivity. Each treatment was divided into 15 replicates and finally autoclaved. Three-month-old callus cultures that were induced from four maize genotypes were splinted into equal weights (200 mg) and planted in each replicate. All treatments were incubated under growth chamber conditions

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

for 30 days. Different parameters were used to evaluate the salinity tolerance (Masuda *et al* 2021). Measurement of the callus dry weight (DW), reduction in DW, and the salt tolerance index

The fresh callus was harvested on the 30th day. Dried samples were obtained using dry oven at 55 ºC for 48 h to calculate the dry weight (DW) (Hannachi *et al* 2022). The relative percent reduction in DW was estimated according to (De Leon *et al* 2015) by the following:

DW reduction (%) = 100 (DW control – DW stress/ DW control)

The salt tolerance index (STI) in dry callus matter was estimated using the method determined by (Hu *et al* 2022).

$$STI\ (\%) = \frac{\text{Total dry weight of plant tissue subjected to salinity}}{\text{Total dry weight of plant tissue in control group}} x100$$

Mineral content: Four mineral ions were analyzed in a callus under salt stress. Sodium (Na^{+}), potassium (K^{+}), chlorine (Cl^{-}), and calcium (Ca^{++}) were determined by atomic absorption spectroscopy after acidic digestion, according to the method described by (Paul *et al* 2014).

Carbohydrates content: The anthrone method was applied to determine the content of soluble carbohydrates in samples of callus as described in (Nikoogoftar-Sedghi *et al* 2024). Briefly, 50 mg of each dried sample was homogenized with 70% ethanol. The centrifugation was carried out for 15 minutes at 4500 rpm. Hundred µl of supernatant was mixed with an anthrone-acid mixture and kept for 10 minutes in a water bath. The photometric method was employed to detect the absorbance at 625 nm with glucose as a standard. Proline content, the content of proline was determined as described in (Dinakar *et al* 2009). Briefly, 100 mg of dried callus cultures were homogenized with 3% of 5-sulfosalicylic acid and centrifuged at 3500 rpm for 10 minutes. The supernatant was mixed with an acid-ninhydrin reagent and boiled for 30 minutes in a water bath. After cooling, the red layer was separated by adding toluene. Proline content (µg/g) was estimated by spectrophotometry (Optima-300, Japan) at 520 nm against standard proline (Sigma-Aldrich, USA).

Pearson correlation analysis: Correlation analysis was performed between callus growth parameters, mineral ion content, carbohydrates, and proline content of the studied maize genotypes under 16 dS/m salinity stress.

Genetic similarity under salt stress: *DNA extraction*: Total DNA was extracted from dried callus using the Geneaid DNA purification kit (Taiwan). The purification and quantification of isolated DNA were verified by a nanodrop spectrophotometer. Gel electrophoresis using 1% agarose was employed to confirm the quality of the isolated DNA (Alwash *et al* 2016).

RAPD-PCR amplification

Seven RAPD primers were chosen to identify the genetic similarity (GS) of the studied maize genotypes, named OPB-01, OPB-06, OPC-08, OPE-13, OPE-16, OPF-20, and OPG-09. Five microliters of template DNA, 2 µL of primer (10 pmole/µl), 1 U of taq polymerase, 200 mM dNTPs, and 1.5 mM $MgCl_2$ were added to Each 20 µL reaction maser mix (Bioneer-Korea). A Biorad thermal cycler (USA) was operated for polymerase chain reaction (PCR) amplification. The program steps were followed as described, which included the initial denaturation step (95 °C for 5 min), then 40 cycles of denaturation (95 °C for1 min), annealing (43 °C for 1 min), and final elongation (72 °C for 8 min). After all, the cooling step was performed at 10 °C. The separation of the final products of PCR was carried out on 1.4% agarose (Oktavianingsih *et al* 2021). Gel was stained with Safe-Red and photographed by the Gel-documentation system (Atta-Japan) under UV illumination.

Genetic diversity: The performance of the utilized RAPD markers was quantified according to (El-bakatoushi *et al* 2017), included estimation of total bands (TB), monomorphic bands (MB), polymorphic bands (PM), polymorphic percentage (P%), primer efficiency (PE), and primer discriminatory

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

power (PDP). The cluster analysis was applied to form a genetic diversity and similarity relationship depending on Jaccard's coefficients using Ward's method by PAST computer software (version 1.94b).

Statistical analysis: A completely randomized design (CRD) was used to analyze the differences among the data in the experimental treatments. All the treatments were replicated twice; ten replications of culture test tubes were used. Three replications of the chemical analysis were tested. The least significant difference (LSD) was used at $p \leq 0.05$ for a multiple comparison (Selvamuthu *et al* 2018).

RESULTS AND DISCUSSION

Establishment of callus cultures: Mature embryos growing on MS medium with 2,4-D 3.0 mg/L and Kin 0.5 mg/L were able to start callus cultures after three weeks in the dark conditions. The callus morphology, formed from four maize genotypes, was friable and a yellowish-white. The callus cultures that were three months old were used later to evaluate different salinity stresses. The present results are forming a good texture of callus in maize, applying a mixture of auxin and cytokinin. (Ma *et al* 2022) demonstrated that high concentration of auxin and relatively low level of cytokinin could directly activate the expression of several genes that induce callus development from various explants.

Moreover, the findings of current study consistent with previous study (Malini *et al* 2018), indicated that *Z. mays* maturing seed or embryos gave a high callus formation percentage

Effect of salinity stress on callus growth

The callus cultures grown under different concentrations of salt stress for 30 days exhibited significant differences in growth parameters. The results illustrated in Figure 1-a show that the growth pattern under salt influence is similar for different studied genotypes; when the salt stress increased, the DW of callus were significantly decreased for all maize genotypes. In this case, the lowest DW were observed at a higher salinity (16 dS/m) compared to the control treatment (2 dS/m). Alfajr and Almaha attained significantly high means of DW (36.9 and 37 mg, respectively), while T1, and T6 recorded low means (15.6 and 16.0 mg, respectively). In order to evaluate the ability of four maize genotypes to withstand high salinity stress, the study particularly analyzed the impact of the highest salt concentration (Hasanuzzaman *et al* 2021) dS/m) on the growth of callus matter. This was achieved by quantifying the percent reduction of DW. The results in Figure (1-b) show that the DW percentage reduction ranged from 34% to 58% among the maize genotypes. The genotype T6 had the highest reduction rate (58%), while the lowest reduction (34%) was obtained from the cultivar Almaha. The salt tolerance index (STI) of cultivars Alfajr and Almaha exposed to the 16 dS/m treatment was higher than that of genotypes T1 and T6 exposed to the same salt concentration (Figure 1-c). Considerably, the highest rate of STI was 66 % attained by Almaha, while the lowest rate of 42% was observed in genotype T6. Additionally, the results mentioned above, that the rates of STI are inversely proportional to the rates of DW reduction. The results confirmed the good ability of the two genotypes, Alfajr and Almaha, to tolerate high salinity comparable to T1 and T6. Maize is considered a moderately cereal crop in terms of its response to salinity stress (Ibrahim *et al* 2022), although the early growth stage is very sensitive to salt stress (Yuan *et al* 2019). The results of our research support this observation, revealing that even under high salt stress (16 dS/m), the reduction of callus growth in sensitive genotypes was under 60%. In contrast, with resistant cultivars, growth reduction performed at 34%. Despite this reduction in cell growth, salt-tolerant cultivars showed better resistance than sensitive genotypes, as reflected in the higher value of STI. According to (Mansour *et al* 2005), salt-tolerant plants can suffer a decrease in growth, allowing them to preserve energy for vital functions. Furthermore, our findings demonstrated that the STI significantly decreased as salt stress increased. (Khan *et al.*2022) showed similar results related to eight pea genotypes under salinity stress. A higher STI value signifies a lower level of influence, while a lower value means a higher level of affect (Aggarwal *et al.*2024). The STI helps identify genotypes that develop mechanisms to

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

mitigate these stresses, like effective ion transport, the generation of osmoprotectants, and reactive oxygen species (ROS) (Yamazaki *et al.*2020).

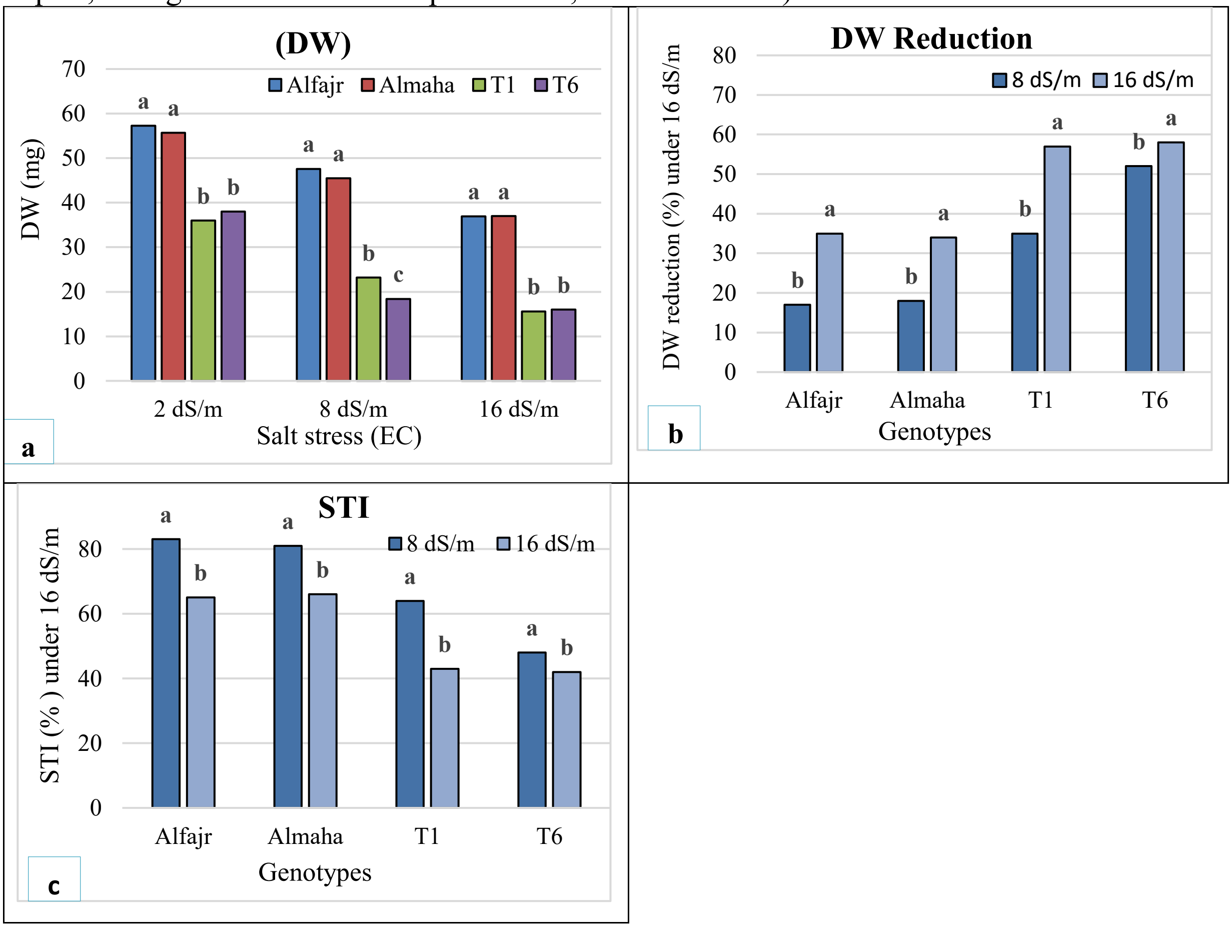

Figure 1. Shows the effect of salt stress on callus growth in four maize genotypes: (a) dry weight of maize genotypes under three levels of salinity; (b) percent of dry weight reduction under salinity 8 and 16 dS/m; (c) percent of salinity tolerance index under salinity 8 and 16 dS/m. Each value is an average of five replicates. "Bars marked with different lowercase letters mean significant differences at a level of P< 5%"

Impact of salinity stress on mineral contentSodium ion (Na^+)

The addition of elevated concentrations of salt to the MS cultural medium, significantly induced the buildup of Na^+ ions in the callus. The content of Na^+ ions intensified with the increase in salt stress. Maize genotypes differed in their ability to retain Na^+ according to their tolerance to salt stress (Figure 2-a). The data indicate that the highest salinity concentration (16 dS/m) resulted in a substantial rise in Na^+ accumulation at extremely high levels in the two salt-sensitive maize genotypes. In detail, we evaluated the Na^+ content at 166.9 mg/g in genotype T1 and 161.9 mg/g in genotype T6. The resistance cultivars, Alfajr and Almaha, had the lowest Na^+ accumulation, with contents reaching 45.1 and 42.5 mg/g, respectively.

Chloride ion (Cl^-)

Increasing salt strength in the culture medium significantly boosted chloride ions (Cl^-) in callus culture (Figure 2-b). Nevertheless, the four genotypes had significantly dissimilar Cl^- concentrations in their cultures. Under 16 dS/m salinity, the highest means (187.3 and 178.4 mg/g) were achieved from T6 and T1, respectively, which were significantly higher than 87.3 and 84.8 mg/g that were attained from Alfajr and Almaha, respectively. The toxicity or ionic imbalance are the main negative effects of salt stress that is made by Na^+ and Cl^-, which could damage plant cells and membranes and inhibit growth more significantly (Hasanuzzama *et al.*2021). In general, Na^+ and Cl^- accumulate in harmful quantities, impairing cellular activities and causing oxidative stress as a consequence of a

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

high level of ROS (Kesawat *et al.* 2023). Another toxic effect of Na^+ and Cl^-, is that they cause a K^+/Na^+ imbalance like seen in maize roots (Hu *et al.* 2022) and compete with K^+ and Ca^{+2} that reduce their concentration, particularly in sensitive genotypes, more than tolerant genotypes (Stavi *et al.*2023).

Potassium ion (K^+)

It is clear that the pattern of K^+ accumulation in callus when it is subjected to two high strengths of salinity levels (8 and 16 dS/m), is not the same as the pattern of Na^+ accumulation (Figure 2-c). The K^+ accumulation exhibited a significant increase in the salt-tolerant cultivars Alfajr and Almaha. Likewise, this accumulation displayed a relative rise with higher salt concentrations. Unlike the sensitive genotypes T1 and T6, the addition salt to the culture medium dramatically decreased K^+ content. When analyzing the findings across different genotypes, particularly at the highest salinity stress of 16 dS/m, it was observed that the K^+ accumulation was 99.0 mg/g in Almaha and 96.0 mg/g in Alfajr. In contrast, the low K^+ concentrations were 37.0 and 33.9 mg/g achieved by genotypes T1 and T6, respectively.

Calcium ion (Ca^{+2})

Following a similar pattern to K^+ accumulation, the amount of Ca^{+2} ions in the culture medium went up as the salt stress level went up in salt-resistance maize cultivars but down in sensitive baize genotypes as the salt stress levels went up. To better understand the pattern, it is important to note that the cultivars Alfajr and Almaha had a lot of Ca^{+2} (23.1 and 22.9 mg/g, respectively), when the salt stress was 8 dS/m. Separately, under 16 dS/m, the genotypes T1 and T6 had the lowest values of 6.1 and 6.7 mg/g, respectively (Figure 2-d). The Ca^{+2} ion is necessary for supporting the integrity of the cell membrane and forming the middle lamellae in the cell wall (Aly *et al.* 2018). Consequently, the elimination of Ca^{+2} from its binding locations in the cell membrane strongly changed the stability of the membranes, contributing to improved salt stress (Farooq *et al.* 2015). Another consideration of contrast is that salt- sensitive genotypes constantly exhibit a greater increase in cell permeability compared to tolerant maize cultivars (Mansour *et al.* 2005). In the present study, cations like K^+ and Ca^{+2} ions decreased with the increase in salinity levels. Furthermore, the concentration of these cations significantly declined in sensitive genotypes as the salinity increased. The *Oryza sativa* displayed similar observations (Srivastavaa *et al.* 2022). These observations are consistent with respective studies revealing how plants respond to salinity by adjusting the levels of ions and metabolites (Yuan *et al.* 2019; Ali *et al.* 2021).

Impact of salinity stress on carbohydrates and proline content

Carbohydrates (CHO) content

The bar chart in Figure 2-e clearly shows that salt-tolerant cultivars demonstrated a rise in their carbohydrates (CHO) content as they were subjected to a higher level of salt stress (600.0 and 630.9 mg/g), respectively, from cultivars Alfajr and Almaha. One o the defensive mechanisms they possess against salinity stress could be responsible for this increase in CHO concentration. At the same time, it is important to mention that sensitive genotypes T1 and T6 exhibited a significant reduction in CHO content (173.9 and 192.0 mg/g), respectively, when subjected to the same salt stress (16 dS/m), comparable to the control treatment (2 dS/m). The graphical illustration clearly indicates an inverse correlation in the manner of CHO content between salt-tolerant and salt-sensitive maize genotypes.

Proline content

The statistical analysis reveals that there are significant variances among four genotypes in their proline content when grown under three salt concentrations (Figure 2-f). The proline content increased proportionally with increasing salt concentrations in all callus cultures for the studied maize genotypes. Obviously, the salt-tolerant cultivars recorded the superiority average means of proline comparable to the sensitive genotypes. The control treatment (2 dS/m) had the lowest content of proline. In contrast, under the highest salt stress of 16 dS/m, the significant superiority value was achieved from cultivar Alfajr, while the lowest means were 14.4 and

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

16.2 mg/g for genotypes T1 and T6, respectively.

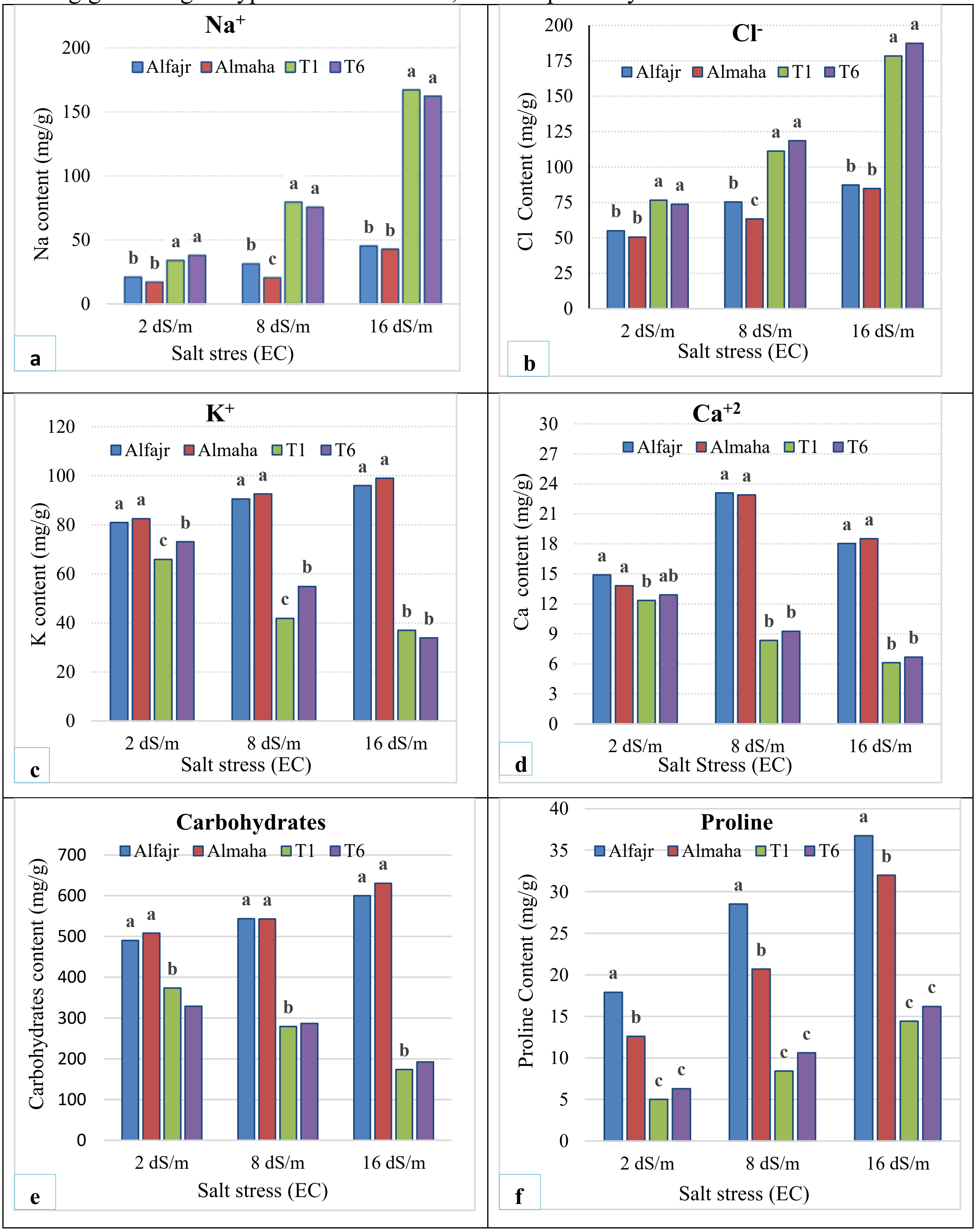

Figure 2. Impact of three levels of salinity stress on the content of: (a) Na^+, (b) Cl^-, (c) K^+, (d) Ca^{+2}, (e) carbohydrates, and (f) proline. Each value is an average of five replicates. "Bars shown with dissimilar lowercase letters indicate significant differences at a level of P< 5%"

Carbohydrate and proline concentrations were found to be significantly higher in tolerant maize cultivars compared to sensitive genotypes under the same salt stress. The presence of NaCl stress leads to an increase in the accumulation of carbohydrates that assist in alleviating stress, ROS, and storing carbon. These directly impact physiological processes like respiration and photosynthesis (Naz *et al.* 2022). Fundamentally, osmoregulation aids

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

plants in mitigating the adverse effects of osmotic stress resulting from salts (Ahmad *et al.* 2017). Proline and carbohydrates are the primary osmolytes that trigger osmoregulation in maize when subjected to salt stress (Farooq *et al.* 2015). The accumulation of proline has been confirmed to be an effective protectant of plasma membrane permeability (Mansour *et al.* 2013). However, another observation showed that in saline conditions, soluble carbohydrates in plants may have a more noteworthy function than proline (Zhang *et al.* 2019). For instance, the elevation of sugar content in maize shoots was higher than the elevation of proline content under high salinity stress compared to low salt stress (Sun *et al.*2018). Another investigation found that raised salt stress resulted in a higher proline accumulation in the calli of a salt-tolerant maize cultivar than in a sensitive one (Sukma *et al.* 2020) which is consistent with the results of our current study.

Pearson correlation analysis

The Pearson correlation coefficient (PCC) evaluates the proximity and intensity of the linear relationship among two factors. As shown in Table 1, the positive and highly PCC were seen between DW and STI, K^+, Ca^{+2}, carbohydrates, and proline content, but had a negative correlation with Na^+ and Cl^- content. On the contrary, DW reduction achieved a positive correlation with Na^+ and Cl^- content but had negative correlation with STI, K^+, Ca^{+2}, carbohydrates, and proline content. The STI was positive and greatly correlated to K^+, Ca^{+2}, carbohydrates, and proline content, while negatively correlated to Na^+ and Cl^- content. The ions Na^+ and Cl^- exhibited a similar pattern of negative correlation with K^+, Ca^{+2}, carbohydrates, and proline content; however, the correlation was positive between them. Additional positive correlation was observed between K^+ content and Ca^{+2}, carbohydrates, and proline content. Ca^{+2} also had a positive correlation with carbohydrates and proline. Finally, carbohydrates were positively correlated with proline. All positive PCCs showed highly significant differences at a level of $P<5\%$. Similar correlations have been reported between ions in various parts of rice under salt stress (Nemati *et al.* 2011). A positive correlation between STI and growth parameters has been observed in salt-tolerant varieties of rice under salinity stress (Zhou *et al.* 2022). Another positive correlation has been reported between proline and carbohydrate in different *Populus euphratica* olive under salt stress (Neko *et al.* 2019).

Table 1. Pearson correlation analysis between callus dry weight (DW), dry weight reduction percentage (DW reduction), salt tolerance index (STI), sodium (Na^+) content, chloride (Cl^-) content, potassium (K^+) content, calcium (Ca^{+2}) content, carbohydrates, and proline content of four maize genotypes in response to 16 dS/m salinity stress.

	DW	*DW reduction (%)*	*STI (%)*	*Na*	*Cl*	*K*	*Ca*	*Carbohydrate*	*Proline*
DW	1								
DW reduction (	-0.99865	1							
STI (%)	0.997479	-0.999729655	1						
Na	-0.99981	0.998093255	-0.99708	1					
Cl	-0.9968	0.999318201	-0.99899	0.995542	1				
K	0.998362	-0.999984804	0.999829	-0.99779	-0.99938	1			
Ca	0.999875	-0.997741787	0.996234	-0.9997	-0.99568	0.997363	1		
Carbohydrate	0.998759	-0.997989888	0.997757	-0.99938	-0.99497	0.997826	0.998245	1	
Proline	0.982755	-0.974290922	0.968843	-0.98126	-0.97296	0.973098	0.985279	0.974012562	1

Genetic diversity

Seven RAPD primers (Table 2 and Figure 3) made a total of 34 bands with an average of 4.8 per primer. Twenty bands were monomorphic, while the remaining 14 were polymorphic. The total products of primers divided into different size groups ranged from 150-2250 bp. Primer OPG-09 showed the highest polymorphic (P) percent (57.1%). The primers OPE-13 and OPG-09 had the highest primer efficiency (20.5). While the maximum value of primer discriminatory power (PDP) was 28.5, that achieved by primer OPG-09. The results of the genetic variation study demonstrated two groups: Alfajr and Almaha combined in one group, while T1 and T6 were arranged in another group. Plant genotypes exhibit significant genetic variation in their ability to adapt to environmental salt stress, as documented by (Mazumder *et al.* 2021). Moreover, previous investigations have shown that certain genes control the essential pathways responsible for salinity stress resistance (Gupta *et al.* 2014). Using maize tissue culture, the zmhkt1,5 gene was identified responsible for Na+ exclusion in response to different salinity stresses. Additionally,

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

(Yu *et al.* 2023) proved the involvement of the *ZmHSP90* gene in maize adaptation to high concentrations of NaCl stress. Whereas two genes proved their relationship with salinity stress in rice at the seedling stage (Kim *et al.* 2023).

Table 2. Shows the RAPD primer sequence that was used for studying polymorphism, along with the size range (SR), total bands (TB), monomorphic bands (MB), polymorphic bands (PB), polymorphic percentage (P%), primer efficiency (PE), and primer discriminatory power (PDP) for each RAPD primers.

No.	Primers	Sequence (5'-3')	SR (bp)	TB	MB	PB	P%	PE	PDP
1	OPB-01	GTTTCGCTCC	250-750	3	2	1	33.3	8.8	7.1
2	OPB-06	TGCTCTGCCC	300-750	4	3	1	25.0	11.7	7.1
3	OPC-08	TGGACCGGTG	250-2000	6	3	3	50.0	17.6	21.4
4	OPE-13	CCCGATTCGG	150-2250	7	4	3	42.8	20.5	21.4
5	OPE-16	GGTGACTGTG	350-750	4	3	1	25.0	11.7	7.1
6	OPF-20	GGTCTAGAGG	500-1000	3	2	1	33.3	8.8	7.1
7	OPG-09	CTGACGTCAC	300-1000	7	3	4	57.1	20.5	28.5
Total bands				34	20	14			
Average/primer				4.8	2.8	2	38.0	14.2	14.2

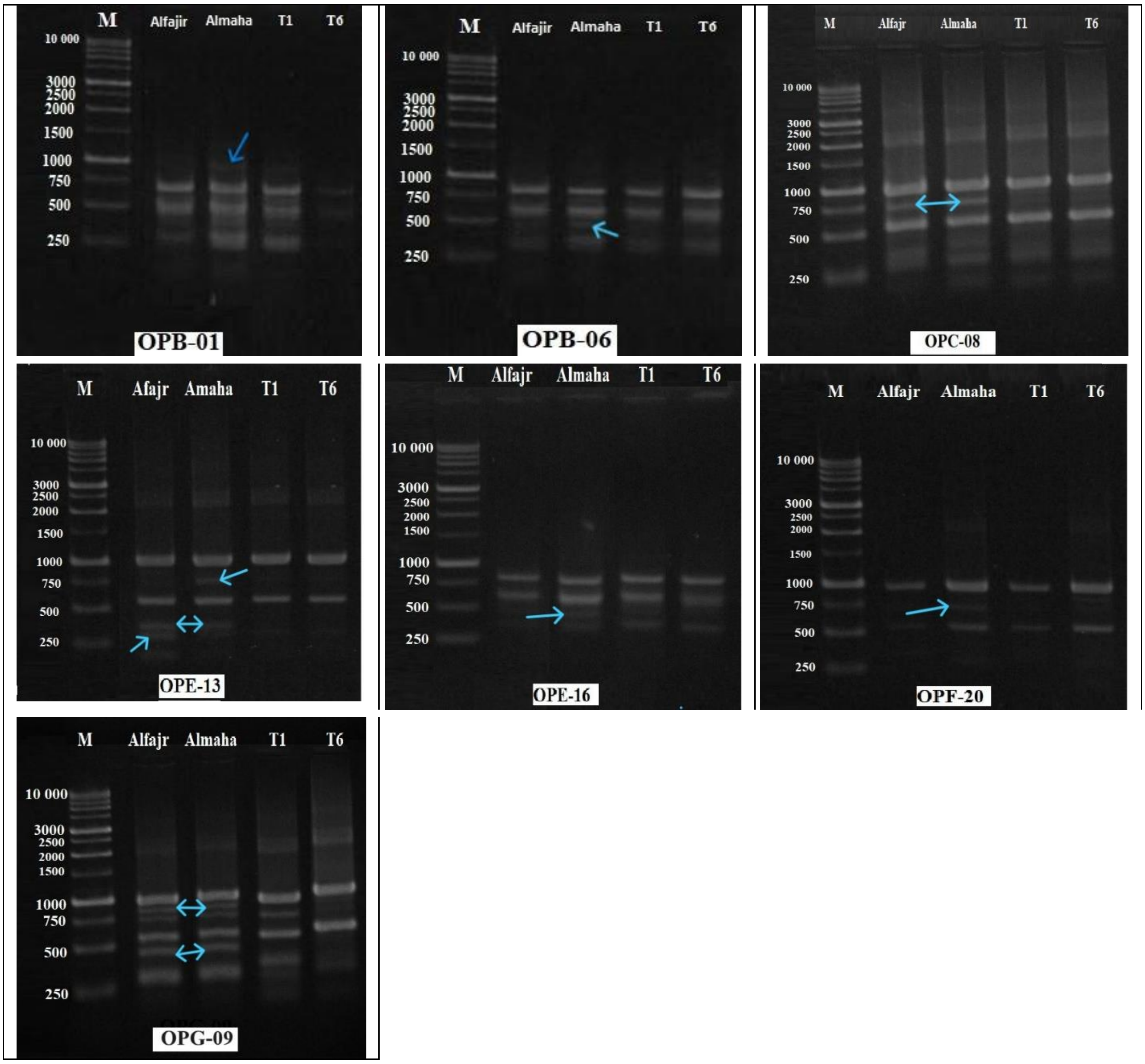

Figure 3. Gel electrophoresis presented the amplification profile of four maize genotypes (Alfajr, Almaha, T1, and T6) using seven RAPD primers: OPB-01, OPB-06, OPC-08, OPE-13, OPE-16, OPF-20, and OPG-09. M = 1 kb DNA Ladder (250-10000 bp).

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

The results presented in Jaccard's similarity matrix (Table 3) and the UPGMA dendrogram (Figure 4) provide a clear feature of the extent of genetic distance between the four genotypes. The cluster tree is divided into two main clusters. The first branch introduced the cultivars Alfajr and Almaha to the clusters. In the similarity matrix, these clusters recorded 0.848 as a Jaccard's distance value, indicating a high measure of similarity between them. The second branch also consisted of two clusters, giving genotypes T1 and T6. Comparatively, a high Jaccard similarity value was 0.880, highlighting their substantial closeness to one another. Eventually, the two main branches merged to create a Jaccard's distance value of 0.300 between these main clusters, indicating a moderate degree of resemblance

Table 3. Diversity and similarity matrix (Jaccard's coefficient) of four maize genotypes based on RAPD primers.

	Alfajr	Almaha	T1	T6
Alfajr	**1**			
Almaha	**0.848**	**1**		
T1	**0.741**	**0.727**	**1**	
T6	**0.700**	**0.687**	**0.880**	**1**

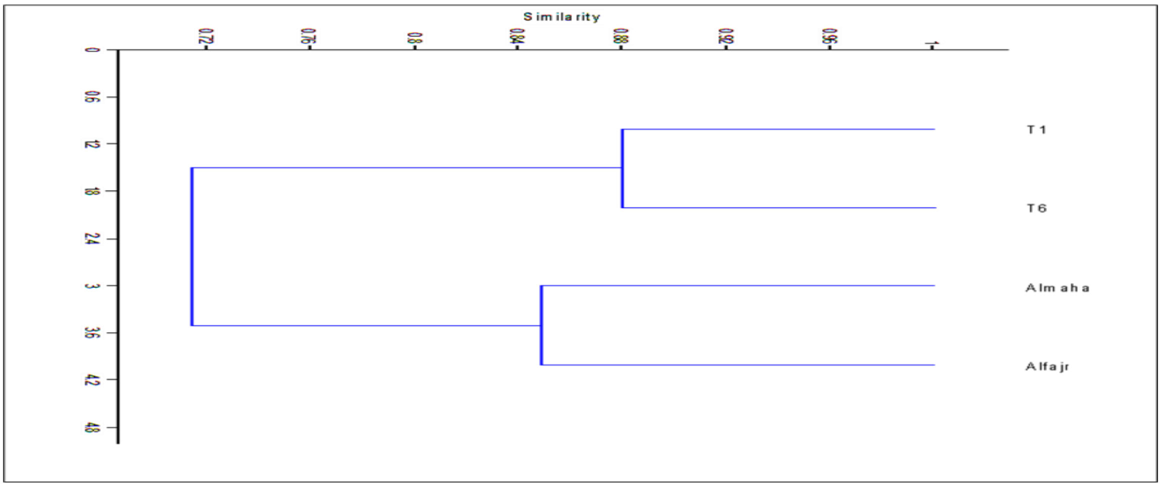

Figure 4. The UPGMA dendrogram of four maize genotypes resulted from seven RAPD primers.

CONCLUSION

The investigation indicates that salinity had an impact on the growth of maize callus cultures, particularly, higher levels of salt led to a decrease in average callus weight. Tolerant maize cultivars showed a higher STI and had better adaptation to salt stress conditions. Ion studies revealed that sensitive genotypes displayed a higher accumulation of Na^+ and Cl^- ions, while tolerant cultivars maintained an optimum equilibrium of ions. Genetic analysis showed separate groups of maize genotypes that were either semi-tolerant or susceptible to salt stress. These results have the potential to inform future breeding plans on creating maize varieties that are more adaptable to grow in salinity conditions.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFERENCES

Aggarwal, G., P. Edhigalla, P. Walia, S. Jinda, and S.S. Sanda. 2024. A method for screening salt stress tolerance in Indian mustard (*Brassica juncea*) (L.) Czern and Coss at seedling stage. Scientific Reports. 14(12705). https://doi.org/https://doi.org/10.1038/s41598-024-63693-6

Ahmad, A., A. Ismun, and M. Taib. 2017. Effects of salinity stress on carbohydrate metabolism in *Cryptocoryne elliptica* cultures. J. Trop. Plant Physiol., 9: 1–13.

Al-Alwani, A.A.M., and M.A. Mohammed. 2023. Propagation of *Chia* plants using plant tissue culture technique. Journal of Biotechnology Research Center, 17(1): 66–80.

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

https://doi.org/https://doi.org/10.24126/jobrc.2023.17.1.702

Ali, M., S. Afzal, A. Parveen, M. Kamran, M.R. Javed, G.H. Abbasi, Z. Malik, M. Riaz, S. Ahmad, M.S. Chattha, M. Ali, Q. Ali, M.Z. Uddin, M. Rizwan, and S. Ali. 2021. Silicon mediated improvement in the growth and ion homeostasis by decreasing Na^{+} uptake in maize (*Zea mays* L.) cultivars exposed to salinity stress. Plant Physiology and Biochemistry, 158: 208–218. https://doi.org/10.1016/j.plaphy.2020.10.040

Alwash, B. M., and H. E. Hussein. 2016. Induction of genetic variation for salt tolerance in tissue culture of *Capsicum annuum* L. Journal of Biotechnology Research Center, 10(1): 15–50.

Aly, A.A., R.W. Maraei, and S. Ayadi. 2018. Some biochemical changes in two Egyptian bread wheat cultivars in response to gamma irradiation and salt stress. Bulgarian Journal of Agricultural Science, 24(1): 50–59.

Cao, Y., X. Zhou, H. Song, M. Zhang and C. Jiang. 2023. Advances in deciphering salt tolerance mechanism in maize. The Crop Journal, 11(4): 1001-1010. https://doi.org/10.1016/j.cj.2022.12.004

De Leon, T.B., S. Linscombe, G. Gregorio, and P.K. Subudhi. 2015. Genetic variation in Southern USA rice genotypes for seedling salinity tolerance. Frontiers in Plant Science, 6(MAY):1–13. https://doi.org/10.3389/fpls.2015.00374.

Dinakar, N., P.C. Nagajyothi, S. Suresh, T. Damodharam, and C. Suresh. 2009. Cadmium induced changes on proline, antioxidant enzymes, nitrate and nitrite reductases in *Arachis hypogaea* L. Journal of Environmental Biology, 30(2): 289–294.

El-bakatoushi, R., D. Gamil, and A. Ahmed. 2017. Evaluation of genetic diversity in wild populations of *Peganum harmala* L., a medicinal plant. Journal of Genetic Engineering and Biotechnology, (pp. 143–151). https://doi.org/10.1016/j.jgeb.2017.11.007

Farooq, M., M. Hussain, A. Wakeel, and K.H.M. Siddique. 2015. Salt stress in maize: effects, resistance mechanisms, and management. A review. Agron. Sustain. Dev., 35: 461–481. https://doi.org/DOI 10.1007/s13593-015-0287-0

Gheith, E.M.S., O.Z. El-Badry, S.F. Lamlom, H.M. Ali, M.H. Siddiqui, R.Y. Ghareeb, M.H. El-Sheikh, J. Jebril, N.R. Abdelsalam, and E.E. Kandil. 2022. Maize (*Zea mays* L.) productivity and nitrogen use efficiency in response to nitrogen application levels and time. Frontiers in Plant Science, (Vol. 13). https://doi.org/10.3389/fpls.2022.941343

Gupta, B., and B. Huang. 2014. Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. International Journal of Genomics, 2014(701596). https://doi.org/http://dx.doi.org/10.1155/2014/701596 Review

Hannachi, S., K. Steppe, M. Eloudi, L. Mechi, I. Bahrini, and M.C. Van Labeke. 2022. Salt stress induced changes in photosynthesis and metabolic profiles of one tolerant ('Bonica') and one sensitive ('Black Beauty') Eggplant cultivars (*Solanum melongena* L.). Plants, 11(590). https://doi.org/https://doi.org/10.3390/plants11050590

Hasanuzzaman, M., M.R.H. Raihan, A.A.C. Masud, K. Rahman, F. Nowroz, M. Rahman, K. Nahar, and M. Fujita. 2021. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. International Journal of Molecular Sciences, 22(9326). https://doi.org/https://doi.org/10.3390/ijms22179326

Hu, D., R. Li, S. Dong, J. Zhang, B. Zhao, B. Ren, H. Ren, H. Yao, Z. Wang, and P. Liu. 2022. Maize (*Zea mays* L.) responses to salt stress in terms of root anatomy, respiration and antioxidative enzyme activity. BMC Plant Biology, 22(602). https://doi.org/10.1186/s12870-022-03972-4

Ibrahim, N.A., W.N.W. Ahmed, and H. Othman. 2022. Distinguishing water salinity threshold through irrigation for maize (*Zea mays* L.) growth and yield. International Conference on Sustainability Agriculture and Biosystem, 1182(012075). https://doi.org/doi:10.1088/1755-1315/1182/1/012075

Jassim, E.H., H.H. Salih, M.I. Salih, and S.F. Hassan. 2021. Influence of glutamine in some alkaloids production from callus of *Peganum*

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

harmala L. Biochemical and Cellular Archives, 21(1): 921-927. DocID: https://connectjournals.com/03896.2021.21.921

Kesawat, M.S., N. Satheesh, B.S. Kherawat, A. Kumar, H.U. Kim, S.M. Chung, and M. Kumar. 2023. Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules current perspectives and future directions. Plants, 1(864). https://doi.org/https://doi.org/10.3390/plants12040864

Khan, M.A.H., M.A.B. Mia, M. Abdul Quddus, K.K. Sarker, M. Rahman, and Etc. 2022. Salinity-induced physiological changes in Pea (*Pisum sativum* L.): germination rate, biomass accumulation, relative water content, seedling vigor and salt tolerance index. Plants, 11(24): 3493. https://doi.org/https://doi.org/10.3390/plants11243493

Kim, T.H., and S.M. Kim. 2023. Identification of candidate genes for salt tolerance at the seedling stage using integrated genome-wide association study and transcriptome analysis in Rice. Plants, 12(6). https://doi.org/10.3390/plants12061401

Li, J., Q. Zhu, F. Jiao, Z. Yan, H. Zhang, Y. Zhang, Z. Ding, C. Mu, X. Liu, Y. Li, J. Chen, and M. Wang. 2023. Research progress on the mechanism of salt tolerance in maize: A classic field that needs new efforts. Plants, 12(2356). https://doi.org/https://doi.org/10.3390/ plants12122356 Academic

Ma, J., Q. Li, L. Zhang, S. Cai, Y. Liu, J. Lin, R. Huang, Y. Yu, M. Wen, and T. Xu. 2022. High auxin stimulates callus through SDG8-mediated histone H3K36 methylation in Arabidopsis. J. Integr. Plant Biol., 64(12): 2425–2437. https://doi.org/https://doi.org/10.1111/jipb.13387

Malini, N., C.R. Anandakumar, R. Gnanam, and S.H. Ramakrishnan. 2018. Effect of harmones on callus induction in Maize (*Zea mays* L.). Journal of Applied and Natural Science. 10 (1): 202 – 209. https://doi.org/10.31018/jans.v10i1.1605

Mansour, M.M.F. 2013. Plasma membrane permeability as an indicator of salt tolerance in plants. Biologia Plantarum, 57(1): 1–10. https://doi.org/10.1007/s10535-012-0144-9

Mansour, M.M.F., K.H.A. Salama, F.Z.M. Ali, and A.F.A. Hadid. 2005. Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. Gen. Appl. Plant Physiology, 31(1–2): 29–41.

Masuda, M.S., M.A.K. Azad, M. Hasanuzzaman, and M.D. Arifuzzaman. 2021. Evaluation of salt tolerance in maize (*Zea mays* L.) at seedling stage through morphological characters and salt tolerance index. Plant Physiol. Rep., 26: 419–427. https://doi.org/10.1007/s40502-021-00611-2

Mazumder, S.R., H. Hoque, B. Sinha, W.R. Chowdhury, M.N. Hasan, and S.H. Prodhan. 2020. Genetic variability analysis of partially salt tolerant local and inbred rice (*Oryza sativa* L.) through molecular markers. Heliyon, 6(e04333). https://doi.org/https://doi.org/10.1016/j.heliyon.2020.e04333

Mukhopadhyay, R., B. Sarkar, H.S. Jat, P.C. Sharma, and N.S. Bolan. 2021. Soil salinity under climate change: Challenges for sustainable agriculture and food security. Journal of Environmental Management, 280(111736). https://doi.org/10.1016/j.jenvman.2020.111736

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15: 473–497.

Naz, T., M.M. Iqbal, J. Akhtar, M. Saqib, M. Ali, M.I. Zafar, B. Dell, R. Datta, M.J. Ansari, S. Danish, and S. Fahad. 2022. Carbohydrate partitioning, growth and ionic compartmentalisation of Wheat grown under boron toxic and salt degraded land. Agronomy, 12(3). https://doi.org/10.3390/agronomy12030740

Neko, H.T., A. Shirvani, M.H. Asareh, and M.R. Morshedloo. 2019. Physiological response to salinity stress in various *Populus phratica* oliv. ecotypes in Iran. Ecopersia.,7(2):97-103. http://ecopersia.modares.ac.ir/article-24-25997-en.html

Nemati, I., F. Moradi, S. Gholizadeh, M.A. Esmaeili, and M.R. Bihamta. 2011. The effect

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (*Oryza sativa* L.) seedlings. Plant Soil Environ., 57(1):26-33. https://doi.org/10.17221/71/2010-PSE

Nikoogoftar-Sedghi, M., V. Rabiei, F. Razavi, S. Molaei, and A. Khadivi. 2024. Fulvic acid foliar application: a novel approach enhancing antioxidant capacity and nutritional quality of pistachio (*Pistacia vera* L.). BMC Plant Biology, 24(1): 241. https://doi.org/https://doi.org/10.1186/s12870-024-04974-0

Oktavianingsih L., E. Suharyanto, B. S. Daryono, and Purnomo. 2021. Genetic diversity of Taro (*Colocasia* spp.) from kalimantan island, borneo, Indonesia, based on RAPD markers. SABRAO Journal of Breeding and Genetics, 53(3): 447–458.

Paul, B.N., S. Chanda, S. Das, P. Singh, B.K. Pandey, and S.S. Giri. 2014. Mineral assay in atomic absorption spectroscopy. The Beats of Natural Sciences, 1(4):1-17.

Selvamuthu, D., and D. Das. 2018. Introduction to statistical methods, design of experiments and statistical quality control. In Instrumentation Design Studies. Springer Nature Singapore Pte Ltd. 1st ed.:63-96 https://doi.org/https://doi.org/10.1007/978-981-13-1736-1

Singh, S., B. S. Tanwer, M. Khan, J. Rajasthan, P. Pathology, and P. Biochemistry. 2011. Callus induction and *in vivo* and *in vitro* comparative study of primary metabolites of *Withania Somnifera*. 2(3): 47–52.

Srivastavaa, S., and P.K. Sharma. 2022. Morpho-physiological and biochemical tolerance mechanisms in two varieties of *Oryza sativa* to salinity. Russian Journal of Plant Physiology, 69(37): 1–12 https://doi.org/10.1134/S1021443722020194

Stavi, I., N. Thevs, and S. Priori. 2021. Soil salinity and sodicity in drylands: A review of causes, effects, monitoring, and restoration measures. Frontiers in Environmental Science, 9. https://doi.org/10.3389/fenvs.2021.712831

Sukma, K.P.W., B.S. Daryono, Purnomo, and I. Suprapti. 2020. Callus response of hybrid and Madura local corn to salt stress. IOP Conf. Series: Earth and Environmental Science, 457(012077). https://doi.org/doi:10.1088/1755-1315/457/1/012077

Sun, Y., C. Mu, H. Zheng, S. Lu, H. Zhang, X. Zhang, and X Liu. 2018. Exogenous Pi supplementation improved the salt tolerance of maize (*Zea mays* L.) by promoting Na^+ exclusion. Scientific Reports, 8(16203). https://doi.org/DOI:10.1038/s41598-018-34320-y

Taratima, W., T. Chomarsa, and P. Maneerattanarungroj. 2022. Salinity stress response of Rice (*Oryza sativa* L. cv. Luem Pua) calli and seedlings. Scientifica (Cairo), Jul 11(5616683). https://doi.org/10.1155/2022/5616683

Yamazaki, K., M. Ishimori, H. Kajiya-Kanegae, H. Takanashi, M. Fujimoto, J.I. Yoneda, K. Yano, T. Koshiba, R. Tanaka, H. Iwata, T. Tokunaga, N. Tsutsumi, and T. Fujiwara. 2020. Effect of salt tolerance on biomass production in a large population of sorghum accessions. Breeding Science, 70(2): 167–175. https://doi.org/10.1270/jsbbs.19009

Yu, X., Y. Zhao, Y. Wang, Z. Zou, and F. Li. 2023. Maize *ZmHSP90* plays a role in acclimation to salt stress. Peer J., 11(e15819). https://doi.org/DOI .7717/peerj.15819

Yuan, C., S. Feng, Z. Huo, and Q. Ji. 2019. Effects of deficit irrigation with saline water on soil water-salt distribution and water use efficiency of maize for seed production in arid Northwest China. Agricultural Water Management, 212: 424–432. https://doi.org/10.1016/j.agwat.2018.09.019

47- Zhang, J., C. Olariu, R. Steel, and W. Kim. 2019. NaCl-responsive ROS scavenging and energy supply in alkaligrass callus revealed from proteomic analysis. BMC Genomics, 20(990).

48- Zhou, X.Y., J.F. Li, Y.Q. Wang, X.Y. Liang, M. Zhang, M.H. Lu, Y. Guo, F. Qin, and C.F. Jiang. 2022. The classical SOS pathway confers natural variation of salt tolerance in Maize. New Phytol., 236: 479–494. https://doi.org/10.1111/nph.18278

Iraqi Journal of Agricultural Sciences –2026:57(5):1328-1341
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/ykkh6v73

استخدام زراعة الأنسجة النباتية في تقييم أربعة تراكيب وراثية لنبات الذرة .*ZEA MAYS* L لتحمل الملوحة

مها ابراهيم صالح [1]، اشواق شنان عبد[2]، ايمان نعمان اسماعيل[2]، سهى فرج[1]
[1]معهد الهندسة الوراثية والتقنيات الإحيائية للدراسات العليا
[2]مركز بحوث التقنيات الإحيائية / جامعة النهرين

المستخلص

الملوحة هي عامل غير حيوي التي لها تاثيرات سلبية على زراعة الذرة، خاصة في المناطق ذات الترب المالحة. تقيم الدراسة الحالية تاثير الملوحة على النمو والفعاليات الفسيولوجية لسبعة من اصناف الذرة *Zea mays* L. تم استبعاد الاصناف عالية التحمل للملوحة، في حين تم انتاج مزارع الكالس من الاصناف معتدلة التحمل للملوحة وانماط وراثية حساسة، على وسط MS مزود بـ 3 ملغم/لتر 2,4-D و0.5 ملغم/لتر Kin. اظهرت النتائج ان المستويات العالية للملوحة قللت معنوياً وزن الكالس. وابدت الاصناف المعتدلة (الفجر والمها)، ارتفاعاً في مؤشر تحمل الملوحة (STI)، مقارنة بالانماط الوراثية الحساسة (1T و 6T). واشار التحليل الايوني ان الانماط الوراثية 1T و6T اظهرت تركيزاً مرتفعاً لايونات Na^+ و Cl^-. والعكس من ذلك، تراكمت في صنفي الفجر والمها تراكيزاً مرتفعة من الكاتيونات K^+ و Ca^{2+}، جنبا لجنب مع ارتفاع محتوى الكربوهيدرات والبرولين، والذي ربما ادى ذلك الى تطور تحملهما للاملاح. اضافة لذلك، فقد قسم التحليل الوراثي بالاعتماد على اختبار RAPD الاصناف المتحملة والانماط الوراثية الحساسة الى مجموعتين منفصلتين، مؤكدة وجود اختلافات وراثية متعلقة بصفة تحمل الملوحة. تسلط النتائج الضوء على دور التنظيم الايوني، التكيف الازموزي، والعوامل الوراثية على تخفيف تاثير الملوحة على الذرة. تقدم الدراسة رؤى مفيدة بامكانها مساعدة عمليات التربية في تطوير انماط وراثية للذرة متحملة للملوحة والتي هي ضرورية للمحافظة على زراعة الذرة في المناطق المتاثرة بارتفاع الشد الملحي.

الكلمات المفتاحية: الذرة، تحمل الملوحة، RAPD PCR، مزارع الكالس