



## Improving Maize production and irrigation water use efficiency using glycine betaine and hydrogen peroxide under drought conditions



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**A** Two Field experiments of sprinkler irrigation system were performed during the 2021 and 2022 summer growing seasons in a sandy soil in the Experimental farm, Ismailia Agricultural Research Station, Egypt (at latitude 30°58' N, longitude 32°23' E, and an elevation of 13 meters above sea level). This study aimed to investigate the effect of 5 levels of external application of GB and H<sub>2</sub>O<sub>2</sub> on IWUE and total yield of hybrid maize 368 under three different irrigation water levels (100%, 80% and 60% of ET<sub>c</sub>). Sprinkler irrigation was used in this study. The experimental design in each season was Split plot in a randomized complete block design. The three irrigation levels were randomly distributed in the main plots, while five levels of (GB) and (H<sub>2</sub>O<sub>2</sub>) were randomly distributed in the subplots. Decreasing irrigation water quantity from 3100 to 1860 m<sup>3</sup>/feddan significantly decreased 100-grain weight and grain yield/feddan, but significantly improved grain protein content and IWUE. Increasing GB or H<sub>2</sub>O<sub>2</sub> levels resulted in a significant increase in all of the above-mentioned traits compared to untreated analogues. The application of 15 mM GB and 80 mM H<sub>2</sub>O<sub>2</sub> preserved approximately 770 and 663 kg grain per feddan of losses under moderate water stress, in order. While under severe drought, the use of these previous levels of GB and H<sub>2</sub>O<sub>2</sub> resulted in gains of approximately 400 and 290 kg of grain yield/feddan. The use of GB and H<sub>2</sub>O<sub>2</sub> saved about 620 m<sup>3</sup>/fad of water without any loss in grain yield. As a final result, we conclude that the grain yield improved significantly for GB and H<sub>2</sub>O<sub>2</sub> under drought conditions and good irrigation.

**Keywords:** Maize, GB, H<sub>2</sub>O<sub>2</sub>, IWUE, drought, yield.

### Introduction

MAIZE (*Zea mays* L.) is the third-most significant cereal grain in Egypt, although it is susceptible to water stress, which results in substantial losses in both productivity and quality (Sayed *et al.*, 2022). Limited irrigation water uses, combined with salinity or unsuitable temperature, and are often considered negative factors for maize cultivation in different regions of the world. In addition, Massacci *et al.*, (2008) confirmed that a large proportion of arable land, perhaps up to one-third of the land worldwide, suffers from a chronic shortage of water for agriculture in these areas. The study conducted by Abo El Kheir and Mekki, (2007) ; Na *et al.*, (2018) showed that corn was somewhat sensitive to water deficiency in the vegetative stage, while the sensitivity was high during the formation of tassels tasseling, silking, and pollination, and its sensitivity was moderate in the grain filling stage. In the same context, maize cultivation under drip irrigation at a rate of 1575 m<sup>3</sup>/feddan, compared to 2625

m<sup>3</sup>/feddan of ET<sub>c</sub> resulted in significant reductions in RWC, total chlorophyll, ear blade area,, 100-grain weight, IWUE and the production of grain yield (Kotb *et al.*, 2009). When corn plants were exposed to water stress at the beginning of silk formation, this had the greatest impact on the crop and its various components, such as 1000-grain weight, grain yield, and biological yields, compared to other growth stages of the corn crop (Qasim *et al.*, 2019).

One of the negative effects of soil water scarcity is its adverse impact on photosynthesis, as it stimulates the excessive formation of reactive oxygen species (ROS) and their free radicals. These increases in ROS and free radicals levels cause significant damage, such as the activation or inhibition of enzymes, damage to DNA, RNA, and proteins, and the oxidation of membrane lipids, all of which can lead to cell death. Increased levels of ROS within cells may stimulate a complex defense

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system called antioxidants, which consists of both enzymatic and non-enzymatic components, in all plant cells to protect the plant (Shao *et al.*, 2008). Younis, *et al.* (2025) studied sixteen distinct maize varieties, cultivated under three varying irrigation levels: 40% ( $\text{m}^3/\text{ha}$ ), 60% ( $\text{m}^3/\text{ha}$ ), and 80% ( $\text{m}^3/\text{ha}$ ), all administered using spray irrigation. The findings indicated that hybrids SC 168, SC 164, and SC 124 are strongly endorsed for maize breeding initiatives focused on enhancing drought resistance in arid areas.

One potential approach to reduce crop losses due to water shortage is to use osmotic compounds that are sprayed externally onto the vegetative system without negatively interfering with enzymes activities. These osmotic compounds normally accumulate in the cytoplasm of plant cells to aid water absorption when soil water shortages reach levels that are harmful to plants (Annunziata *et al.*, 2019). The accumulation of soluble compounds such as proline, sugars, amino acids, and betaine, whether actively or passively, is an important adaptation for plants in response to various stresses, such as drought, to help plants cope with difficult conditions. Glycine betaine (hereinafter referred to as betaine) is synthesized by many species of the Poaceae, Amaranthaceae, and Malvaceae families. Glycine betaine has been found to be a compound secreted in many different organisms, including higher plants, as a defensive response to various stressors (Hussain *et al.*, 2008 and 2009 and Shala and Mahmoud, 2018). Interestingly, although maize has a relatively low capacity to accumulate glycine betaine, it can take up and accumulate glycine betaine at a remarkably high level after external foliar application and transport it immediately after application to almost all parts of the plant (Yang and Lu, 2006). Therefore, external spraying of the leaves of maize genotypes unable or unwilling to synthesize this compound may increase glycine betaine levels in these plants. More importantly, spraying glycine at an appropriate concentration may be an easy, simple, and low-cost method for increasing net yield while using insufficient amounts of water. In the same vein, Brand *et al.*, (2007) noted that external spraying of GB could be an easy method with a cost of less than \$25/ha to avoid the negative effects of water stress. External spraying of betaine on the leaves or roots of natural and non-accumulating betaine plants has been shown to be effective in enhancing the ability of these plants to withstand various stresses (Mäkelä *et al.*, 1996). Experiments have shown that GB, when sprayed externally on plant leaves, is transported after several hours to other parts of the plants (Mäkelä *et al.*, 1996), where it acts as a non-toxic cytoplasmic compound and intervenes in a pivotal way in protecting important components of plant cells, such as proteins, their compounds and cell membranes, under water stress conditions

(Jagendorf and Takab, 2001). It has also been shown that GB applied to wheat, barley, maize and sorghum increased photosynthesis, total chlorophyll, leaf area, RWC, LWP, IWUE and overall grain yield (Naryyar and Walia, 2004, Abd Alla Kotb 2005, Abd Alla Kotb and Gaballah 2007). In maize, exogenous application of 100 mM GB increased growth, yield and the activities of antioxidant enzyme under water stress (Anjum *et al.*, 2011). The results obtained in the study of Shafiq *et al.*, (2021) demonstrate the role of foliar bio stimulants (sugar beet extract (SBE) and glycine betaine (GB) in improving the growth and productivity of maize varieties by modifying the oxidative defense system and accumulating osmotic stress-protective substances under water deficit conditions. Hence, foliar application of GB may be an economically viable route to avoid the negative effects of water stress on both growth and yield of maize.

Hydrogen peroxide has been conclusively proven to play a pivotal role as a signaling molecule in photosynthesis, translocation, transpiration, respiration and to buffer against numerous abiotic and biotic stresses, such as cold, drought, salinity and heat. It is an environmentally friendly molecule at the appropriate concentration and is produced mainly during photosynthesis and photorespiration and at a lower concentration during respiration in plant cells (Slesak *et al.*, 2007). Therefore, adding it in the appropriate way may lead to increased crop productivity. It has become clear that the best time to use hydrogen peroxide is by mixing it with irrigation water or spraying it on plant leaves. Foliar spraying of hydrogen peroxide during the early stages of plant growth also had significant enhancing effects (Cavusoglu and Kabar, 2010). Khandaker *et al.*, (2012) stated that fruit development could be improved by spraying the crop of wax apple with  $\text{H}_2\text{O}_2$  treatment. It showed a significant increase in the rates of photosynthesis, stomatal conductivity, transpiration, chlorophyll % and dry matter of leaves. It was also found that treating rice seeds with 10 mM hydrogen peroxide significantly protected chlorophyll from cold, significantly improved the activities of both catalase and ascorbate peroxidase enzymes, and produced high-quality seedlings (Afrin *et al.*, 2019). It was also found that treating corn plants with hydrogen peroxide stimulated the antioxidant system, which led to improved drought tolerance of corn plants in terms of increasing the mass of fresh and dry shoots and roots (Ashraf *et al.*, 2015). In the same direction, under severe water stress, foliar application yellow corn hybrid 352 (three way cross) by 60mM  $\text{H}_2\text{O}_2$  significantly improved ear leaf blade area, total chlorophyll, RWC, ear length, ear diameter, 100-grain weight and grain yield/fad in comparison with untreated plants (Frage, 2020 and Kotb *et al.*, 2021). Youssef *et al.* (2025)

recommended using 300 ppm of citric acid with a water supply level of 75% ET<sub>c</sub> because it led to increase corn growth and increase its productivity higher than the comparison treatment, which may save 25% of irrigation water while achieving a higher yield and quality than the control treatment. The aim of this research is to estimate the effect of irrigation water quantities and external application of GB and hydrogen peroxide on growth, yield and IWUE of maize under water stress conditions

## Materials and Methods:

### Research Site and Conditions:

Two experiments using sprinkler irrigation were conducted for maize cultivation during the 2021 and 2022 summer seasons on sandy soil at the experimental farm of the Ismailia Agricultural Research Station, Egypt (at latitude 30°58' N, longitude 32°23' E, and an elevation of 13 meters above sea level). The climate of this area is semi-arid, with an average annual rainfall of 20 mm from December to March, an average temperature of 29.5°C in summer, and 46.5% RH. The monthly climate data predicted for the Ismailia region during the 2021 and 2022 seasons are shown in Table 2. To estimate the physical and chemical properties of the experimental field, soil samples were taken from depths ranging from 0 to 60 cm (Table 1). The soil composition at this site was 68.17% coarse sand, 23.45% fine sand, 4.08% silt, and 4.30% clay, making it a mostly sandy soil. The bulk density of the soil was estimated according to Grossmann and Reinsch (2002). The wilting point and field capacity were estimated according to the method of Cassel and Nielsen (1986).

### Experimental Design, Treatments and Agronomic Practices:

The experimental design in each season was a Split plot in a randomized complete block design with three replicates. A sprinkler irrigation system was used where three irrigations treatments and five levels of external application of hydrogen peroxide and glycinebetaine were randomly distributed on the main and sub plots, in order. To measure the amount of irrigation water, a pressure valve and a flow meter were used to control the operating pressure. The three irrigation treatments were W1: 1.00, W2: 0.8 and W3: 0.60 of ET<sub>c</sub>. Twenty-five days after planting, the three irrigation rates were applied. For both growing seasons, the water requirement for each irrigation was calculated based on both K<sub>c</sub> and ET<sub>o</sub>. ET<sub>o</sub> was estimated according to the Penman-Monteith equation (Allen *et al.*, 1998) based on the maize plant growth stages and the expected climatic conditions during the different irrigations. The FAO K<sub>c</sub> coefficient for

corn was 0.40 for the initial stage (20 days), 0.80 for the crop growth stage (35 days), 1.15 for the mid-season stage (40 days), and 0.70 for the final season stage (30 days). At the end of irrigation, the amount of water applied to the plants was calculated for each irrigation treatment during the two growing seasons. The average water quantities during the two growing seasons were found to be 3,100, 2,480, and 1,860 cubic meters per feddan for the three irrigation treatments, respectively. Five levels of 5 and 15 mM GB and 40 and 80 mM H<sub>2</sub>O<sub>2</sub> plus water (control) were randomly placed within each main plot as sub plots. All spray solutions, including the water treatment, were applied with 0.1% Tween-20 using a back-mounted pressurized sprayer that was calibrated to deliver 15 ml s<sup>-1</sup> at a pressure of 1 bar. External spray treatments were applied with three equal doses 34, 54 and 75 days after planting with a volumetric spray of 200 liters/feddan. Maize was planted on May 6 in both the 2021 and 2022 seasons in hills 25 cm apart by using yellow corn hybrid 368 (three way cross). The sub-plot was 14 square meters with 5 rows of 4 meters each, 70 cm apart. The previous crop was beans in 2021 and 2022 seasons. To ensure high germination rates, all test plots were irrigated with 27 mm fad<sup>-1</sup> of water at planting with an additional irrigation of 37 mm fad<sup>-1</sup> after 20 days to ensure full seedling development. Subsequently, sprinkler irrigation was applied every three days during planting. Twenty days after planting, the corn plants were thinned to one plant per hill. Agricultural practices were implemented in accordance with maize cultivation practices in this area.

**Table 1. Some physical and chemical properties of the soil in the experimental field as an average for the 2021 and 2022 seasons**

Soil depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture class	Bulk density (g cm <sup>-3</sup> )
0-60cm	68.17	23.45	4.08	4.30	Sand	1.52
Soil depth (cm)	Field capacity (%)	Wilting point (%)	pH	Organic matter (%)	EC (dS m <sup>-1</sup> )	
0-60cm	7.40	1.39	7.6	0.26	2.06	

**Table 2. The predicted monthly climatic data at Ismailia Governorate during the growing periods of corn in 2021 and 2022 seasons**

Mon.	Average temperature °C				Average RH (%)		Average wind speed (Km/hr)	
	Max.	Min.	Average				2021	2022
2021	31	18	28	26	40	45	12	13
May	31	18	28	26	40	45	12	13
June	33	20	29	30	44	46	13	14
July	37	23	31	32	44	45	12	13
Aug.	38	23	32	31	46	49	12	11
Sep.	34	21	29	29	53	53	10	11

Source: Meteorological Station of the Agricultural Research Center in Ismailia  
RH: Relative Humidity

### Sampling and Traits Studied

At harvest, yield and some of its components were estimated from the third and fourth row plants in each subplot.

- 1- Weight of 100 kernels in grams
- 2- Grain yield per feddan (kg). The grain moisture content was adjusted to 15.5%, and then the grain yield was estimated.
- 3- Protein content of kernels (%): It was estimated by multiplying the nitrogen content of grains by 6.25, while the nitrogen content of grains (%) was calculated using a modified microkijeldahl apparatus as shown by A.O.A.C (1980).
- 4- Irrigation Water Use Efficiency (IWUE) in kg/m<sup>3</sup>: It was calculated as follows:  $IWUE = GY / IR \times 100$ , where GY is the grain yield (kg/fad) and IR is the quantity of irrigation water used (m<sup>3</sup>/fad) for each of the three irrigation treatments.

### Statistical Analysis

All data were analyzed using the CoStat software, version 6.311 (CoHort software, Berkeley, CA 94701). Split plot in a randomized complete block design. Means followed by the same alphabetical letters are not statistically different according to Duncan's Multiple Range Test at the 5% level of significance (Duncan, 1955). To compare the differences between the means of the interactions, the least significant difference (LSD at  $P \geq 0.05$ ) was applied (Steel *et al.*, (1997).

### Results and Discussion:

#### Weight of 100 kernels (g)

The results obtained from Table 3 showed the effect of irrigation rates and foliar spray treatments, whether with glycine betaine or hydrogen peroxide, on the weight of 100 grains at harvest. This trait demonstrated significant responds for the two studied factors in both seasons. Regarding the effect of irrigation amounts, data revealed that increasing irrigation amount gradually and significantly increased the values of this trait in the two seasons. In comparison with severe water stress (60% ETc), the relative increasing percentages due to using full irrigation (100% ETc) were about 27% in 2021 and 2022 seasons. These results are largely consistent with those published by Abo-Marzoka *et al.*, (2016), Qasim *et al.*, (2019) and kotb *et al.*, (2021). The results of Table (3) explained that the foliar application either GB or hydrogen peroxide had very significant effects on the weight of 100 grains in both years. The maximum level of exogenous application of GB and hydrogen peroxide significantly gave the maximum weights of 100-grain in both seasons followed by the weights obtained from the lowest level of GB and hydrogen

peroxide in both seasons. Meanwhile, the lower values were obtained from untreated plants in both seasons.

From our data of the first season (Table 3), it appeared that using higher levels of GB or H<sub>2</sub>O<sub>2</sub> with good irrigation gave significantly higher 100-grain weights (31.34g and 31.29g), respectively, without significant differences between them. Meanwhile, untreated plants and treated by lower level of H<sub>2</sub>O<sub>2</sub> under full irrigation gave 26.03g and 27.93g in 2021 season insignificant differences between them, respectively. On the other hand, the lowest 100-grains weight (19.48g) was obtained from the interaction between severe water stress and without treatment. On average, using 15mMGB and 80mM H<sub>2</sub>O<sub>2</sub> protected about 7 grams of loss per 100 grain weight, under moderate drought (0.8ETc). Also, the same GB and H<sub>2</sub>O<sub>2</sub> levels under severe drought (0.6 ETc) maintained approximately 6 g of loss per 100 grain weight. It can be concluded that under all conditions (stress and non-stress), external spraying of these compounds on the leaves led to a significant improvement in the weight of 100 grains compared to normal plants that were sprayed with water. Our results for the weight of 100 grains are very similar to those obtained from Frage, (2020), Kotb *et al.*, 2021, Abd Alla Kotb and Gaballah (2007) and Shafiq *et al.*, (2021).

**Table 3. Effect of water stress (W) and foliar spray (S) treatments by GB and H<sub>2</sub>O<sub>2</sub> on 100-grain weight (g) of maize at harvest in 2021 and 2022 seasons.**

Items	2021				2022			
	W1	W2	W3	Mean	W1	W2	W3	Mean
S0	26.03	21.58	19.48	22.36 <sup>c</sup>	23.73	19.04	16.62	19.80 <sup>d</sup>
S1	27.93	26.49	22.94	25.79 <sup>b</sup>	25.16	23.09	19.41	22.55 <sup>c</sup>
S2	31.29	28.65	25.04	28.33 <sup>a</sup>	28.01	25.05	24.20	25.76 <sup>a</sup>
S3	30.88	25.96	22.60	26.48 <sup>b</sup>	27.80	23.96	21.41	24.39 <sup>b</sup>
S4	31.34	28.59	25.73	28.55 <sup>a</sup>	28.76	25.78	23.51	26.02 <sup>a</sup>
Mean	29.50 <sup>a</sup>	26.25 <sup>b</sup>	23.16 <sup>c</sup>		26.69 <sup>a</sup>	23.39 <sup>b</sup>	21.03 <sup>c</sup>	
Interaction (W x S)	L.S.D <sub>0.05</sub> =2.30				NS			

W1: well-watered (3100 m<sup>3</sup>/fad); W2: 0.8 W1, W3: 0.6 of W1. S0: untreated plants, S1:40mM H<sub>2</sub>O<sub>2</sub>, S2: 80mM H<sub>2</sub>O<sub>2</sub>, S3: 5mM GB, S4: 15mM GB

#### Grain yield/fad

The results in (Table 4) indicated the presence of highly statistically significant differences in the two seasons. Our results indicate that decreasing amounts of irrigation from 3100 to 1860m<sup>3</sup>/fad gradually and significantly decreased grain yield/fad in the two seasons. Compared with unstressed plants which had 2833.1 and 2626.5 kg/fad in both seasons, respectively, corn plants exposed to moderate and severe water stress showed a statistically significant reduction on grain yields (2395.2 and 2065.5 kg /fad in first season) and (2184.5 and 1872.2 kg/fad in second season).

Due to severe water stress, the reduction reached to about 27 and 29% in 2021 and 2022 seasons, respectively. To confirm the results we obtained, Aslam *et al.*, (2013) demonstrated that the different stages of corn growth are greatly affected by water stress, which leads to prolonged flowering and silk formation, decreased chlorophyll content, reduced photosynthesis and consequently a significant decrease in corn productivity. Muhammad *et al.*, (2001) also demonstrated that water stress significantly decreased the number of grains/ear, thousand-grain weight, and grain production. In the same vein, water stress had significant negative effects on the production of grains compared to these traits under unstressed conditions (Kotb *et al.*, (2009), Qasim *et al.*, (2019), Moharramnejad *et al.*, 2019 and kotb *et al.*, 2021).

Exogenous application of GB or H<sub>2</sub>O<sub>2</sub> to corn plants showed a significant increase in grain yield compared with untreated plants. So we can summarize that GB and H<sub>2</sub>O<sub>2</sub> application alleviated the adverse effect of water stress. Meanwhile, the maximum values of grain yield were significantly obtained from 15mM GB at harvest followed by the treatment of 80mM H<sub>2</sub>O<sub>2</sub> with significant differences between them (Table 4). Grain productions were significantly ameliorated by about 605 and 590 kg/fad in GB-treated plants in both seasons, respectively. Foliar spraying of glycine betaine also resulted in significant improvements in IWUE and grain production at suitable concentrations on wheat, barley, maize, and sorghum (Naryyar and Walia, 2004, Abd Alla Kotb 2005, Abd Alla Kotb and Gaballah 2007).

Our data showed that corn plants subjected to 0.6 ETc without GB or with 40mM H<sub>2</sub>O<sub>2</sub> significantly gave the minimum values of yield in ratio to the other treatments (Table 4). While in the case of good irrigation, the highest average grain yield values were obtained significantly using 15mM GB and 80 mM H<sub>2</sub>O<sub>2</sub> insignificant differences among them. On average over the two seasons, grain yields were improved by about 622 and 509 kg/fad in 15mM GB and 80mM H<sub>2</sub>O<sub>2</sub>-treated plants under normal irrigation. Also, 15mM GB and 80mM H<sub>2</sub>O<sub>2</sub> treatments protected about 770 and 663 kg/fad grains/fad from lose under 0.8ETc, in order. Under severe drought (0.6 ETc), the same level of GB and H<sub>2</sub>O<sub>2</sub> resulted in 400 and 290 kg/fed higher grain yields compared to the untreated ones. Application of GB and H<sub>2</sub>O<sub>2</sub> saved approximately 620 m<sup>3</sup>/fad of irrigation water without yield reduction. Confirming our findings, Hydrogen peroxide has been conclusively proven to play a pivotal role as a signaling molecule in photosynthesis, translocation, transpiration, and respiration and to buffer against numerous abiotic and biotic stresses, such as cold, drought, salinity and heat. It has also been proven that H<sub>2</sub>O<sub>2</sub> has positive and regulatory effects on the stages of plant growth, development, quality and

improving productivity (Zhou *et al.*, 2012). Also, Khandaker *et al.*, (2012) stated that fruit development could be improved by spraying the crop of wax apple with H<sub>2</sub>O<sub>2</sub>. While the results of both Guler and Pehlivan, (2016) confirmed that low dose H<sub>2</sub>O<sub>2</sub> pre-treatment alleviated water loss and H<sub>2</sub>O<sub>2</sub> content and increased drought stress tolerance by inducing the antioxidant system. Furthermore, wax apple fruits treated with 5 mM hydrogen peroxide gave raises the component of yield in comparison with untreated plants (Khandaker *et al.*, 2012) while, Geros *et al.*, (2012) reported that adding H<sub>2</sub>O<sub>2</sub> to fruit can improve the ripening process. In the same direction, under well-watered or water stress, foliar application yellow corn hybrid 352 (three way cross) by 60mM H<sub>2</sub>O<sub>2</sub> significantly improved 100-kernel weight, and grain production in ratio to untreated plants (Frage, 2020 and Kotb *et al.*, 2021). On the other hand, Exogenous application of GB on two maize cultivars improved plant growth, yield characteristics. The results obtained in this study demonstrate the role of foliar bio stimulants in enhancing the growth and yield of maize varieties by up regulating the oxidative defense system and accumulating osmotic stress-protective substances under water-deficit conditions (Shafiq *et al.*, 2021).

**Table 4. Effect of water stress (W) and foliar spray (S) treatments by GB and H<sub>2</sub>O<sub>2</sub> on grain yield (kg/fad) of maize at harvest in 2021 and 2022 seasons.**

Items	2021				2022			
	W1	W2	W3	Mean	W1	W2	W3	Mean
S0	2470.1	1913.3	1860.0	2081.1 <sup>d</sup>	2316.7	1693.3	1680.0	1896.7 <sup>d</sup>
S1	2641.2	2265.8	1976.8	2294.6 <sup>c</sup>	2421.3	2065.7	1697.0	2061.3 <sup>c</sup>
S2	3012.1	2582.2	2124.3	2572.9 <sup>b</sup>	2792.3	2349.0	1994.0	2378.4 <sup>b</sup>
S3	2916.7	2541.1	2107.3	2521.7 <sup>b</sup>	2697.0	2341.0	1911.0	2316.3 <sup>b</sup>
S4	3125.5	2673.3	2259.0	2685.9 <sup>a</sup>	2905.3	2473.3	2079.0	2485.9 <sup>a</sup>
Mean	2833.1 <sup>a</sup>	2395.2 <sup>b</sup>	2065.5 <sup>c</sup>		2626.5 <sup>a</sup>	2184.5 <sup>b</sup>	1872.2 <sup>c</sup>	
Interaction (W x S)	L.S.D <sub>0.05</sub> =120.50				L.S.D <sub>0.05</sub> =125.90			
W1: well-watered (3100 m <sup>3</sup> /fad); W2: 0.8 W1, W3: 0.6 of W1. S0: untreated plants, S1:40mM H2O2, S2: 80mM H2O2 , S3: 5mM GB, S4: 15mM GB								

#### Grain protein content (%)

The obtained results from Table 5 showed the effects of irrigation amounts and foliar spraying treatments either GB or hydrogen peroxide on grain protein content at harvest. This trait demonstrated significant responds for the two studied factors in both seasons. Regarding the effect of irrigation regime on this character, data revealed that gradually reducing irrigation rates led to a significant increase in the protein content of grains. In comparison with irrigation by 100% of ETc, the relative increasing percentages due to application

60% of ETc were about 25% and 17% for first and second seasons, respectively. Kotb *et al.*, (2009) confirmed that water deficit significantly decreased yield and its components of maize plants but increased grain protein content as compared to normal irrigation.

Our results of Table 5 explained that the exogenous application either GB or hydrogen peroxide had a significant impact on the protein content of the grain in first and second seasons. Meanwhile, the highest values of grain protein content were significantly gained by the exogenous application by highest level of GB followed by the lowest level of GB with significant differences between them in both seasons. The relative increasing percentages due to application 15mM GB were about 36 and 26% in both seasons, respectively. Meanwhile, untreated plants S0 gave the minimum values of this trait. In the same vein, using of 15mM ameliorated grain yield, grain N content and protein yield by 28.47 and 25.30%, 19.38 and 17.96% and 54.53 and 47.25% in comparison with untreated plants in the two seasons, in order (Kotb *et al.*, 2009).

The results in Table (5) revealed that using high concentrations of GB and H<sub>2</sub>O<sub>2</sub> with severe water stress significantly gave the highest grain protein content (15.43%) in first season. Meanwhile, the minimum grain protein content (9.53%) were taken from the interaction between untreated plants and well-watered. Under severe water stress, the foliar application of 15mM GB and 80mM H<sub>2</sub>O<sub>2</sub> significantly ameliorated grain protein content by approximately 35 and 19% in compared with untreated plants in 2021 season, respectively. It turned out that our results were in good agreement with those obtained by (Kotb *et al.*, 2009). H<sub>2</sub>O<sub>2</sub> has also been shown to have positive and regulatory effects on growth, development, quality and productivity of plant. (Zhou *et al.*, 2012).

**Table 5. Effect of water stress (W) and foliar spray (S) treatments by GB and H<sub>2</sub>O<sub>2</sub> on grain protein content (%) of maize at harvest in 2021 and 2022 seasons.**

Items	2021				2022			
	W1	W2	W3	Mean	W1	W2	W3	Mean
S0	9.53	10.17	11.43	10.38 <sup>c</sup>	9.80	10.33	11.70	10.61 <sup>c</sup>
S1	10.10	10.57	13.00	11.22 <sup>d</sup>	10.10	11.47	12.73	11.43 <sup>d</sup>
S2	10.80	12.43	13.63	12.29 <sup>c</sup>	11.43	12.30	13.40	12.38 <sup>c</sup>
S3	11.43	13.33	14.37	13.04 <sup>b</sup>	12.03	13.00	13.57	12.87 <sup>b</sup>
S4	12.53	14.47	15.43	14.14 <sup>a</sup>	12.73	13.17	14.23	13.38 <sup>a</sup>
Mean	10.88 <sup>c</sup>	12.19 <sup>b</sup>	13.57 <sup>a</sup>		11.22 <sup>c</sup>	12.05 <sup>b</sup>	13.13 <sup>a</sup>	
Interaction (W x S)	L.S.D <sub>0.05</sub> =0.51				NS			

W1: well-watered (3100 m<sup>3</sup>/fad); W2: 0.8 W1, W3: 0.6 of W1. S0: untreated plants, S1: 40mM H<sub>2</sub>O<sub>2</sub>, S2: 80mM H<sub>2</sub>O<sub>2</sub>, S3: 5mM GB, S4: 15mM GB

### Irrigation water use efficiency (IWUE)

The effects of irrigation rates and foliar spray (S) treatments by GB and H<sub>2</sub>O<sub>2</sub> and their interactions on irrigation water use efficiency were significant in both years (Table 6). IWUE significantly increased in response to severe water stress (1.110 and 1.110 kg/m<sup>3</sup>) or moderate stress (0.966 and 0.881 kg/m<sup>3</sup>) relative to controls (0.914 and 0.847kg/ m<sup>3</sup>) in both years, respectively. Also, foliar application of high GB level significantly ameliorated IWUE in both years followed by the highest level of H<sub>2</sub>O<sub>2</sub> with significant differences between them compared to their untreated counterparts. Under severe water stress treatments, the data recorded in revealed that using 15mM GB gave highly significant values from IWUE (1.215 and 1.118 kg/m<sup>3</sup>) followed by the values obtained from 80mM H<sub>2</sub>O<sub>2</sub> in 1<sup>st</sup> season and 2<sup>nd</sup> season relative to the other treatments, respectively. Generally, under good irrigation and water stress conditions, foliar application of GB and H<sub>2</sub>O<sub>2</sub> on yellow corn hybrid 368 (three way cross) significantly ameliorated IWUE in both seasons. It can be summarized that the water use efficiency of plants was better when GB and H<sub>2</sub>O<sub>2</sub> were sprayed on plants under stress conditions as well as good irrigation. Our previous results were in good agreement with those published by Kotb *et al.*, 2009, Kotb and Elhamahmy, 2013, Kotb, 2014 and Kotb *et al.*, 2021).

**Table 6. Effect of water stress (W) and foliar spray (S) treatments by GB and H<sub>2</sub>O<sub>2</sub> on Irrigation water use efficiency (IWUE) in kg m<sup>-3</sup> of maize at harvest in 2021 and 2022 seasons**

Items	2021				2022			
	W1	W2	W3	Mean	W1	W2	W3	Mean
S0	0.797	0.772	1.000	0.856 <sup>d</sup>	0.747	0.683	0.904	0.778 <sup>d</sup>
S1	0.852	0.914	1.063	0.943 <sup>c</sup>	0.781	0.833	0.912	0.842 <sup>c</sup>
S2	0.972	1.041	1.142	1.052 <sup>b</sup>	0.901	0.947	1.072	0.973 <sup>b</sup>
S3	0.941	1.025	1.133	1.033 <sup>b</sup>	0.870	0.944	1.027	0.947 <sup>b</sup>
S4	1.008	1.078	1.215	1.100 <sup>a</sup>	0.937	0.997	1.118	1.017 <sup>a</sup>
Mean	0.914 <sup>c</sup>	0.966 <sup>b</sup>	1.110 <sup>a</sup>		0.847 <sup>b</sup>	0.881 <sup>b</sup>	1.007 <sup>a</sup>	
Interaction (W x S)	L.S.D <sub>0.05</sub> =0.035				L.S.D <sub>0.05</sub> =0.030			

W1: well-watered (3100 m<sup>3</sup>/fad); W2: 0.8 W1, W3: 0.6 of W1. S0: untreated plants, S1: 40mM H<sub>2</sub>O<sub>2</sub>, S2: 80mM H<sub>2</sub>O<sub>2</sub>, S3: 5mM GB, S4: 15mM GB

### Conclusion

our results indicate that drought significantly reduced maize plant production. While the external application of GB and H<sub>2</sub>O<sub>2</sub> at appropriate levels improved grain protein content and irrigation efficiency in maize plants. Under water stress conditions, the application of high levels of GB and H<sub>2</sub>O<sub>2</sub> gave positive results, indicating that the used non-enzymatic antioxidants played an important role in mitigating the negative effects of water

stress, thus improving growth, productivity and IWUE under the new soil conditions.

#### Consent for publication:

All authors declare their consent for publication.

#### Author contribution:

The manuscript was edited and revised by all authors.

#### Conflicts of Interest:

The author declares no conflict of interest.

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