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The role of osmotic and hormonal modification in the physiological and anatomical traits and water use efficiency of bread wheat (*Triticum aestivum* L.) grown under drought conditions

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Abstract

A field experiment was done on wheat crop (*Triticum aestivum* L.) in the experimental farm field of the Guidance and Training Center in Babylon, 81 km north of Babylon, on the wheat crop in latitude (^{O}32.61 North) and longitude (^{O}44.30 East) above sea level. during the winter growing season of 2025-2026, with the aim of supporting the wheat crop by providing a package of components to withstand drought stress and the stresses associated with and arising from it. A randomized complete block design (RCBD) with a split plot design was employed and three replications were used. The most important factor was water stress with the control treatment (S_1) using 50% of the water available, and 75% of irrigation water plus 50% of the water (S_2) added to the control treatment. These were given the symbols S1, S_2 and S_3, respectively. Subplot treatments were: spraying plants with glutamic and citric acids (T_1), soil injection with a baker's yeast extract, Saccharomyces cerevisia (T_2), the antitranspirant, kaolin (T_3), a combined treatment (T_4) comprising all the above, and a control treatment (T_5) in which only distilled water was sprayed onto the plants. The water stress treatment S_1 was significantly superior for all the studied traits compared to the 75% and 50% of the added water from the control treatment which were the lowest average treatments. In the same way, the average seasonal water consumption at treatment S_1 was 374.25 mm whereas the water consumption for the remaining two treatments were 303.00 mm and

231.75 mm, respectively. Except for water use efficiency, where the 75% and 50% tensile treatments excelled the control treatment, with averages of 1.49 and 1.55 kg m^{-3}, respectively, compared to the control treatment S_1, which had the lowest average at 1.44 kg m^{-3} .

Treatment T_4 significantly excelled treatment T_2 for most of the studied traits, with no significant difference between them, except for anatomical traits. All treatments combined significantly improved anatomical traits, with no significant difference between them, as they all contributed to reducing the average length and width of the stomatal opening of the flag leaf and recorded the lowest averages, compared to treatment T_5, which recorded the highest averages for these traits at 26.68 and 2.26 µ.

Keywords: Wheat, water tension, osmotic modification, hormonal modification, water use efficiency.

Introduction

Wheat (*Triticum aestivum* L.) is a strategic crop crucial to global food security and is cultivated on vast areas. It ranks first in terms of cultivated area and production among cereal crops, and most agricultural studies and research have focused on it due to the pressing need for it resulting from the steady increase in population.[11] Irrigation from Tigris and Euphrates is very crucial for wheat production in Iraq, especially in the center-south. Generally, the agricultural share of water from these two rivers is decreasing due to increasing human and industrial water use, as well as the policies of Iraq's neighboring countries, which have begun to propose what is called water redistribution in the region. This compels us to seriously consider how to utilize available water resources scientifically and thoughtfully to reduce waste resulting from misuse. Furthermore, we must invest in all possible means to increase the development and production of this crop, especially in conditions of water scarcity, accompanied by rising temperatures and low rainfall. The absence of modern irrigation technologies and the lack of strategic planning in the long-term are all risks associated with wheat cultivation, with soil water availability being regarded as a key factor. One of the main determinants of this crop's production, located in the arid and semi-arid regions of the world, which suffer from low and fluctuating annual rainfall [4], necessitates the use of field methods and practices derived from scientific research to mitigate the negative impacts of this effect. These include accurately estimating the crop's water requirements to increase water use efficiency according to actual needs, thereby allowing for the expansion of agricultural areas, especially in lands with limited water resources [2].Other field practices that reduce water consumption for this crop include soil and plant osmotic and hormonal modification.

These include the use of mechanical solutions to reduce water loss and the spraying of antitranspirants, such as kaolin, to minimize water loss from the plant. Antitranspirants partially control stomatal closure by forming a layer on leaf surfaces, increasing water use efficiency and the relative water content of the leaves when soil moisture is low. Amino acids, which are biostimulants, also influence the plant's enzymatic activity and play a vital role in cell membrane formation, nucleotide synthesis, enzyme and vitamin production, and growth regulation, while also promoting vegetative growth. The root and strongly help in resisting environmental stresses such as drought, salinity and heat, in addition to its role in nitrogen assimilation and protein synthesis. Citric acid stimulates photosynthesis and increases carbohydrate synthesis and accumulation, as it is effective in the growth and development of the morphological and physiological characteristics of the plant [16]. The amino acid glutamic acid, which is an organic carboxylic acid and is the first amino acid formed after nitrogen absorption, improves nitrogen metabolism and helps in the absorption of other amino acids. Spraying the plant with it leads to a decrease in the content of proline and antioxidant enzymes because it scavenges reactive oxygen species harmful to the plant [17].Biofertilizers have gained significant attention in recent years due to their low environmental pollution and the increased diversity of microorganisms they contain, which play a major role in enhancing soil fertility. Baker's yeast, for example, promotes vegetative growth and the production of growth regulators such as cytokinins, auxins, and gibberellins, while also increasing chlorophyll content [19]. Therefore, the presence of any one of these components alone offers a partial solution to the problem, while the combined presence of these components provides a complementary approach to mitigating stresses resulting from drought. The aim of this study were:

To approximate the water requirement of wheat for various stresses during the season.

-To deliver a group of parts which allow the plant to resist water stress.

Materials and Methods

The experiment was carried out over the winter of 2025-2026 in Al-Sadda Al-Hindiya district of Al-Sayed Abu Al-Hudain region of the experimental farm in Al-Mahnawiya area of the Babylon province. This farm belongs to a training and extension center in Babylon, 81 km north of Babil city, Iraq, at altitude of 32.61 N, 44.30 E above sea level. This study had two factors. The first factor was at three levels of water stress: 50% (control treatment), 75%, and 50% (control treatment). These levels were in the main plot and were symbolized as S1, S2 and S3 respectively. The following was added as a subplot:

T_1: Spraying the plants with glutamic acid and citric acid.

T_2: Inject the soil with yeast extract (baker's yeast, Saccharomyces cerevisia) at a concentration of 8 g/L, applied at a rate of 20 ml/plant, and injected to a depth of 15-20 cm.

T_3: Use the antitranspirant kaolin at a concentration of 6%.

T_4:Combine the above treatments T_1, T_2, and T_3 (the substances were added sequentially to avoid any reaction between them if mixed together).

T_5: Spray with distilled water only.

The spraying process was conducted twice: once at elongation and again at flowering.

Random soil samples were taken from the field before planting, from different locations and depths ranging from 0 to 30 cm. The samples were then mixed, homogenized, air-dried, finely ground, and passed through a sieve with 2 mm openings. A homogeneous sample was taken for analysis to determine its physical and chemical properties at the Central Laboratory for Soil Analysis – General Authority for Agricultural Research/Abu Ghraib, as shown in Table (1).

The experimental plot was plowed twice perpendicularly using a moldboard plow, and the smoothing process was conducted using disc harrows. Then the leveling process was conducted and the plots were divided. The area of the plot was 2 x 3 = 6 m^2. Phosphate fertilizer in the form of triple superphosphate containing 45% P_2O_5 was added to the soil according to the recommendation of 80 kg.ha-1 a few days before planting when preparing the soil [13]. Urea fertilizer was used as a nitrogen source (N 46%) and was added in three equal doses: the first immediately after emergence, the second at the beginning of the tillering stage, and the third at the flowering stage (50%) at a level of 120 $kg.ha^{-1}$ [14]. Seeds of Mawada variety were sown on November 22, 2025, and weeding was conducted as needed. Harvesting took place after the plants reached full maturity on May 5, 2026. A randomized complete block design (RCBD) with a split-plot arrangement and three replications was used. Each replication was divided into 15 experimental units, for a total of 45 experimental units for the entire study. A 2-meter distance was maintained between replications to create irrigation channels and paths. Approximately 0.75 meters were left between experimental units to ensure no overlap between treatments. The plots were then lined with 10 rows, 2 meters each, spaced 25 centimeters apart.

Table (1) Some Physical and Chemical Properties of the Field Soil

traits		values
Soil separators	Sand	**50**
	Clay	**31**
	silt	**19**
pH		**7.61**
Soil texture EC($ds.m^{-1}$)		**Silty-clay- sandy**
	Apparent density $Mg.m^{-3}$	**1.33**
elements available	**N**	**25.33**
	P	**7.2**
	K	**205**
Organic matter (%)		**1.4**
Field capacity		**0.432**
Permanent wilting point cm3 cm-3		**0.228**
Available water		**0.204**

Available water content in the soil was estimated by calculating the difference between the moisture content at 33kPascals (field capacity) and 1500kPascals (PWP) of the soil, assuming that the relationship between the structural tension of the soil sample and the moisture content at the listed tensions (33, 100, 500, 1000, 1500, 2000 and 5000) kPascals is known (Figure (1)).

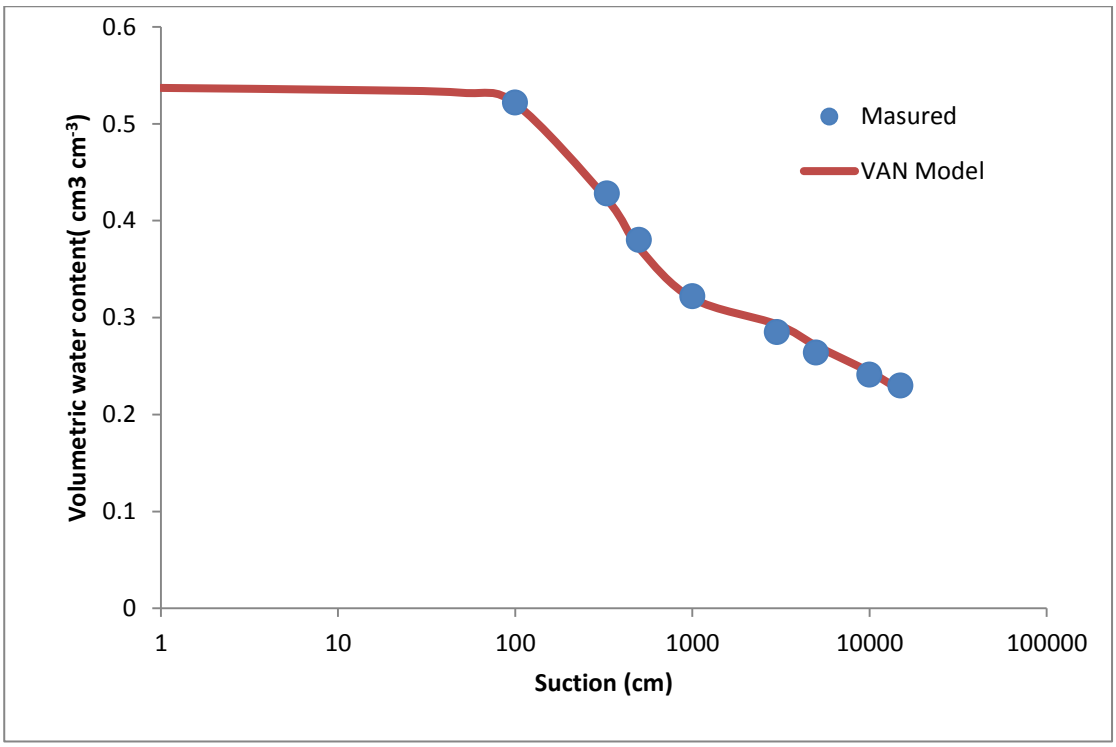

Figure (1). Shown that Soil Moisture Characteristic Curve for the Experiment.

Irrigation Method:

Irrigation was done through plastic pipes attached to a constant flow electric pump. A meter was fastened to the pipe to see how many litres of water was flowing through it. All plots were irrigated at planting (first irrigation) and up to field capacity to obtain field emergence with equal amounts of water.

Method for Measuring Soil Moisture Content:

The volumetric method was used to measure soil moisture content. Before irrigation, soil samples were taken to the depth of 30 cm with core sampler. Then the sample was weighed immediately by the wet weight method. It was then microwaved for 10 minutes to dry it out and weighed again after drying. Then the moisture content was estimated by using Equation [12].

$$PW = \frac{MW - DW}{DW} \times 100$$

Where: DW * = Dry weight (g), PW * % = Moisture content based on dry weight, MW * = Wet weight (g)

Then the volumetric moisture content was calculated based on the bulk density according to equation [15]:

$$Qv = Qw \times \partial b$$

Where: Qw = Weight moisture content, Qv = Volumetric moisture content and ∂b = Apparent density of the soil (mg/m³).

The plants were irrigated when 50% of the available water was depleted, as monitored by the soil moisture profile curve (Figure 1). Immediately after planting, the plants were irrigated again using plastic pipes, based on the depletion of their moisture content and the replenishment of the soil to field capacity. The depth of added water was calculated using the moisture profile curve (Figure 1) and equation [6].

$$d = (\theta f.c - \theta w)D$$

Where: D = depth of root system (mm), d = depth of added irrigation water (mm), θf.c = volumetric moisture at field capacity ($cm^3\ cm^{-3}$), Θw = volumetric moisture before irrigation ($cm^3\ cm^{-3}$)

Then, the volume of water in liters was calculated based on the area of the experimental unit using the following equation:

$$VW = d \times A$$

where:

VW = volume of water (liters)

d = depth of added water (mm)

A = area of the experimental unit (m^2)

The collected and tabulated data were then analysed by the available-made statistical software GenStat V.20, according to the ANOVA table used in the previous work [1]. The means were compared using Least Significant Difference test at 0.05 probability level [13].

Preparation of Concentrations:

1- Spray Treatments: Glutamic acid (250 $mg.L^{-1}$) and citric acid (50 $mg.L^{-1}$) were prepared in small flasks using a magnetic stirrer hotplate to ensure complete dissolution. The volume was then increased to 1 liter with distilled water. Kaolin (6%) was used as an antitranspirant. The plants were sprayed twice: once during the vegetative growth stage and again at the beginning of flowering.

The control group was sprayed with distilled water only. The spraying process continued until the plants were wet. A surfactant was added before spraying to reduce the water's surface tension.

2- Soil Treatment: For this treatment, baker's yeast at a concentration of 8 g/L was used. It was a commercially available dry yeast of Turkish origin. It was dissolved in warm water and mixed with 1 g of sucrose to activate it. It was then placed in an incubator at 37°C for two hours. The yeast was injected into the soil at a rate of 20 ml only once during the completion phase of the research at the beginning of the planting, as it is a biological agent capable of multiplying to a equilibrium stage with the medium. The injection was administered at a depth of 20 cm [19].

Study traits:

- **Plant Height (cm):** This was measured from the soil surface to the base of the spike of the main branch using a metal tape measure. The average of ten randomly selected plants was taken from the midlines of each experimental unit.

- **Flag Leaf Area (cm^2):** Ten plants were randomly selected from the midlines of each experimental unit according to the equation adopted by [20] as follows:

Flag Leaf Area = Flag Leaf Length × Maximum Width × Correction Factor (0.95).

- **Flag leaf chlorophyll index (SPAD):**

This property was estimated using a Japanese-made SPAD Model-502 Chlorophyll Meter, as the average of five readings per flag leaf from a random sample of ten plants in the experimental unit [28].

- **Relative water content in flag leaves (%):** Estimated using the following equation:

$$R.W.C = \frac{FW - DW}{TW - DW} \times 100$$

Where:

R.W.C = represents the relative water content.

FW = represents the fresh weight (g).

DW = represents the dry weight (g).

TW = represents the heaped weight (g).

Some new leaves were cut off and put into nylon bags to avoid moisture loss. Immediately after cutting and after 12-14 hours in distilled water at room temperature and under light, they were weighed. The leaves were then dried with the help of blotting paper and the dried leaves were weighed again and the heaped weight was recorded. Lastly, they were dried in an oven at 85°C until the weight became constant and the dry weight was determined.[22]

- Plasma membrane stability index in flag leaf: a sample of ten plants was collected from the flag leaf at each experimental unit before irrigation and at the flowering stage in

order to determine the stability of the flag leaf plasma membranes.The sample was cut into small pieces with a diameter of 2 cm, then 20 pieces were removed, washed several times with distilled water, and divided into two bottles, each containing 10 pieces. Then 20 ml of distilled water was added to them, and they were left on the shaker for 24 hours. This represents C_2. As for the second bottle, it was placed in an autoclave at 120°C for 20 minutes, which represents C_2. Then the aqueous solution was extracted, and the electrical conductivity (EC) of both C1 and C_2 was measured at room temperature according to [23], according to the following equation:

$$MSI = 1- (EC_1 /EC_2) \times 100$$

Where:

MSI: Leaf cell membrane stability index.

EC_1: Electrical conductivity of the first bottle.

EC_2: Electrical conductivity of the second bottle.

- **Number of grain.spike (grain per spike^{-1}):** Calculated as the average of ten spikes from a random sample taken for each experimental unit.

- **Weight of 1000 grains (g):** Randomly taken from the grain yield for each experimental unit, weighed, and returned to the yield.

- **Grain yield (ton.ha^{-1}):** Detached from the harvested plants for an area of 1 m², weighed, and returned to the yield.

- **Actual water consumption ETa**: The water balance equation was used to calculate the actual crop water consumption [6]

$$\Delta S = (I+P+C) – (ETa+D+R)$$

where: C: Capillary rise (mm) assuming 0 because the groundwater depth is more than 1 meter. I: Depth of added irrigation water (mm). P: Rainwater depth (mm), ETa: Actual evapotranspiration (mm). D: Drainage depth (mm) assuming 0. Deep seepage losses = (0). R: Surface runoff (mm) assuming 0 because the slabs are flat and bounded by shoulders that do not allow surface runoff.

Change in soil moisture content at the beginning and end of the season (ΔS) = 0 because– the soil moisture content at the beginning of the season is similar to the soil moisture at the end of the season. Thus, the water consumption equation is:

$$ETa = I + P$$

-**Water efficiency for grain yield (WUE) (kg m^{-3}):** as calculated using equation [10].

$$W.U.E = \frac{GY}{ETa}$$

Where: WUE = Water use efficiency (kg m^{-3}), GY = Grain yield (kg ha^{-1}), ETa = Total water consumption of the crop (m^3 ha^{-1}).

-**Number of stomata per flag leaf (stomata mm²):** Calculated for three flag leaves and ten readings per experimental unit using a

graduated lens divided into squares of known area, as follows:

Average number of stomata per mm² of epidermis = Average number of stomata divided by the area of the microscopic field.

- Stoma length and width (µ): Based on the average of ten readings. For the leaves where the number of stomata was measured, the dimensions of the stoma opening (stomata length and width) were measured using an ocular micrometer after calibration under the microscope to average the ten readings for the flag leaf. The stripping method was used on whole leaves (flag leaves) at the 100% flowering stage. The underside of the leaf was cleaned to remove any adhering dust. Cross-sections were then taken from the fresh leaf samples, and the number of stomata and stomatal dimensions (stomata length and width) were measured according to method [24]. These measurements were performed in the laboratories of the College of Science, University of Baghdad.

Results and Discussion:

1 -Plant Height (cm):

Results in Table (2) show that the two study factors and their interaction had significant effect on stem height.The table indicates that the control (S_1) was excelled with average height of 86.90 cm while the other two tension treatments reduced the height of the stems. This is because under drought conditions, the number of cells and their size decreases, and their elongation is inhibited [25]. Moreover, low soil moisture reduces the uptake of nutrients, especially nitrogen, known to be important for cell elongation [5].Average lengths of 84.75 cm were recorded in treatment T_4 which was significantly larger than treatments T_3 and control treatment (averages 84.22 and 83.37 cm, respectively), but was not significantly different from treatments T_1 and T_2 (averages 84.58 and 84.38 cm, respectively). The combination of the treatments seems to have a synergistic effect, making the plant more tolerant to water stress. This was shown by the increase in stem length which was stated to be an indicator of plant growth. Some balance in the plant's internal physiological regulation was obtained by the combination of additives, which is disturbed by drought. This latter aspect can be considered a stress absorber due to the numerous other stresses that accompany drought. Moreover, the effect of the amino acid glutamic acid increased the amount of chlorophyll synthesized and decreased the cell's osmotic potential (made it more negative upon spraying) when the plant was sprayed with glutamic acid and citric acid. It was the treatment T_4, which was the most effective and its combination of stress-resistance factors that significantly outstood the others in the water stress interaction, showing its high effectiveness**.**

Table (2) the effect of the two study factors and their interaction on plant height (cm).

Water tension levels	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	
S_1	89.25	88.98	82.75	89.42	84.08	86.90
S_2	84.52	84.42	80.08	84.75	82.42	83.24
S_3	79.98	79.75	75.08	80.08	77.75	78.53
average	84.58	84.38	79.31	84.75	81.42	
LSD	Water tension 1.37		Osmotic and hormonal modification 0.55			Interaction 1.43

.4-

Leaf Area Index (cm^2)

From the results presented in Table (3) it is observed that the two factors of the study and their interaction have significant effect on the flag leaf area.

As the water stress increased the amount of leaf area decreased significantly until the highest degree of water stress (S_3) which had an average of 40.43 cm^2. Water shortage causes a decrease in leaf area because of stomatal closure, leaf curling and reduction in photosynthesis. This is not only because of reduced availability of photosynthetic materials for growth, but because of the increased free radical production under water stress and a change in cell membrane permeability due to physical factors such as reduced swelling pressure required for growth. These factors act together to diminish the vegetative growth – in particular the leaf area – which is a drought sensitive factor. This is in line with previous studies [26].Table shows that flag leaf area was highest in treatment T_2 (46.34 cm^2) than the least in T_5 (control) (39.72 cm^2). This is due to the high protein content, vitamin B, thiamine, riboflavin and pyridoxine content of the yeast, which are also natural growth promoters. Moreover, yeast secretes vitamins, amino acids, cytokinins, auxins, gibberellins etc., which have stimulatory effects on cell division [27].Significant differences between the two types of study factor and interaction. $S_2 \times T_2$ combination gives highest mean leaf area as 46.50cm^2 which is not significantly different than $S_3 \times T_5$ combination but significantly different than $S_3 \times T_1$ combination. The areas were lowest for both combinations (39.17 cm^2).

Table (3) shows the effect of the two study factors and their interaction on flag leaf area (cm^2).

Water tension levels	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	
S_1	41.17	48.70	45.83	43.17	39.83	43.74
S_2	41.50	46.50	45.77	43.50	40.17	43.49
S_3	39.17	43.83	40.17	39.83	39.17	40.43
average	40.61	46.34	44.16	42.17	39.72	
LSD	Water tension 1.40		Osmotic and hormonal modification 1.46			Interaction 2.47

3-

Flag Leaf Index of Chlorophyll (SPAD):

The data presented in Table (4) showed that both study factors and interaction of the two factors had significant effect on flag leaf index of chlorophyll.

It is observed from the table that as the water stress increases the flag leaf index of chlorophyll decreases and it is maximum under (S_3) stress treatment with an average value of 38.71 (SPAD). Soil moisture reduction has a negative impact on the plant as well as its pigment, including chlorophyll. The pigment can break down because of the action of the chlorophyllase enzyme, the removal of the magnesium atom by the dechelalase enzyme, the breakdown of the porphyrin ring by the dioxygenase enzyme, and the oxidation of the iron by the iron enzyme .This is also done by the iron oxidase enzyme. As can be seen in the table, the treatment T_2 significantly outperformed T_4 according to the chlorophyll flag leaf index (SPAD) no different than T_4, with averages of 45.86 and 45.73 respectively. This is compared with the lowest average of 40.09 (SPAD) with the control treatment. The yeast raises the level of macro and micronutrients including iron and magnesium that are involved in the synthesis of chlorophyll molecules. It also raises the level of nitrogen, phosphorus and potassium in the leaves. In addition, the growth regulators secreted by the yeast, like cytokinins, have a function in the process of photosynthesis and in the protection of the chlorophyll from degradation. These cytokinins delay senescence of leaves by being involved in the formation of chlorophyll molecules and by controlling protein and RNA synthesis.[29]

Treatment T_2 showed significantly higher flag leaf chlorophyll index than did treatment T4 and was not significantly different than treatment T_4. There was also a large interaction between the two study factors, as indicated by the table. The $T_2 \times S_1$ combination had the highest mean score of 49.68 (SPAD) while the $T_5 \times S_3$ and $T_3 \times S_3$ combinations had the lowest mean score of 35.75 (SPAD) for this trait.

Table (4) shows the effect of the two study factors and their interaction on the science leaf index (SPAD) measured from the chlorophyll.

Water tension levels	**Osmotic and hormonal modification treatment**					**average**
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	
S_1	**47.38**	**49.68**	**44.58**	**49.45**	**44.81**	**47.18**
S_2	**44.31**	**45.98**	**41.71**	**45.81**	**39.71**	**43.51**
S_3	**38.21**	**42.35**	**35.75**	**41.95**	**35.75**	**38.71**
average	**43.3**	**45.86**	**40.68**	**45.73**	**40.09**	
LSD	**Water tension 0.75**		**Osmotic and hormonal modification 0.59**			**Interaction 1.07**

4

- Relative Water Content in Leaf:(%)

The effect of the two factors of the study and their interaction on the relative water content of leaves is significantly different as revealed by the results in Table.(5)

As the level of water stress was increased gradually, the relative water content of the leaves decreased gradually as compared to the treatment S_1 (the controlled treatment) whose average leaf relative water content was the highest, when the data were presented (Table 1). This is because as the water stress increases, there is a decrease in water potential of soils and thus, a decrease in water uptake capacity of the roots and an imbalance in water absorption and transpiration which has a negative effect on the plant water status [30]. Additionally, the reduced plant cover in treatment S_3 , due to the smaller plants, may be the reason for this, leading to increased evaporation from the soil surface, which negatively impacts the plant.Treatment T_4 showed a significant improvement over T_2 and T_1 with averages of 82.94%, 82.57%, and 82.50%, respectively, while the lowest average was observed for treatment T_5 with an average of 76.61%. This is likely to be due

to their effect on internal physiological factors like the permeability of membranes and to their stimulatory effect on root development.Note that there are also significant differences between the two experimental factors (rows and columns) in the table. Treatment T_4 outperformed all other water stress treatments while the other treatment (T_5) yielded the lowest averages in all water stress treatments.

Table (5) The effect of the two study factors and their interaction on relative water content (%) of science leaves

Water tension levels	**Osmotic and hormonal modification treatment**					**average**
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	
S_1	**84.83**	**84.50**	**83.50**	**85.17**	**81.17**	**83.83**
S_2	**82.23**	**82.37**	**77.50**	**82.50**	**76.17**	**80.15**
S_3	**80.43**	**80.83**	**73.83**	**81.17**	**72.50**	**77.75**
average	**82.50**	**82.57**	**78.28**	**82.94**	**76.61**	
LSD	**Water tension 1.84**		**Osmotic and hormonal modification 1.43**			**Interaction 2.60**

5-

Plasma membrane stability index in flag leaf :

Table (6) shows a significant effect of water tension levels, while the Osmotic and hormonal modification treatment and the interaction between the two factors were not significant .The table shows that the control treatment (S_1) was excelled, with no significant difference from the tension treatment (S_2), with averages of 55.49 and 55.12 respectively. In comparison, the tension treatment (S_3) had the lowest average at 47.24. This may be because increased water tension leads to increased ROS, the most significant damage of which is the oxidation of the cell's plasma membranes, as well as the membranes of cell organelles, thus damaging the plasma membranes [31].

Table (6) shows the effect of the two study factors and their interaction on the plasma membrane stability index in flag leaf.

Water tension levels	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	
S_1	53.81	54.05	56.99	52.42	57.20	55.49
S_2	50.95	55.98	56.95	52.44	57.30	55.12
S_3	46.59	50.33	39.30	47.38	52.61	47.24
average	40.45	53.45	51.08	50.75	55.70	
LSD	Water tension 4.56		Osmotic and hormonal modification no			Interaction no

6-

Number of grain.spike (grain.spike^{-1}):

The results in Table (7) show a significant effect of water stress, osmotic adjustment, and hormonal treatment, but no significant interaction effect on grain per spike trait.

The table shows that the control treatment S_1 was significantly excelled, with an average of 48.63 grain.spike^{-1} compared to the other treatments, which showed a decrease in the average of this trait. This decrease may be due to the fact that water stress, when applied during the later stages of plant growth (node elongation and the tillering stage, before spike emergence), affects the growth of florets and spikelets, potentially causing floret abortion. This result is consistent with [32], who demonstrated that the low number of grain.spike^{-1} is related to the availability of irrigation water before and during the flowering stage.

The table also shows that treatment T_4 was excelled treatment T_2, with averages of 47.96 and 47.05 grain.spike^{-1} respectively, compared to the control treatment T_5, which had the lowest average of 39.19 grain.spike^{-1} . The combined treatments achieved a significant increase in this trait, supporting the research hypothesis that combining multiple stress tolerance factors yields better results than considering individual factors.

Table (7) shows that the study factors and their interaction significantly affected the grain.spike^{-1}.

Water tension	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	

levels						
S_1	48.95	50.10	47.33	51.58	45.21	48.63
S_2	44.25	47.77	41.55	47.96	38.55	44.07
S_3	40.86	43.29	40.35	44.34	43.29	40.52
average	44.69	47.05	43.06	47.96	39.19	
LSD	Water tension 2.07		Osmotic and hormonal modification 2.27			Interaction no

7-

Weight of 1000 grains (g):

Table (8) shows a significant effect of water stress, osmotic adjustment, and hormonal adjustment treatments, while the interaction between these treatments had no significant effect.

The interaction between these treatments was not statistically significant but there was significant effect of water stress, osmotic adjustment and hormonal adjustment treatments (Table (8)). The average weight of 1000 grains for the average of the other treatments was smaller than that of the control treatment (S_1) with an average weight of 38.43 g. This loss was due to the negative response to water stress which caused a reduction in vegetative growth and hence flag leaf area, as shown in Table 3. Such a reduction in flag leaf area (vegetative growth) reduced the ability of the flag leaves to intercept light and thus their vital role in providing the grains. This in turn resulted in less dry matter that is later remobilized to the grains and consequently less grain size. This is particularly true in the presence of high temperatures, low relative humidity and high wind speeds that hasten leaf senescence and lower their capacity to produce photosynthetic products for the grain, which in turn results in grain weight loss [7].It is observed from the table that treatment T_2 was superior in which the average weight was 38.53 g and there was no significant difference between the two treatments T_2 and T_4 with an average weight of 38.12 g respectively. This was in contrast to the control treatment T_5 that had the lowest mean weight for this trait of 34.15 g. This could be related to the provision of several mineral nutrients and amino acids by the yeast treatment T_2 and T_4 and additional amino acids to support osmotic adjustment, and hormones to overcome hormonal imbalance in the plant. These include enhanced ABA and ethylene as growth inhibitors and as aging agents, and destruction of chlorophyll and nucleic acids due to water stress. In addition, they were able to reduce transpiration mechanically, but did not study the molecular basis of the regulation.

Table (8) shows that the two factors and their interaction affected the weight of 1000 grains

Water tension levels	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	
S_1	37.90	40.40	37.76	39.60	36.50	38.43
S_2	35.33	38.66	35.03	38.33	34.30	36.33
S_3	34.33	36.53	34.00	36.43	31.67	34.59
average	35.85	38.53	35.60	38.12	34.15	
LSD	Water tension 0.40		Osmotic and hormonal modification 0.60			Interaction no

8

- Grain Yield (tons.ha^{-1}):

As seen in Table (9) the two study factors and their interaction have significant effects.The results indicated that treatment S_1 (control) significantly excelled with the highest average grain yield of 5.38 tons.ha-1 while treatment S_3 had the lowest average grain yield of 3.60 tons.ha^{-1}. The reduction in grain yield resulting from the higher water stress could be due to reduced yield components. Due to water stress, the number of grain.spike^{-1} and the weight of 1000 grain decreased (Tables 7 and 8) which also resulted in a decline in grain yield. In addition, the combination of water deficit, high temperature and low relative humidity at the grain filling stage led to a reduction in the amount of dry matter that was ultimately deposited in the grain. All of these factors have a negative influence on the yield [33]. It was also found that significantly excelled treatment T_4 (with an average yield of 5.00 tons.ha^{-1}) is better than the control treatment T5 with an average yield of 3.66 tons.ha^{-1}. This is in line with the research hypothesis which states that multiple stress tolerance factors (compared with single factors) will show better results. Furthermore, the increased yield components in this treatment positively impacted this trait, which is a result of these components. In terms of interaction, the $T_4 \times S_1$ combination was significantly outstripped with an average yield of 5.91 tons.ha^{-1} while the $T_5 \times S_3$ combination had the lowest average yield of 3.01 tons.ha^{-1}.

Table (9) The results of two factors and interaction of these factors on grain yield (ton.ha^{-1}).

Water tension levels	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	
S_1	5.59	5.70	5.26	5.91	4.43	5.38
S_2	4.83	4.98	4.42	5.00	3.54	4.55
S_3	3.50	3.89	3.53	4.09	3.01	3.60
average	4.63	4.86	4.40	5.00	3.66	
LSD	Water tension 0.15		Osmotic and hormonal modification 0.11			Interaction 0.21

9 -

Actual Water Consumption (ETa):

Table (10) presents the average actual water consumption (ETa) of various levels of water stress. The water stress treatment (S_2) had the highest water consumption rate (374.25 mm) in Season followed by the control treatment (S_1) (303.00mm). Treatment (S_3) was most water efficient in Season with a water use of 231.75 mm.

The table shows that water consumption in the depletion treatments S_1 and S_2 was the highest compared to the depletion treatment S_3, which had the lowest water consumption. This is because the moisture content in the depletion treatments S_1 and S_2 was near the field capacity which led to more water loss due to evapotranspiration. This is a natural phenomenon because the more moisture content it has, the better it is for the growth of the plant canopy, which would lead to an increase of water loss due to evapotranspiration.

Table (10) shows the actual water consumption (mm) and number of irrigations for the water tension coefficients.

Tension levels	Number of irrigations	Rainwater depth (mm)	Added water depth (mm)	Actual water consumption mm Season-1	Water used (m^3/ha)

S_1	11	89.25	285.00	374.25	3742.5
S_2	11	89.25	213.75	303.00	3030.0
S_3	11	89.25	142.50	231.75	2317.5

10

-Water Efficiency for Grain Yield (WUE) (kg m^{-3}):

The effect of the two study factors as well as the interaction between them on the water efficiency characteristic of grain yield is significant as presented in Table (11).Table shows that the higher the water stress the higher the water use efficiency.. The highest average was observed with the S_3 stress treatment, reaching 1.55 kg m^{-3} water, while the lowest average was observed with the S_1 stress treatment, reaching 1.44 kg m^{-3} water. When soil moisture is close to field capacity, water consumption increases compared to water stress treatments. This is confirmed by the results of studies conducted by [34] and [35], who demonstrated increased water use efficiency when soil moisture content is low due to the physiological nature of the plant. The plant exerts maximum effort to utilize the available water to achieve normal yield.Treatment T_4 significantly excelled treatment T_4 for this trait, with an average of 1.65 kg m^{-3} water, and did not differ significantly from treatment T_2, which averaged 1.61 kg m^{-3} water. This was in contrast to the lowest average, achieved by the control treatment T_5, which averaged 1.21 kg m^{-3} water. This indicates the importance of the interaction between drought tolerance factors and the crucial role of yeast in supplying plant roots with essential compounds such as amino acids, growth-stimulating hormones, and nutrients. This, in turn, encourages root growth, which is the primary tissue responsible for absorption. Increased root activity may be due to improved water utilization efficiency when effective transpiration control factors are present .The interaction also had a significant effect on this trait, as the $T_4 \times S_3$ combination excelled it with an average of 1.76 kg m^{-3} water, compared to the lowest average of 1.16 kg m^{-3} water for the $T_5 \times S_2$ combination.

Table (11) The water efficiency of grain yield (WUE) (kg m^{-3}) was affected by the two study factors as well as their interaction

Water tension levels	**Osmotic and hormonal modification treatment**					**average**
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	

S_1	**1.48**	**1.53**	**1.41**	**1.56**	**1.17**	**1.44**
S_2	**1.58**	**1.64**	**1.45**	**1.65**	**1.16**	**1.49**
S_3	**1.51**	**1.67**	**1.52**	**1.76**	**1.29**	**1.55**
average	**1.44**	**1.61**	**1.46**	**1.65**	**1.21**	
LSD	**Water tension 0.05**		**Osmotic and hormonal modification 0.13**			**Interaction 0.32**

11

- Number of Stomata per Flag Leaf (stoma mm^2):

Table (12) shows a significant effect of water stress on this trait, but osmotic, hormonal, and interaction factors had no significant effect.

Water stress treatment S_1 showed the highest number of stomata (44.62 stomata mm^2) in comparison to treatment S_3 which had the lowest number of stomata (36.74 stomata mm^2). This reduction can be explained by the fact that stomatal response is directly associated with soil water content, because water deficiency-induced chemical signals like ABA are produced by the roots and used to respond to water deficiency. This is similar to the observations of [36] who found that water-stressed wheat leaves reduced both number and density of stomata.

Table 12 shows the effect of the two study factors and the interaction among them on the number of stomata per flag leaf (stoma mm^2).

Water tension levels	**Osmotic and hormonal modification treatment**					**average**
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	
S_1	**41.94**	**44.48**	**46.45**	**46.80**	**43.45**	**44.62**
S_2	**41.92**	**44.55**	**46.49**	**46.70**	**40.31**	**43.99**
S_3	**36.88**	**39.83**	**28.80**	**42.11**	**36.09**	**36.74**
average	**40.25**	**42.95**	**40.58**	**45.20**	**42.95**	
LSD	**Water tension 4.55**		**Osmotic and hormonal modification no**			**Interaction no**

12-

Stomatal length and width (µ):

The two study factors and the interaction of these factors were significant in influencing the stomatal length and width characteristics of flag leaf as shown in Tables 13 and 14.The S_1 stress treatment had the longest average stoma length (27.04 µ) and the widest average stoma width (2.30 µ) and no significant difference was observed between this and the S_2 stress treatment (26.56 µ and 2.26 µ, respectively). The S_3 stress treatment had the smallest averages for these two traits at 23.10 and 1.89 µ respectively. The increased depletion level led to a reduction in stoma length and width, which affected carbon dioxide uptake and reduced its fixation in photosynthesis, thus impacting plant growth and yield. The result is in-line with that of [37] and [38] who noticed that the length and width of stoma decreased when wheat plants were under water stress.

The table also indicates that all treatments for hormonal and osmotic additions significantly influenced the stoma length reduction; no significant difference was found among the treatments as compared to the control treatment which showed the highest mean for this trait with 26.68 and 2.26 (µ) for length and width of the flag leaf stoma, respectively. For all the treatments, it is noted that the stomatal opening length and width is reduced. This resulted in reduced water loss through transpiration, and enhanced water use efficiency through mechanical (physical) or physiological barrier/inhibitions to water loss. The barriers are nondestructive and basically close the stomas without compromising the efficiency of carbon metabolism, resulting in an increase of the relative water content and hence the drought resistance [7]. Moreover, the treatments used together have a positive impact on water stress since it is associated with enhanced antioxidant defense mechanisms such as higher activity of antioxidant enzymes (SOD, POD, CAT). They also regulate the permeability of cell membrane, which helps to reduce the ROS production at the time of water stress and thus reduces the oxidative damage of functional molecules of cell membrane. This keeps many of the physiological processes intact in stressed plants, and also decreases the stomatal area to ensure that the plant cells are not damaged by water stress [8].It was also found that the $T_5 \times S_1$ combination was significantly excelled in both length and width of stoma, with the averages being 28.71 and 2.52 micrometers, respectively, compared to the other combinations, which were not significantly different in stoma length or flag leaf width.

Table (13) The effect of the two study factors and their interaction on the length of the stoma in the flag leaf (µ) is presented

Water tension levels	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	
S_1	26.39	26.67	26.53	26.89	28.71	27.04
S_2	26.29	26.79	26.50	26.90	26.32	26.56
S_3	22.69	22.44	22.69	22.66	25.00	23.10
average	25.13	25.30	25.24	25.48	26.68	
LSD	Water tension 0.61		Osmotic and hormonal modification 0.36			Interaction 0.74

Table (14) The effect of the two study factors and the interaction between them on the width of the stoma opening in flag leaf (µ)

Water tension levels	Osmotic and hormonal modification treatment					average
	T_1	T_2	T_3	T_4	T_5	
S_1	2.27	2.24	2.22	2.27	2.52	2.30
S_2	2.26	2.22	2.20	2.26	2.38	2.26
S_3	1.83	1.93	2.00	1.80	1.88	1.89
average	2.12	2.13	2.14	2.11	2.26	
LSD	Water tension 0.05		Osmotic and hormonal modification 0.04			Interaction 0.08

References:

[1] **Al-Asadi, M. H. S. (2019).** GenStat for Agricultural Experiment Analysis. Al-Qasim Green University – College of Agriculture. Dar Al-Warith Printing and Publishing House. Iraq. Pages: 304.

[2] Abdelraouf, R. E; El-Shawadfy, M. A., Dewedar, O. M., & Hozayn, M. (2021). Field and modelling study for deficit irrigation strategy on roots volume and water productivity of wheat. *Journal of Water and Land Development*. No. 49 (IV–VI): 129–138.

[3]Al- Raoie, K.M; and A.A.M. Khalaf Allah (2000). Design and Analysis of

Agricultural Experiments, Dar Al Kutub Foundation for Printing and Publishing. University of Al Mosul. Iraq. 488 p.

[4] Al-Dulaimi, Z. S.; Al Ubori, R. S., & Ahmed, S. A. (2024). Effects of Water Stress on Water Consumption, Water Use Efficiency of Different Wheat Varieties. Ecological Engineering & Environmental Technology (EEET), 25(10).

[5] Al-Dulaimi, Z. S., & AH, A. S. (2024). Effect of Amti-transpiration and potassium on water use efficiency and water consumption under water stress conditions to climate change resistance. *journal of environmental and earth sciences*, *6*(3), 124-132.

[6] Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. *Fao, rome*, *300*(9), D05109.

[7] Al-Muaini, I. H. A & Al-Ubaidi, M, A,G. (2018). Scientific Foundations for the Management, Production and Improvement of Field Crops, Dar Al-Warith for Printing and Publishing. Pages: 1067.

[8] Al-Rubaie, B. J. H. (2021). Antioxidants. Al-Muthanna University. College of Agriculture. Iraq. First Edition. Pages: 410.

[9] Al-Rubaie, B. J. H. (2022). Drought Stress on Plants. First Edition. Republic of Iraq. Pages: 592.

[10] Ehdaie, B., & Waines, J. G. (1993). Variation in water-use efficiency and its components in wheat: I. Well-watered pot experiment. *Crop Science*, *33*(2), 294-299.

[11] FAO (2018) . Crop and livestock products . ww.fao.org/faostat/en/data/tp.

[12] Hillel, D. (1980). Application of Soil Physics. Academic press. Inc. New York.116 – 126.

[13] Silva, J. A. D., Goi, C. J., Fernandes, S. B., Mantai, R. D., Scremin, O. B., & Pretto, R. (2016). Nitrogen efficiency in oats on grain yield with stability. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *20*(12), 1095-1100.

[14] Ratan, N., Singh, U. N., & Pandey, H. C. (2016). Yield and quality of oat (Avena sativa L.) as influenced by nitrogen and varieties in Bundelkhand region (UP) India. *Agricultural Science Research Journal*, *6*(1), 27-30.

[15] Zein, A. K. (2002). Rapid determination of soil moisture content by the microwave oven drying method. Sudan engineering society journal, 48(40): 43-54.

[16] Abd El-al, F. S. (2009). Effect of Urea and Some Organic Acids on Plant Growth, Fruit Yield and its Quality of Sweet Pepper (*Capsicum annum*). Research Journal of Agriculture and Biological Sciences, 5(4): 372-379.

[17] Al-Nimrawi, S. K. H. & Wael, M. J.(2021) . The effect of glutamic acid spraying on the growth characteristics, yield, and its components of several bread wheat varieties *Triticum aestivum* L. Journal of Educational and Scientific Studies. College of Education, Iraqi University.3(7):63-82.

[18] Abu Dhahi, Y. M. & Al-Yunis, M. A. (1988). A Guide to Plant Nutrition. Theoretical Part. Ministry of Higher Education and Scientific Research. University of Baghdad. Pages: 291.

[19] Majid Kawthar Khalid.(2018). The role of osmotic and hormonal modification in the drought tolerance of maize plants. Master's Thesis. College of Agriculture. University of Baghdad.P: 85 .

[20] Thomas , H. (1975). The growth response of weather of simulated vegetative swards of single genotype of Lolium perenne. J. Agric. Sci. Camb. 84 : 333-343.

[21] Zhang, J., & Kirkham, M. B. (1996). Antioxidant responses to drought in sunflower and sorghum seedlings. *New phytologist*, *132*(3), 361-373.

[22] Barnes, D. L., & Woolley, D. G. (1969). Effect of Moisture Stress at Different Stages of Growth I. Comparison of a Single-Eared and a Two-Eared Corn Hybrid 1. *Agronomy Journal*, *61*(5), 788-790.

[23] Deshmukh, P. S., Sairam, R. K., & Shukla, D. S. (1991). Measurement of ion leakage as a screening technique for drought resistance in wheat genotypes. Indian J. Plant Physiolgicall.34:89-91.

[24] Al-Attar, A. A., Al-Allaf, S. M., & Al-Mukhtar, K. A. (1990). Microscopic Preparations. Ministry of Higher Education and Scientific Research - College of Science - University of Basra. Higher Education Press.

[25] Sallah, P. Y. K., Obeng-Antwi, K., & Ewool, M. B. (2002). Potential of elite maize composites for drought tolerance in stress and non-drought environments. Afric.Crop Sci. 10(1):1-9.

[26] Al-Dulaimi, Z. S. A.(2024). Physiological and anatomical responses of some wheat varieties to water stress and antitranspirants in growth and yield. PhD thesis. College of Agriculture. Al-Qasim Green University. Pages: 254.

[27] Atiya, H. J. A.& Jadou, K. A. (1999). Plant Growth Regulators: Theory and

Application. Ministry of Higher Education and Scientific Research. Baghdad. Printing House for Books. Pages: 25.

[28] Reynolds, M. P., Singh, R. P., Ibrahim, A., Ageeb, O. A. A., Larque-Saavedra, A., & Quick, J. S. (1998). Evaluating physiological traits to complement empirical selection for wheat in warm environments. *Euphytica*, *100*(1), 85-94.

[29] Al-Rubaie, Baqir Jalab Hadi. (2022). Drought Stress on Plants. First Edition. Republic of Iraq. Pages: 592.

[30] Qayyum, A., Al Ayoubi, S., Sher, A., Bibi, Y., Ahmad, S., Shen, Z., & Jenks, M. A. (2021). Improvement in drought tolerance in bread wheat is related to an improvement in osmolyte production, antioxidant enzyme activities, and gaseous exchange. *Saudi journal of biological sciences*, *28*(9), 5238-5249.

[31] Saqr, M. T. (2008) Plant Physiology - Stress Physiology and Plasma Membrane Permeability. Faculty of Agriculture - Mansoura University.

[32] Farooq, S., Shahid, M., Khan, M. Á., Hussain, M., & Farooq, M. (2015). Improving the productivity of bread wheat by good management practices under terminal drought. *Journal of Agronomy and Crop Science*, *201*(3), 173-188.

[33] Bandgar, A., Bharad, S. G., Potdukhe, N. R., Tayade, S. D., & Amarshettiwar, S. B. (2020). Effect of moisture stress on morpho-physiological traits of wheat (*Triticum aestivum* L.) genotypes. *Journal of Pharmacognosy and Phytochemistry*, *9*(1), 361-364.

[34] Faleh, A. S. & Saleh, A. T. (2012). Estimating the water requirements of yellow corn crop based on deficit irrigation and comparing it with climatic equations and evaporation measurements. Diyala Journal of Agricultural Sciences. 4(1):62-75.

[35] Phene, C.J., R.J.Reginato, B. Itier & Tanner, B.R. (1990). Sensing irrigation needs. P p.207-261. In G.J. Hoffman, T.A. Howell, and K.H. Solomon (eds.) Management of Farm Irrigation Systems, American Society of Agricultural Engineers, Vol.54 No 1.

[36] Li, Q., Gao, Y., Hamani, A. K. M., Fu, Y., Liu, J., Wang, H., & Wang, X. (2023). Effects of warming and drought stress on the coupling of photosynthesis and transpiration in winter wheat (Triticum aestivum L.). *Applied Sciences*, *13*(5), 2759.

[37] Alam, A. M. S., Kabir, G., Ud-Deen, M. M., & Hoque, E. (2011). Effect of water stress on stomatal characters of twenty one near isogenic lines of wheat (*Triticum aestivum* L.). *Bangladesh*

Journal of Agricultural Research, *36*(1), 173-181.

[38] Hashim, I. K. (2017). The effect of abscisic acid on the growth, yield, and quality of bread wheat grown under water stress conditions. PhD dissertation. College of Agriculture. University of Baghdad. Pages: 156.