# Cu/Zn and MnSOD gene expression induced by ethyl methanesulfonate and drought stress in maize

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# Cu/Zn and MnSOD Gene Expression Induced by Ethyl Methanesulfonate and Drought Stress in Maize

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**Abstract.** Dehydration is one of the most important determinants to plant growth and productivity in general, and maize in particular. Therefore, the current study aimed to induce genetic variation for drought tolerance in two maize inbred lines and their $F_1$ hybrid with aid of ethyl methanesulfonate EMS mutagenesis. Furthermore, the simultaneous effect of EMS was investigated in the expression of MnSOD and Cu/Zn-SOD genes. The study was conducted in the fields of Abu Gharib Station- Agricultural Research Department- Baghdad in the spring season of 2019. The results showed that the genotypes differed significantly in the levels of the expression of both MnSOD and Cu/Zn-SOD genes. The gene pool of 18 and 5 parental lines and their single hybrid (Nahrain) reflected on the expression level of Cu/Zn-SOD gene, that produced 39.54, 17.63 and 24.37 copies, for the three genotypes, respectively at the 7-day irrigation interval. There was an increase in the expression level of the previously mentioned gene in most genotypes at a concentration of 70 mM EMS under the longest irrigation interval (11 days). The plants of inbred 18 were the best at the level of expression of the Cu/Zn-SOD gene with 18.61 copies, followed by the single hybrid and the parental inbred 5 with 11.99 and 7.79 copies, respectively. The investigate genotypes revealed clear differences in the expression of MnSOD gene in response to the two studied factors, as hybrid plants obtained the highest level of gene expression reached 120.59 copies at the EMS concentration of 70 mM and irrigated with the longest interval. It was followed by untreated plants of inbred 18 (control treatment) with 98.33 copies, then inbred 5 which produced 61.92 copies of the same gene at the EMS concentration of 60 mM and under the shorter irrigation interval (7 days). The overall performance of the $F_1$ hybrid and parental line 18 in respect of MnSOD gene expression was nearly identical with 41.83 and 41.81 copies, respectively.

**Keywords**: MnSOD, Ethyl, Maize, Cu/Zn

## INTRODUCTION

Plants like other living organisms, faces a growing challenge of environmental stress, that threatens the existence of several plant species and the food security. Thus, there is a necessary to pay more attention to the improving of new maize genotypes (Zea maize L.) since this crop is one of the five main food sources [1]. This crop has multiple uses ranged between direct consumption by human and animals and for industrial purposes like antifreeze materials and pharmaceuticals. Furthermore, the new emerged use as a source for biofuel production [2]. Most problems that face plant cultivation come from the lack of "mobility", which makes the crop vulnerable to inappropriate growth conditions termed stress, that determine yield potential and keeping it at the minimum level [3]. Depending on causative element, stress can be classified into biotic and abiotic stresses. Extreme values of humidity, light, salts, nutrients, temperature, etc. are among the factors of abiotic stresses [4]. Plants have developed various mechanisms to deal with stress in general and dehydration in particular, such as modifying gene expression [5]. Therefore, the use of gene products as indicators to determine the genetic efficacy for drought tolerance is more accurate to formulate recommendations about such concerns [6]. Naturally, mutations are rare unless they are induced by physical factors (ɣ, β, gama and beta rays etc.) or chemically stimulated by sodium azide SA, colchicine, methyl methanesulfonate (MMS) and Ethyl methanesulfonate (EMS) mutagens, which are used to produce the desired variants of specific characters. Ethyl methanesulfonate (EMS) is one of the most important point chemical mutagens

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used for notifying heterogeneity without causing widespread chromosomal abnormality with which the progeny survival rate may decrease [7]. Like other stress condition, drought produces Reactive Oxygen Species (ROS) that have a key role in triggering programmed cell death after their release of free hydroxyl radicals and thus oxidation of fat within the cell membranes, carbohydrates and protein decomposition, disturbing the enzymatic system and damaging DNA. Therefore, induction of heterogeneity in the encoded genes of the group of SOD enzymes (Fe, Cu/Zn, and Mn) is effective in improving the defense system against ROS and contributes significantly to plants' tolerance to drought [8]. The study aims to evaluate the performance of three maize genotypes (inbred lines of 18 and 5 and their individual two hybrids) under field conditions by the effect of soaking seeds in different concentrations of EMS and its reflection on the level of the Cu/Zn-SOD and MnSOD genotypes under irrigation intervals.

## MATERIALS AND METHODS

The experiment was conducted during the spring season 2019 at the Abu Gharib Experiments Station - Agricultural Research Department - Baghdad - Iraq. The study included three maize genotypes (two inbred lines, 18 and 5, in addition to $F_1$ single hybrids), when their seeds were soaked for 5 hours in three concentrations of EMS (0, 60 and 70 Mm) in dark room at 25±2 temperature. The investigated EMS concentrations were prepared by diluting each concentration in 1 liter of phosphate buffered saline (PBS). The pH of the prepared concentrations was adjusted to 7, 6.6 and 6.47, respectively. exposed to irrigation intervals 7, 11 days. The genotypes were planted on a furrows with three replicates, each of 18 experimental units with an area of 3 x 3 $m^2$, composed of 4 lines and the application of drought stress started at 4-5 leaves stage.

## GENE EXPRESSION OF CU/ZN-SOD AND MNSOD

### Plant Sample Collection

Fresh maize leaves from all treatment were collected at the end of vegetative growth and dipped in trizol solution (600 µl) for RNA extraction by using One-Step RT-qPCR System (Promega, USA). The supplier instructions were followed.

### QPCR Conditions

The thermal profile of qPCR was as follows: Reverse transcription was at 37 ºC, for 15 min., one cycle; Hot-start activation was at 95 ºC for 10 min. in 1 cycle. Denaturation, Annealing and Extension were at 95 ºC, 60 ºC and 72 ºC for 10, 30 and 30 sec., respectively, and all were run for 40 cycles. The last step of the dissociation was at 60 - 95ºC in 1 cycle. The expression of Cu/Zn-SOD and MnSOD genes was studied with the aid of four specific primers, forward 5`-TGTTGCAAATGCTGAGGGCATAGC-3`and reverseprimers 5`- CCAACAACACCACATGCCAGTCTT-3` for Cu/Zn-SOD gene and forward 5`-TTGTGTACCTGCTGGACCAAGTGT-3` and reverse 5`-ACTACGAGCAGCAGAAAGTGGAGT-3` for MnSOD gene. After obtaining the (Ct) averages of the genes used under the influence of EMS concentration and drought stress, the following calculations are made for estimating gene expression [3]

$$\text{Cu-Zn-SOD: } y = -3.33\,x + 34.65 \quad X = y - 34.65/-3.33$$
$$Y = ct \quad X = \log con \quad copy = 10^{Log\ con}$$
$$Y = ct \quad X = \log con \quad copy = 10^{Log\ con} \tag{1}$$

## RESULTS AND DISCUSSION

### Cu/Zn-SOD Gene Expression

Results of the Cu/Zn-SOD gene expression (TABLE 1 and Fig 1) exhibited a marked difference in expression levels for this gene between genotypes (two inbred lines and their $F_1$ hybrid), EMS (mM) concentrations, and irrigation intervals. As inbred line 18 showed the highest level of gene expression among other genotypes reached 39.54 copies (TABLE 1), inbred line 5 revealed the minimum gene expression with only 17.63 copies of the targeted gene. The effect of EMS concentrations varied according to the different genotype, where the plants of inbred line 18 gave the highest gene expression reached 29.33 copies at a concentration of 70 mM EMS, while inbred line 5 showed the lowest expression of the same gene at the concentration of 60 mM of the mutagens (2.99 copies). The level of the Cu/Zn-SOD gene expression differed in response to the watering intervals, as plants of inbred line 18 at 11 days irrigation interval showed the highest level of this gene expression with 14.34 copies, while the plants of inbred line 5 gave the lowest gene expression at the same interval reached 4.88 copies. The results also indicated a clear variation in genotypes performance effected by EMS concentrations and irrigation intervals. Hybrid plants showed the highest gene expression reached 18.88 copies at 70 mM concentration of EMS cultivated under the longest irrigation interval (11 days). Untreated plants of the single hybrid (Nahrain) showed the highest level of gene expression reached 24.37 copies with a general performance across the practiced factors scored 11.99 copies of Cu/Zn-SOD gene. On the other hand, the untreated plants of inbred line 5 produced the minimum copies of the investigated gene (17.63 copies) with a general performance reached 7.79 copies. The previous results indicated a clear fluctuation in the expression of Cu/Zn-SOD gene in all genotypes, in response to the subjected factors. The overall performance approved the superiority of inbred line 18 in the Cu/Zn-SOD gene expression with 18.61 copies, as the highest performance, followed by (Nahrain) hybrid and inbred line 5 with an expression level of11.99 and 7.79 copies of the investigated gene in the two genotypes, respectively. Previous studies by [9] Displayed similar findings of fluctuating gene expression between in response to EMS concentrations. While [10]. Confirmed the positivity of EMS treatments in stimulating dehydration tolerance in the treated maize genotypes. [11]. Reported that the level of Cu/Zn-SOD gene expression was not affected by drought stress, while it was differed under low temperature.

**TABLE 1.** Expression of Cu/Zn-SOD gene affected by EMS and irrigation intervals in maize genotypes

| Sample No. | Genotypes | EMS Con. (Mm) | Irrigation Intervals (day) | Cu/Zn-SOD Expression | Average |
|---|---|---|---|---|---|
| 1 | 18 | 0 | 7 | 39.54±4 | 18.61 |
| 2 | | | 11 | 14.34±2 | |
| 3 | | 60 | 7 | 11.24±2 | |
| 4 | | | 11 | 14.12±2 | |
| 5 | | 70 | 7 | 29.23±3 | |
| 6 | | | 11 | 3.21±1 | |
| 7 | 5 | 0 | 7 | 17.63±2 | 7.79 |
| 8 | | | 11 | 4.88±1 | |
| 9 | | 60 | 7 | 2.99±1 | |
| 10 | | | 11 | 7.77±2 | |
| 11 | | 70 | 7 | 4.19±1 | |
| 12 | | | 11 | 9.28±2 | |
| 13 | Nahrain | 0 | 7 | 24.37±3 | 11.99 |
| 14 | | | 11 | 7.54±2 | |
| 15 | | 60 | 7 | 4.11±1 | |
| 16 | | | 11 | 6.14±2 | |
| 17 | | 70 | 7 | 10.92±3 | |
| 18 | | | 11 | 18.88±3 | |
| Average | 27.18 | 10.44 | 8.92 | Values are expressed as means ± standard error | |

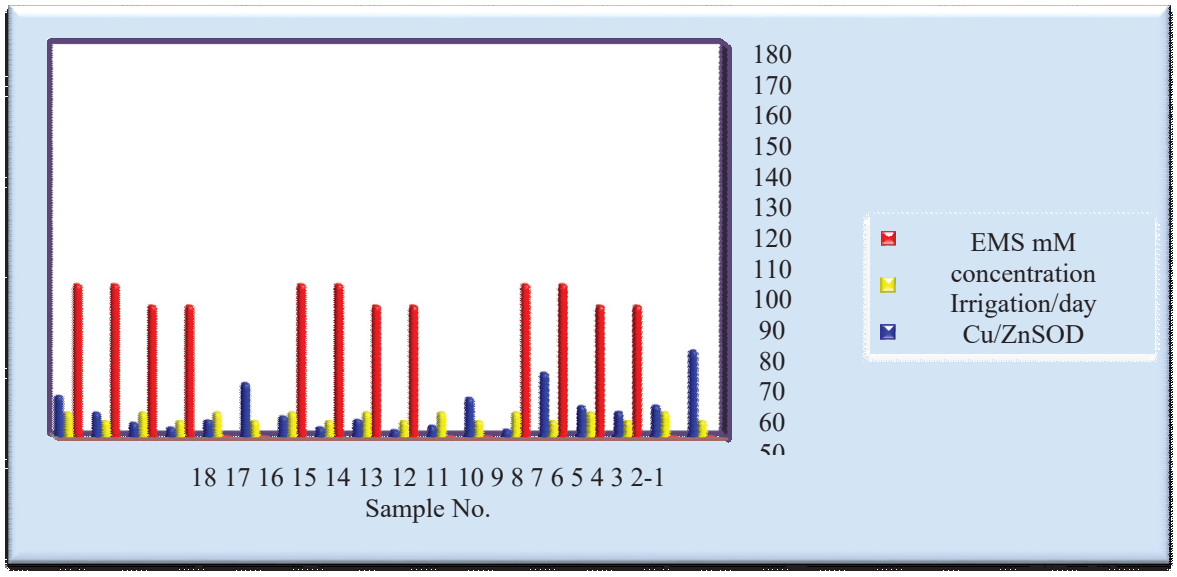


**FIGURE 1.** Expression of Cu/Zn-SOD gene affected by EMS and irrigation intervals in maize genotypes.

## MNSOD GENE EXPRESSION

Results presented in TABLE 2 and Fig 2 showed a clear difference in level of MnSOD gene expression between the two inbred lines and their single hybrid, EMS concentrations (mM), and the irrigation intervals. The plants of inbred 18 showed the highest genetic expression among other genotypes that reached 98.33 copies

TABLE 2. However, inbred line 5 gave the minimum copies of the targeted gene (14.87 copies). The results introduced a clear evidence for the effect of EMS concentrations and the different genotypes, as the plants of inbred line 5 gave the highest level of gene expression reached 61.92 copies at a concentration of 60 mM EMS, while the same inbred showed the lowest level of expression for the previously mentioned gene (12.60 copies) at 70 mM of EMS. Maize plants under the effect of irrigation intervals revealed different levels of MnSOD gene expression, where inbred line 18 at the longest irrigation interval (11 days) gave the highest gene number of gene copies reached

copies, while plants of inbred line 5 gave the lowest expression at the same interval (12.42 copies). The tested genotypes also varied in response to EMS concentrations and irrigation intervals, where hybrid plants at 70 EMS concentration and the longest irrigation interval gave the highest level of gene expression (120.59 copies), while the lowest number of copies was obtained from the plants of inbred 18 treated with same concentration of mutagen and subjected to the same interval of irrigation (6.02 copies). Through the performance of the genotypes, Nahrain hybrid was outperformed at the concentration of 70 mM and irrigate with the longest irrigation interval by giving the highest expression of MnSOD gene reached 120.59 copies. The untreated plants of inbred line 18 expressed the highest copies number of the investigated gene (98.33 copies) with a general performance reached 41.81 copies. Whereas, the performance of inbred line 5 was completely different as its plants produced the lowest number of copies amounted to 61.92 copies at a concentration of 60 mM EMS irrigated according to the shorter interval (7 days). Results indicated the importance of the genetic background of any genotypes that may significantly determine the norm of response to growth conditions in general and stress in particular.

Generally, the single hybrid (Nahrain) exhibited very close performance to the parental line 18, in respect of MnSOD gene expression that evidenced by their with copies reached 41.83 and 41.81, respectively. This indicates the extraordinary capability of some genotypes in passing their key genes to their progeny which in turn will result in a state of genetic regulation towards the desired direction regarding the targeted gene expression. Furthermore, the superiority of the hybrid plants at a concentration of 70 mM under the longest irrigation interval gave 120.59 copies of the target gene.

The state of low gene expression of a specific gene, may be due to the influence of other environmental factors and their interactions, hence lack of control as they are purely external factors. These results supported by previous findings [12], [13] and [14] which indicated the increase of MnSOD gene expression in maize genotypes in response to abiotic. Also, the current results are consistent with some reports about the importance of EMS in improving maize tolerance to drought [15],[ 16].

**TABLE 2.** Expression of MnSOD gene affected by EMS and irrigation intervals in maize genotypes.

| Sample No. | Genotypes | EMS Con. (Mm) | Irrigation Intervals (day) | MnSOD Expression | Average |
|---|---|---|---|---|---|
| 1 | | | | 98.33±6 | |
| 2 | | 0 | 7 | 71.84±5 | |
| 3 | | | 11 | 13.91±3 | |
| 4 | | 60 | 7 | 18.44±3 | |
| 5 | 18 | | 11 | 42.34±4 | 18.61 |
| 6 | | 70 | 7 | 6.02±2 | |
| 7 | | | 11 | 14.87±3 | |
| 8 | | 0 | 7 | 12.42±3 | |
| 9 | | | 11 | 61.92±5 | |
| 10 | | 60 | 7 | 21.73±3 | |
| 11 | 5 | | 11 | 12.60±2 | 7.79 |
| 12 | | 70 | 7 | 19.11±2 | |
| 13 | | | 11 | 47.74±4 | |
| 14 | | 0 | 7 | 32.27±3 | |
| 15 | | | 11 | 15.65±3 | |
| 16 | | 60 | 7 | 11.28±2 | |
| 17 | | | 11 | 23.46±3 | 11.99 |
| 18 | | 70 | 7 | 33±6 | |

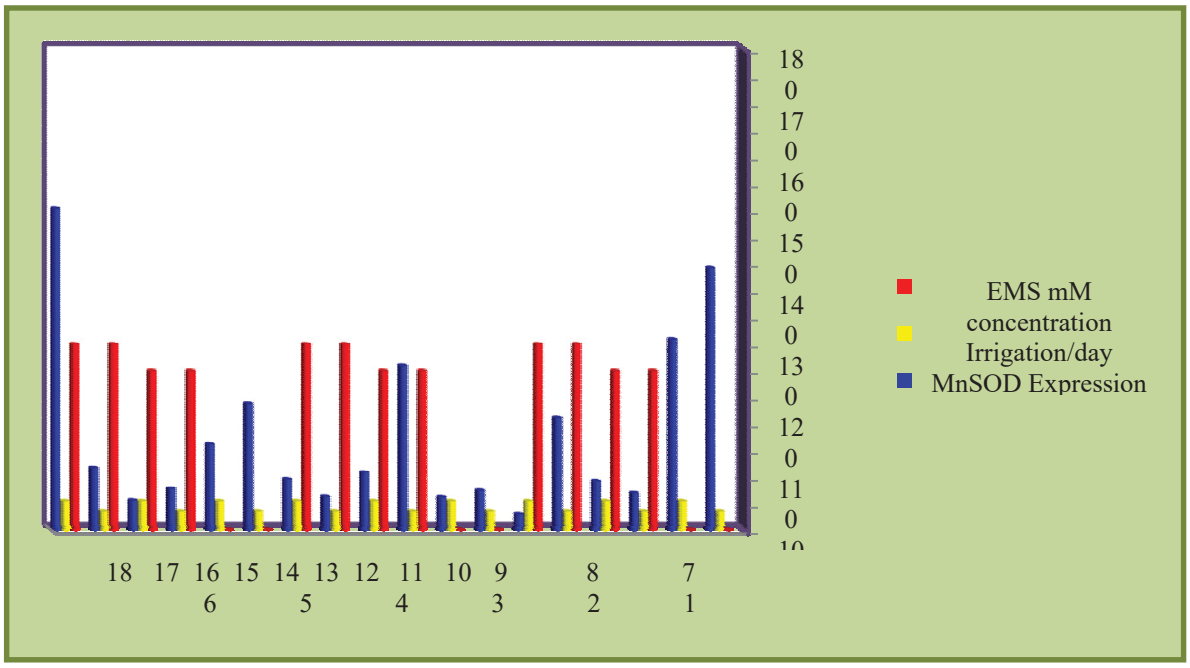


**FIGURE 2.** Expression of MnSOD gene affected by EMS and irrigation intervals in maize genotypes.

## CONCLUSION

The study of gene expression in maize genotypes increases the awareness of the true role of the SOD family induced by chemical mutagens and moisture deficiency, the studied genotypes varied in their response to EMS concentrations and irrigation intervals via high and low performance of maize genotypes regarding Cu/ZnSOD gene expression. The superiority of inbred line 18 can be noted, meanwhile the entire genotypes tend to produce more copies from both targeted genes at the higher EMS concentrations and the longest intervals of irrigation. Although, the hybrid gave the highest gene expression at the highest concentration of EMS and the longest irrigation interval, followed by inbred line 5 at the EMS concentration of 60 mM in response to the shorter irrigation interval, with high convergence in the general performance of the inbred line 18 and its progeny. Thus, the morphological traits will not be enough to speculate the genetic structure, simply because every genotype has a wide range of morphological plasticity depending on the appropriate conditions to reactivate silence genes under stress in general and drought in particular. Hence, the investigation of SOD role in regulating abiotic stress tolerance is informative.

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