Molecular Detection of the Gene (Glutathione S-Transferase Zeta Class-Like) Responsible for Stress Tolerance and Studying Its Genetic Expression in a Number of Snake Melon Cultivars

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Abstract: The experiment was conducted in the winter agricultural season 2020 in the greenhouses of the Al-Fares Company in Al-Zubair district, with the aim of investigating one of the important genes responsible for tolerating Armenian cucumber for stress conditions, which is the gene (glutathione S-transferase zeta class-like) in 21 Armenian cucumber cultivars. Isolation of RNA from leaves after 100 days of cultivation at the saline level 5 dSm⁻¹, as well as a control treatment of 1 dSm⁻¹ and studying their gene expression using RT-qPCR (Real Time-quantitative Polymerase Chain Reaction) technique. The results showed the excelled of the cultivars under the influence of salt stress, Egypt, Babylon, Mosul (Carmelis), Kirkuk, Diyala and Karbala, as they gave the highest expression of their gene expression (25.12, 21.87, 19.04, 21.87, 19.04 and 20.40), respectively, so they can be considered salt-tolerant cultivars. This is because the expression values for the gene (glutathione S-transferase zeta class-like) were high compared to the rest of the cultivars. As for the cultivars whose sensitivity to salinity was confirmed by the experiment, they are Iranian (Ghani), Italian, Baghdad and Amara. These cultivars gave the lowest gene expression values (2.73, 4.46, 4.14 and 3.49), respectively.

1. Introduction
Snake melon (Cucumis melo Var.flexuosusis) is a summer important crop of the Cucurbitaaceae family [1]. Salinity is one of the most prominent abiotic environmental stresses, and the accumulation of salts in agricultural lands reduces the percentage of germination and plant growth and its inability to complete its life cycle. Where, the inhibition of plant growth or its death due to the presence of high concentrations of salts is due to their influencing role in various physiological and biochemical processes in plants [2]. Gene expression is also one of the important tools in the plants' response to all environmental changes occurring on the plants, and that the plant has the ability and adaptation to change the degrees of gene expression according to the environmental variables, and these adaptations are caused by the plant's tolerance to those conditions, while this mechanism does not possess other plants. Where it has been proven through studies that the gene expression can be observed in both tolerant and non-tolerant plants. By studying the response of plants to these variables in the environment, they lead to a specific mechanism for gene expression that will contribute to maintaining the metabolic processes to improve their tolerance to stress, not to mention other factors such as groups of genes that affect through transcription factors and gene regulation [3]. The difference in gene expression values facilitated the identification of many genes that are stimulated when plants are exposed to environmental stresses [4]. For this reason, the cultivars differ in their tolerance to salt stress according to their
different genotypes [5]. And that there are three main factors at the level of gene expression that control the quality of salt tolerance, which are ionic balance, damage control and growth regulation. These factors must be controlled by the regulation of three aspects of functional genes: transport, defend cells, detoxification, and photosynthesis [6]. [7] found in their study, molecular methods investigation of the SKC1 gene responsible for salt stress tolerance and the study of gene expression in some local cultivars of rice, which are seven local cultivars which are Anbar Al-Barakah, Anbar-33, Yasmine, Furat-1, Bohuth, and Meshkhab-1 and Mishkhab-2, and they were grown at the salinity level (15 dSm⁻¹) in addition to the control treatment (3 dSm⁻¹) to study their gene expression using SYBR Real time PCR molecular technique. The results showed that the two cultivars, Meshkhab-1 and Mishkhab-2, excelled on the rest of the cultivars in giving them the highest average of "SKC1 gene expression values associated with the salinity tolerance traits compared to the other studied cultivars. As for the two cultivars Furat-1 and Bohuth, they can be considered relatively tolerant to salinity or semi-tolerant. This is because the expression values of the SKC1 gene were relatively high compared with the control treatment. As for the cultivars whose sensitivity to salinity was confirmed by the experiment, Anbar Al-Barakah, Anbar-33 and Yasmine, The amount of gene expression in them did not give a difference "or a perceptible expression after being exposed to salt tension compared with the control treatment. [8] during his study of stress-responsive genes that enhance the tolerance of genetically modified Arabidopsis under conditions of drought and cold stress, found that drought and cold are the main factors that limit plant growth and the AmNAC11 gene was identified as being sensitive to stress. This gene also showed a genetic expression as a result of plant exposure to drought, cold and high salinity conditions. The concentration of this protein in the nucleus has also been observed, which plays an important role in tolerance of drought, cold and salinity. Therefore, our current study aimed to investigate the glutathione S-transferase zeta class-like gene responsible for some of the salinity-carrying mechanisms of Armenian Cucumber and to study its gene expression to know the cultivars that are more tolerant to salinity, and thus the possibility of developing and producing new varieties of hybrid Armenian cucumber, which are distinguished by their ability to withstand salt stress and water shortage, to contribute to improving the local cultivation of this crop.

2. Materials and Methods
The experiment was conducted in the winter agricultural season 2020 on one of the farms of Al-Faris Agricultural Company in the desert area of in Al-Zubair district in Basra province with the aim of studying the effect of irrigation water salinity on growth and the yield of twenty-one types of Armenian cucumber, where the experiment included two main factors, namely, two types of salinity of irrigation water, they are 1, 5 dSm⁻¹ and 21 cultivars of Armenian cucumber, of these, 16 localities belong to the following province: Basra, Amarah, Nasiriyah, Kut, Diwaniyah Karbala, Najaf and Samawah, Baghdad, Diyala, Tikrit, Kirkuk (Abu Zugaib cultivar), Mosul (Abu Akaf and Dory cultivar) and Al-Ramadi, and 5 imported cultivars are Iranian, Tarouz, Taarzi, Egyptian and Italian hybrids, and 5 imported cultivars are the Iranian cultivar Ghani and the Tarouz hybrid produced by the company French Flemorin and Taarzi hybrid produced by the Dutch company Simins, and from Egyptian by the International Andalusian Company, and Italian by Pagano Costantino, Relative gene expression data were analyzed according to a randomized complete block design (RCBD) with three replicates for the purpose of estimating the gene expression of the glutathione S-transferase zeta class-like gene responsible for the salinity-tolerance trait of 42 samples, 21 Irrigation with fresh water, and the other with saline water of 5 dB/m. At the end of the growing season, it was done. A leaves was taken for each cultivar for the purpose of RNA extraction. [9] method were used to estimate the relative gene expression of the glutathione S-transferase zeta class-like gene. The elongation factor gene was used as a reference gene through the following equations:

As: Ct test is the cycle threshold of tested samples for a target gene (glutathione S-transferase zeta class-like). Ct elongation factor is the cycle threshold for the reference gene (elongation factor).
CT Control is the cycle threshold of a control sample for a target gene (glutathione S-transferase zeta class-like).

RNA was extracted using the kit provided by the American company Zymo, and RNA purity was assessed by means of a Nano Drop device. As the absorbance of the RNA sample is calculated at the wavelength of 260 nm (O.D260), then the absorbance of the sample itself is calculated at the wavelength of 280 nm (O.D280). As the ratio between the wave reading (260 nm) to (280 nm) helps to evaluate RNA purity [10].

2.1 Converting RNA to cDNA

Using the PrimeScript™ RT reagent Kit (Perfect Real Time) # RR037A from Takara, the RNA was converted into cDNA as well as the laboratory water with Diethylpyrocarbonate (DEPC). Two types of primers were used: the first type (15 Oligo (dT), a volume of g20, and its concentration after dilution with sterile distilled water (DW) was 0.5 µg/ µl. Given the permeation of the material, the second type of primer was used, the Random hexamer with a volume of 24 µg and its concentration after dilution. 0.2 µg / µl, The frozen materials (RNA, Water DEPC and Primer) were thawed before use.

| Table (1) Program of reaction conditions for converting RNA into cDNA |
|-------------------------|--------|--------|
| Step                    | Temperature | Time   |
| Primer annealing        | 35 °C    | 3 min  |
| cDNA Synthesis          | 60 °C    | 30 min |
| Heat inactivation       | 95 °C    | 5 min  |
| No. of cycles           |          | 1 cycle|

2.2 RT-qPCR technique for gene expression determination

The instantaneous reflex reaction test (RT-qPCR) for the study treatments was performed using the GoTaq® Probe RT-qPCR Master Mix kit provided by Promega, the components of which are attached in Table (2).

| Table (2) several components for preparing the Quantitative polymerase Chain Reaction (GoTaq® Probe RT-qPCR Master Mix) |
|-----------------------------------------------------------------------------------------------------------------------------|
| Components                                                                                                                 |
| GoTaq® Hot Start Polymerase                                                                                                 |
| MgCl2                                                                                                                        |
| dNTPs                                                                                                                        |
| proprietary reaction buffer                                                                                                 |

2.3 Primer sequences used

1-glutathione S-transferase zeta class-like
Forward TTGTGAAAAACCATCGGGGA
Reverse ACAGAGCCTATTGAGGACG
product length = 155

2-elongation factor
TCTTCTGCCTATCGCACA
Forward TGCTGAGCCTGTGAAG
Reverse TGCTGAGCCTGTGAGAAG
product length = 102

The volume required for all components of the Quantitative polymerase Chain Reaction.

The above components were mixed with a rotary mixer device at a speed of 3000 revolutions / min for 10 seconds, then placed in the instantaneous thermal polymerization device(Thermocycler) and the program was conducted as in Table (3).
Table (3) Program of Reaction Conditions (RT-qPCR)

| Step            | Temp. (°C) | Time       | Cycle | Scaning |
|-----------------|------------|------------|-------|---------|
| Enzyme activation | 95 °C      | 05:00 min  | Hold  |         |
| Denaturation    | 95.0 °C    | :00:20     | 40    |         |
| Annealing/Extension | 60.0 °C | :00:20 sec |       |         |
|                 | 72.0 °C    | :00:20 sec |       |         |

After the reaction ended, the data were analyzed and the samples were placed in the freezer.

3. Results and Discussion:

3.1 Isolation of non-deoxyribonucleic acid (RNA) from the leaves of Armenian cucumber plants.

After using the diagnostic kit equipped by the American company Zaimo RNA extraction ZR Plant RNA No. R2024, ZYMO To extract the RNA genetic material from the leaves of all 42 cultivars of Armenian cucumber. Twenty-one samples irrigated with fresh water. Twenty-one irrigated with salt water at a concentration of 5 dDm-1.

![Figure (1): RNA migration products isolated from leaves for all studied cultivars in acarose gel at a concentration of 1.5% (1.30 hr, 5V / cm, 1X TBE) imaged under ultraviolet U.V. After staining with ethium bromide pigment.](image)

3.2 Concentrations and purity values of RNA from Armenian cucumber samples.

The values of the Armenian cucumber samples irrigated with fresh water are between (ng / μl 89.8 - 0.327), while the purity of the samples themselves varied between (2.03 - 0.6). As for the samples irrigated with salt water, their concentrations varied between (ng / μl 25 - 1.2). The purity of the samples is between (2.09 - 0.79). The reading of the purity and the concentrations was taken by a Nano Drop (Quantustm Fluorometer) and afterwards the concentrations of the samples were reduced to ng / μl 20 for use in the reaction mixture.

3.3 cDNA construction for the leaves of the studied cultivars.

After isolating RNA and ensuring its integrity, purity and concentration from the leaves of the Armenian cucumber plants under study, it was converted into cDNA by using a kit provided by the American Takara PrimeScriptTM RT Reagent Kit (Perfect Real Time) # RR037A and after completing the process of converting
RNA into cDNA, the cDNA was migrated by gel method Acarose at a concentration of 1.5%, and this migration succeeded by showing it in the form of a clear and pure package for all cultivars. The reading of purity and concentrations was also taken by a nano drop (Quantum fluorometer) for cDNA as it was a clear transfer concentrations which are pure packages in order to enter the stage of studying gene expression using a device Real time PCR.

Figure (2): RNA-to-cDNA conversion products isolated from leaves for all studied cultivars in acarose gel at a concentration of 1.5% (1.30 hr, 5V / cm, 1X TBE) imaged under UV ultraviolet U.V. After staining with ethium bromide pigment.

3.4 Gene expression (Glutathione S-transferase zeta class-like)

The expression of the glutathione S-transferase zeta-class-like gene was measured using the RT-qPCR (Real Time-Quantitative Polymerase Chain Reaction) technique to study the gene expression of the salt tolerance traits of a number of Armenian cucumber cultivars under the influence of salt stress.

The results in Table (4) showed that irrigation with salt water has a significant effect compared to irrigation with fresh water, where gene expression increased in plants irrigated with salt water compared to plants irrigated with fresh water, and this is consistent with what [11].found. As it was shown that GSKs3 genes in Arabidopsis are stimulated under the influence of osmotic and salt stress, and the same table also shows that the cultivars differed among themselves in gene expression, which refers to the gene expression values represented by the value of the Cycle threshold (ct), which indicates the degree of gene expression inversely. As it gave the cultivars Egyptian, Babylon, Mosul (Karmles), Kirkuk, Diyala and Karbala, the ct was (21,550, 21,125, 22,150, 21,250, 20,500 and 19,850), respectively. Consequently, it gave the highest gene expression (13.06, 11.44, 10.02, 11.44, 0.701 and 10.02), respectively, compared to the rest of the cultivars, and this may be due to the genetics of the cultivated and the extent of its adaptation to environmental conditions. This was confirmed by some of the results of vegetative growth, flowering growth, chemical components of leaves in plants and total production, and these are consistent with what [12].found that tolerance genes differ in the amount of their expression in wheat cultivars, especially in sensitive genotypes. Therefore, the detection of these genes is very important to
distinguish between susceptible and tolerant genotypes. Previously, the method used to distinguish between genotypes tolerant to salinity grown in saline soils for several generations in a breeding program that relied on the traits of the backline to compare the genotypes. Therefore, a new distinction between genotypes was introduced in the gene expression technique. It was made by discovering salinity genes using the Quantitative polymerase Chain Reaction, in order to shorten the time and cost, which would facilitate the work of researchers. As the difference in the gene expression of the cultivars grown under salt conditions is a genetic fingerprint of the cultivar for you that is distinguished compared to the rest of the cultivated cultivars in future field experiments.

Table (4): Cycle threshold (CT) and relative gene expression of the glutathione S-transferase zeta class-like gene in coughing Armenian cucumber cultivars.

| Treatments          | Ct of gene glutathione S-transferase zeta class-like | Ct of elongation factor | ΔCt   | ΔΔCt   | relative gene expression |
|---------------------|------------------------------------------------------|-------------------------|-------|--------|--------------------------|
| Water quality       |                                                      |                         |       |        |                          |
| fresh water         | 24.769                                               | 19.164                  | 5.605 | 0.0000 | 1.00                     |
| Salt water          | 17.988                                               | 15.802                  | 2.186 | -3.4190| 12.86                    |
| Lsd                 |                                                      |                         |       |        | 0.255                    |
| Armenian Cucumber cultivars |                                              |                          |       |        |                          |
| Basra               | 21.050                                               | 16.725                  | 4.325 | -1.8750| 7.23                     |
| Amarah              | 20.600                                               | 18.050                  | 2.550 | -0.9000| 2.25                     |
| Nasiriyyah          | 22.350                                               | 18.350                  | 4.000 | -1.2000| 3.15                     |
| Wasit               | 20.075                                               | 16.325                  | 3.750 | -1.9000| 7.47                     |
| Baghdad             | 21.525                                               | 18.950                  | 2.575 | -1.0250| 2.57                     |
| Diwaniya            | 21.950                                               | 16.950                  | 5.000 | -1.6000| 5.11                     |
| Samawah             | 22.925                                               | 17.150                  | 5.775 | -1.5750| 4.94                     |
| Najaf               | 24.250                                               | 16.425                  | 7.825 | -1.9250| 7.72                     |
| Karbala             | 19.850                                               | 17.025                  | 2.825 | -2.1250| 10.02                    |
| Diyala              | 20.500                                               | 17.175                  | 3.325 | -2.1750| 10.70                    |
| Alramadi            | 21.525                                               | 17.500                  | 4.025 | -1.5750| 4.94                     |
| Tikrit              | 22.125                                               | 16.900                  | 5.225 | -1.3750| 3.88                     |
| Kirkuk              | 21.250                                               | 17.125                  | 4.125 | -2.2250| 11.44                    |
| Mosul (Karamles)    | 22.150                                               | 16.325                  | 5.825 | -2.1250| 10.02                    |
| Babylon             | 21.125                                               | 18.750                  | 2.375 | -2.2250| 11.44                    |
| Iranian             | 19.225                                               | 18.150                  | 1.075 | -0.7250| 1.87                     |
| French              | 20.625                                               | 18.650                  | 1.975 | -2.0250| 8.79                     |
| Dutch               | 22.000                                               | 18.525                  | 3.475 | -2.0250| 8.79                     |
| Italian             | 21.975                                               | 16.950                  | 5.025 | -1.0750| 2.73                     |
| Egyptian            | 21.550                                               | 17.125                  | 4.425 | -2.3250| 13.06                    |
| Mosul (Akaf)        | 20.325                                               | 18.025                  | 2.300 | -1.9000| 7.48                     |
| Lsd                 |                                                      |                         |       |        | 0.825                    |

Table (5) showed that the data analysis results for the same cultivars under the influence of salt stress shows that stress has an effect on the expression of the glutathione S-transferase zeta class-like gene. The highest
gene expression was shown in the salt tolerant cultivars. This indicates that the salt tolerant cultivars possessed the 155bp glutathione S-transferase zeta class-like gene responsible for the high salinity tolerance trait which was induced by the presence of salt NaCl and showed high expression under salt stress. As the value of the threshold line ct in the traits of Egypt, Babylon, Mosul (Karmles), Kirkuk, Diyala and Karbala (17.300, 16.300, 19.400, 19.100, 16.800 and 16.20), respectively, as in figure (3) that shows the value of the color frequencies and the values of the genes expression were high for these consecutive cultivars, where their gene expression was reported (25.12, 21.87, 19.04, 21.87, 19.04 and 20.40), respectively .While we note from the same table that the cultivars Iranian (Ghani), Italian, Baghdad and Amara recorded a value of ct amounting to (15,550, 19,400, 18,050 and 17,850) respectively, These cultivars gave the lowest gene expression values (2.73, 4.46, 4.14 and 3.49), respectively, for the Salt water treated cultivars. As the degree of gene expression depends on the degree of salt stress, and the higher the salinity, the greater the gene expression, because the gene expression of any gene is an interaction between the environment (salinity) and Genetics (gene ) [13].Based on that, the results of the analysis indicate that the glutathione S-transferase zeta class-like gene is present in all cultivars, and its quantity has varied. Therefore, this gene increases plant tolerance to salinity and this helps the plant grow well under conditions of high salinity. Therefore, the investigation of any A gene from a salinity-tolerant gene must be accompanied by an estimate of its gene expression for the purpose of using it in the future when the salt cultivars are transferred to the salt-sensitive cultivars. This result agree with [7.]) and also with[14].found that increased gene expression of a gene associated with salt tolerance of wheat Tasc enhances the salt tolerance of Arabidopsis and that the transcription process of the Tasc gene in the salt tolerance of wheat .Since it was observed that Tasc gene expression regulates growth after exposure to stress conditions, and by using Real-Time Pcr and analyzing the results, salinity and Ipsiisic acid were found to be the catalysts for this expression in the transgenic plant Arabidopsis, and that the increase in gene expression is the results of an increase in saline tolerance, and transgenic plants appear. Tasc and Col-0 are significantly highly expressed as well as for. AtFry1, AtSad1 and AtCDPK2,and a number of researchers indicated that the salt tolerance gene stimulates its presence of higher salinity, compared with the susceptible cultivars that were less expressive. This is consistent with what [14].found, that it obtained an increase in the gene expression of a gene associated with salt tolerance of wheat Tasc, which enhances the salt tolerance of Arabidopsis .The figure also shows differences in the number of threshold cycles (CT) of the salt tolerance gene for the studied cultivars, where some high lines were found starting from one point, i.e. the threshold cycle ranged between 25-28 indicating the presence of amplification of the gene and thus this gene works to express equal values for the studied cultivars.

Table (5): Comparison cycle threshold and relative gene expression of the glutathione S-transferase zeta class-like gene in Armenian cucumber cultivars, under salt stress.

| Water quality | Armenian cucumber cultivars | Ct of gene glutathione S-transferase zeta class-like | Ct of elongation factor | ΔCt | ΔΔCt | relative gene expression |
|---------------|-----------------------------|---------------------------------------------------|------------------------|-----|------|--------------------------|
| Fresh water   |                             |                                                   |                        |     |      |                          |
| Basra         | 23.700                      | 17.500                                            | 6.200                  | -   | 1.00 |                          |
| Amarah        | 23.350                      | 19.900                                            | 3.450                  | -   | 1.00 |                          |
| Nasiriyah     | 26.100                      | 20.900                                            | 5.200                  | -   | 1.00 |                          |
| Wasit         | 25.400                      | 19.800                                            | 5.600                  | -   | 1.00 |                          |
| Baghdad       | 25.000                      | 21.400                                            | 3.600                  | -   | 1.00 |                          |
| Diwaniya      | 25.300                      | 18.700                                            | 6.600                  | -   | 1.00 |                          |
| Samawah       | 26.500                      | 19.150                                            | 7.350                  | -   | 1.00 |                          |
| Najaf         | 27.050                      | 17.300                                            | 9.750                  | -   | 1.00 |                          |
| City       | Lsd | Lsd  | Lsd  | Lsd  | Lsd  |
|------------|-----|------|------|------|------|
| Karbala    | 23.500 | 18.550 | 4.950 | -    | 1.00 |
| Diyala     | 24.200 | 18.700 | 5.500 | -    | 1.00 |
| Alramadi   | 22.900 | 17.250 | 5.650 | -    | 1.00 |
| Tikrit     | 25.100 | 18.500 | 6.600 | -    | 1.00 |
| Kirkuk     | 23.400 | 17.050 | 6.350 | -    | 1.00 |
| Mosul (Karamles) | 24.900 | 16.950 | 7.950 | -    | 1.00 |
| Babylon    | 25.950 | 21.350 | 4.600 | -    | 1.00 |
| Iranian    | 22.900 | 21.100 | 1.800 | -    | 1.00 |
| French     | 24.950 | 20.950 | 4.000 | -    | 1.00 |
| Dutch      | 26.550 | 21.050 | 5.500 | -    | 1.00 |
| Italian    | 24.550 | 18.450 | 6.100 | -    | 1.00 |
| Egyptian   | 25.800 | 19.050 | 6.750 | -    | 1.00 |
| Mosul (Akaf) | 23.050 | 18.850 | 4.200 | -    | 1.00 |
| Basra      | 18.400 | 15.950 | 2.450 | 3.7500- | 13.46 |
| Alramadi   | 17.850 | 19.900 | 1.650 | 1.8000- | 3.49 |
| Nasiriyah  | 18.600 | 20.900 | 2.300 | 2.4000- | 5.29 |
| Wasit      | 17.650 | 15.200 | 2.450 | 3.1500- | 8.88 |
| Baghdad    | 18.050 | 21.400 | 1.550 | 2.0500- | 4.14 |
| Diwaniya   | 18.600 | 15.200 | 3.400 | -3.2000- | 9.21 |
| Samawah    | 19.350 | 15.150 | 4.200 | 3.1500- | 8.88 |
| Najaf      | 21.450 | 15.550 | 5.900 | 3.8500- | 14.43 |
| Karbala    | 16.200 | 15.500 | 0.700 | 4.2500- | 19.04 |
| Diyala     | 16.800 | 18.700 | 1.150 | 4.3500- | 20.40 |
| Alramadi   | 19.150 | 17.250 | 1.850 | -3.8000- | 13.93 |
| Tikrit     | 19.150 | 15.300 | 3.850 | 2.7500- | 6.76 |
| Kirkuk     | 19.100 | 17.200 | 1.900 | -4.4500- | 21.87 |
| Mosul (Karamles) | 19.400 | 15.700 | 3.700 | 4.2500- | 19.04 |
| Babylon    | 16.300 | 16.150 | 0.150 | 4.4500- | 21.87 |
| Iranian    | 15.550 | 15.200 | 1.800 | 1.4500- | 2.73 |
| French     | 16.300 | 16.350 | 0.050- | 4.0500- | 16.87 |
| Dutch      | 17.450 | 16.000 | 5.500 | 4.0500- | 16.57 |
| Italian    | 19.400 | 15.450 | 3.950 | 2.1500- | 4.46 |
| Egyptian   | 17.300 | 15.200 | 2.100 | 4.6500- | 25.12 |
| Mosul (Akaf) | 17.600 | 17.200 | 0.400 | 3.8000- | 13.96 |

4. Conclusions
From the aforementioned results, the cultivars of Egypt, Babylon, Mosul (Karmles), Kirkuk, Diyala and Karbala are tolerant to salinity because the expression values of the glutathione S-transferase zeta class-like gene were relatively high compared to the other studied cultivars. As for the cultivars that have confirmed their sensitivity to salinity, Iranian, Italian, Baghdad and Amara, because the amount of gene expression for them was low and high, it can be concluded that there is a high percentage of functional variation between the studied samples with regard to their ability to withstand salt stress and this leads to the presence of important genetic and functional variation between these cultivars. Although field experiments provide important information about the extent of genetic variation between cultivars in their tolerance to environmental stress, modern technologies at the level of molecular studies provide accurate and detailed information about the most important mechanisms that plant cultivars follow to protect themselves from environmental stresses, especially salt stress, therefore, isolating and studying these genes is considered a major issue to improve global agriculture and hope for the future to transfer some genetic sites to sensitive cultivars.

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