The effect of interaction between humic acid and water stress in the content of wheat leaves from proline and some of its components

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ABSTRACT

A field experiment was conducted for the cultivation of wheat crop *Triticum aestivum* L. in one of the fields belonging to the extension farm experiments in Al-Mahnawiya belonging to the Extension Center in Babylon province during the winter season (2017-2018) to study the effect of adding humic acid in wheat bearing to water stress during branching stage and grains filling stage, In the experiment, three levels of humic acid were used (0: The control treatment, 20 kg.ha^{-1}: Added to soil, 10 ml.L^{-1}: Sprayed on the plant) which are symbolized by (H_0, H_1, H_2), respectively. Three water stresses were also used: S_0 is the control treatment, (S_1) stopping irrigation in the branching stage, (S_2) stopping irrigation in the grain filling stage. The experiment was applied within the classification of the Randomized Complete Blocks Design (R.C.B.D), in the order of split plots, with three replicates. Wheat seeds (Aba 99 cultivar) were cultivated which obtained from Agricultural Research Station in Babylon. Concentrations of nutrient elements have estimated of nitrogen, phosphorus and potassium in post-harvest soil. Plant height, number of branches, weight of 1000 grains, Biologic yield, grain yield and harvest index, concentrations of nutrient elements (N, P, K) in grain, Protein and Leaves content of K were also estimated. The depth of irrigation water and its date were determined depending on the moisture of the soil and according to the depth of the plant root system . the results were as follows: The spraying treatment with humic acid (H_2) was significantly excelled in most of the growth traits of wheat yield compared with the control treatment which gave (14.55 tons.h^{-1}). The water stress at the grain filling stage (stopping irrigation) led to a significant increase in the leaves content of the proline compared to the two stages of water stress in the branches stage (S_1) and non-stress (S_0). Water stress (S_0) led to a significant increase in grain yield of (6.33 tons.h^{-1}), which did not differ significantly from the treatment of water stress in the branching stage (S_1), which amounted to (5.89 tons.ha^{-1}) while the increase in the S0 treatment was compared to the stopping irrigation treatment S_3 in the grain filling stage, which amounted to (5.50 tons.h^{-1}), an increase of 15.09%. The interaction treatment (H_2S_0) gave the highest values and for most of the study indicators of (16.10 and 6.60 tons.ha^{-1} Biologic yield and grain yield, respectively, 38.61 g weight of 1000 grains). The interaction treatment (H_2S_3) gave the highest harvest index of 42.99% while the H_1S_3 treatment gave the highest leaves content of proline acid (3.10 mg.g^{-1}).

Research paper from MSC thesis for the first Author.

التاثير المتداخل لحامض الهيوميك والاجهاد المائي في محتوى اوراق الحنطة من البرولين وبعض مكوناته

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الخلاصة

اجريت تجربة حقلية لزراعة محصول الحنطة *Triticum aestivum* L. في احدى الحقول التابعة لتجارب المزرعة الارشادية في المهناوية التابع الى المركز الارشادي في بابل خلال الموسم الشتوي (2017-2018) لدراسة تاثير اضافة حامض الهيوميك في تحمل الحنطة للاجهاد المائي في مرحلتي التفرعات ومرحلة امتلاء الحبوب , حيث استعملت في التجربة ثلاث مستويات من حامض الهيوميك وهي (0 معاملة السيطرة , 20 كغم اضافة ارضية .هـ$^{-1}$و10 مل / لتر رشا على النبات) وهي معاملة المقارنة واضافة حامض الهيوميك الى التربة والرش بحامض الهيوميك ورمز لها (H_0 وH_1 وH_2) بالتتابع , كما استعملت ثلاثة اجهادات مائية هي (S_0) وهي معاملة المقارنة , (S_1) قطع الري في مرحلة التفرعات , (S_2) قطع الري في مرحلة امتلاء الحبوب طبقت التجربة ضمن تصنيف القطاعات المعشاة بالكامل بترتيب الالواح

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المنشقة وبثلاث مكررات RCBD , زرعت زرعت بذور الحنطة صنف (اباء 99) , الذي تم الحصول عليه من محطة البحوث الزراعية في بابل قدرت تراكيز العناصر الغذائية لكل من النتروجين والفسفور والبوتاسيوم في التربة بعد الحصاد كما قدر ارتفاع النبات وعدد التفرعات ووزن الف حبة والحاصل البايلوجي وحاصل الحبوب ودليل الحصاد , كما قدرت تراكيز المغذيات N , P , K في الحبوب , البروتين ,K في الاوراق . وحدد عمق ماء الري موعد الريات اعتمادا على الاستنفاد الرطوبي للتربة وبحسب عمق المجموع الجذري للنبات وكانت النتائج كالاتي :- تفوقت معاملة الرش بحامض الهيوميك H_2 قياسا بمعاملة المقارنة الى زيادة معنوية في اغلب صفات النمو لمحصول الحنطة وبلغت (14.55 طن . هـ$^{-1}$ الحاصل البايلوجي , 6.10 طن . هـ$^{-1}$ حاصل الحبوب). وحقق الاجهاد المائي في مرحلة امتلاء الحبوب (قطع الري) الى زيادة معنوية في محتوى الاوراق من البرولين قياسا بمرحلتي الاجهاد المائي في مرحلة التفرعات (S_1) وعدم الاجهاد (S_0) . وادى الاجهاد المائي (S_0) الى زيادة معنوية في حاصل الحبوب بقيمة قدرها 6.33 طن . هـ$^{-1}$ والتي لم تختلف كثيرا عن معاملة الاجهاد المائي بمرحلة التفرعات (S_1), والتي بلغت قيمتها 5.89 طن . هـ$^{-1}$ في حين بلغت نسبة الزيادة لمعاملة المقارنة (S_0) عن معاملة قطع الري في مرحلة امتلاء الحبوب S_3 والتي بلغت قيمتها 5.50 طن . هـ$^{-1}$ وبزيادة قدرها 15.09 %. واعطت معاملة التداخل H_2S_0 اعلى القيم ولاغلب مؤشرات الدراسة بلغت (16.10 و6.60 طن . هـ$^{-1}$ حاصل بايلوجي وحاصل الحبوب بالتتابع , 38.61 غم وزن الف حبة). واعطت معاملة التداخل H_2S_3 اعلى دليل حصاد وبلغت قيمته 42.99 % في حين اعطت معاملة التداخل H_1S_3 اعلى محتوى للاوراق من حامض البرولين بلغت قيمته 3.10 ملغم.غم$^{-1}$.

البحث مستل من رسالة ماجستير للباحث الاول.

1. INTRODUCTION

Wheat (*Triticum estivum L.*) is the first seed crops in the world in terms of importance, cultivated area and global production. It is considered the main food for more than one-third of the world's population (Jamali, 2000). Iraq, as in most other countries of the world, suffers from the reduction of rain and the scarcity of water resources, which has led to the reduction of water levels in the Tigris and Euphrates rivers, As well as the misuse of the sources of this water in agriculture by following a random style in the number of irrigation during the growing season, which requires reconsideration of how to ration water and exploitation in an optimal manner and increase attention to the rationalization water use in agriculture, and not to waste them, the creation of new technologies enable the crop to withstand the lack of water to expand the agricultural area when water is the determining factor, Because decrease in the supply of water to Iraq from neighboring countries, the adoption of scientific and accurate irrigation practices is a crucial issue and is very important. Wheat plants are exposed at certain times of their growth stages to the effects of water stress caused by the lack of moisture of the soil availability for the plant as a result of the lack of adequate water supply at the time of request, or not follow the schedule of scientific irrigation controlled when practicing irrigation or there may be a desire to apply deficit irrigation method to increase the efficiency of water use, which is an essential target for irrigation in dry and semi-dry regions of the world (Ghany Abd-El, 2012, Khan and Naqvi, 2011). Jones and Jones (1989) were more accurate in defining water stress as they defined stress as any force or adverse effect that disrupts normal activity of any plant. Humic acid is one of the commercial products of economic and effective and quick and harmless to humans and animals and plants (Anonymous, 2005) and contains many nutrient elements that lead to increase the growth and yield of the plant and It contains in its composition a carbon, nitrogen, hydrogen and oxygen in varying proportions, led to the formation of compounds of varying molecular weights. Humic acid is used to reduce the harmful effect of mineral fertilizers in soils (Hartwigson and Evans, 2000). The main purpose of the use of humic acid is because it is a humic substance that is nutritious to the plant (Anonymous, 2005). Zhang and Ervin (2004) demonstrated that adding the humic acid to the plant increases the internal Cytokinin with increased oxygen.

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Therefore, the objective of this study is: the effect of adding of humic acid by spraying and added directly to the soil in increasing the tolerance of wheat for water stress in the branches stage and the grain filling stage considering as two critical stages and their effect on the wheat yield and its components and some traits of soil.

2. MATERIALS AND METHODS

A field experiment was conducted for the cultivation of wheat crop (*Triticum aestivum* L.) in the experiments field of the extension farm in Al-Mahnawiya belonging to the extension training center in Babylon province during the winter season (2018-2017), 8 km north of Babylon province, located at Latitude 32 ° 31 'north and longitude 44 °21' To the east in a silty clay loam soil that falls within the level of the Typic Torrifluvent aggregates according to the US modern classification (Soll survey staff, 2006). The leveling and adjustment processes were conducted; the field was divided into three main replicates according to the order of the split-split plots in the Randomized Complete Blocks Design (R.C.B.D), with experimental area 3 × 3. The samples were taken from the field soil represented by 10 samples for the depth 0-0.3 m, the soil samples were mixed and obtained from a compound sample. The soil samples were aerated, then grinded and sieved with a 2 mm diameter sieve. These samples were used to estimate the physical, chemical and fertility properties of field soil before cultivating.

The experiment included the following treatments:

Irrigation treatments (water stress): S_0 without stress, S_1 Stress in the branches stage, S_2 Stress in the grain filling stage.

Addition of humic to soil treatment (20 $kg.h^{-1}$), H_2 Spraying the humic to the plant (10 $ml.L^{-1}$).

Treatments have become 3 × 3 × 3 = 27 experimental units.

Wheat seeds were cultivated (99 aba cultivar) (which was obtained from the Agricultural Research Station in Babylon) on 25 November 2017, with amount of seed 120 $kg.ha^{-1}$ on the lines, It utilized 25 cm between the lines. Each experimental unit contained 8 lines. The tri-superphosphate fertilizer was added, with 100 kg $P.ha^{-1}$ at in one batch with the soil during cultivation.

Nitrogen fertilizers were added by 200 kg.N g^{-1} in the form of urea fertilizer (46% N) and potassium by 120 $kg.Kg^{-1}$ in the form of potassium sulphate (52% K_2O) in the two stages, first at the branches stage and the second at the elongation stage.

The thickets were manually treated and the crop was harvested on April 5, 2018.

Table 1: Physical properties of soil before cultivating.

Properties	Unit	Value
Sand	$g.kg^{-1}$	155
Silt		370
Clay		475
Texture		Silty clay loam
Bulk density	$\mu g.m^{-3}$	1.31
Real density	=	2.65

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Table 2: Chemical properties of soil before cultivating.

Properties	Unit	Value
Electrical conductivity	$ds.m^{-1}$	3.55
pH		7.69
Soil Organic matter	$g.kg^{-1}$	6.44
Carbonate minerals		247.41
interchangeable capacity of positive ions	$Cmol.cal.kg^{-1}$ soil	27.22
Gypsum	$g.kg^{-1}$	7.30
Calcium	Dissolved ions $mmol.L^{-1}$	8.20
Magnesium		7.00
Sodium		8.14
Potassium		0.66
Chloride		14.45
Sulfates		9.33
Carbonates		Nill
Bicarbonates		6.21
Nitrogen availability	$mg.kg^{-1}$ soil	41.41
Phosphorus availability		14.55
Potassium availability		220

Studied traits

1- Weight of 1000 grain (g)

The 1000 grain was randomly taken from the previously harvesting yield of m^2 of each sub-plot experimental unit and weighed by a sensitive balance to represent the weight of 1000 grain and then returned to the product.

2- The biologic yield ($tons.ha^{-1}$)

It was obtained from the dry matter yield (grain yield + straw yield) of the area per m^2 harvested from each sub-plot experimental unit and the weight turned to $tons.ha^{-1}$ (Donaldson, 1996).

3- Grain yield ($tons.ha^{-1}$)

At maturity, 1 m^2 of each experimental unit was harvested, dried, and then take the total weight of the yield and isolate the spikes from the straw and extracted the weight of the grain after the separation.

4- Harvest index (%)

It was calculated from the following equation (Gouzalez, 2007):

$$\textbf{Harvest index (\%)} = \frac{\text{Total grain yield}}{\text{Dry matter yield}} \times \mathbf{100}$$

5- Extraction and estimation of amino acid (proline) in plant leaves

A method of (Bates etal., 1973) was used. Proline was extracted by adding 10 ml of Sulfosalicylic Aqueous acid, its concentration of 30% to fresh sample (5 g) of plant, the sample was squashed then filtered and taken 2 ml of leachate and added 2 ml glacial acetic acid. The sample was then heated with the reagent in a water bath for 1 h after which the sample was refrigerated and 4 mL of Toluene was added and the tube was well churned for 20 seconds and leave at room temperature. The Toluene layer and what contain it of the proline are separated from the mixture. From this upper layer, 1 ml is taken and the absorption degree is measured using a spectrophotometer at a wavelength of 520 nanometers using the equation:

$$\textbf{Proline } (\mu g^{-1} \textbf{ fresh weight}) = \frac{\text{reading} \times 20}{\text{weight of plant sample (g)}} \times 1.47$$

3. RESULTS AND DISCUSSION

Biologic yield

The results of the statistical analysis indicated that the addition of humic acid and water stress and their interactions had a significant effect on the increase of the Biologic yield. Table (3) shows that the adding the acid of the humic has led to the increase of the biologic yield ($tons.ha^{-1}$), which gave the highest value of biologic yield (14.55 $tons.ha^{-1}$) compared to the (H_1

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and H_0) treatments which gave (14.16, 13.60 tons.ha^{-1}), with an increase of (2.75, 6.99%), respectively. It is also noted from the same table that the water stress treatments have significantly affected the biologic yield trait, its highest value was in the control treatment (H_0), which amounted to 15.31 tons.ha^{-1} in terms of the two stages of water stress in the branching stage and the grain filling stage which amounted to (14.04, 12.97 tons.ha^{-1}). The results also showed that the bi-interaction between water stress and the humic acid has led to increase the biologic yield trait, where the interaction treatment H_2S_0 recorded the highest value of (15.31 tons.ha^{-1}) while the interaction treatment H_0S_2 gave the lowest value, which amounted to (12.61 tons.ha^{-1}), with an increase of 27.68%.

Table 3: Effect of the addition of humic acid and water stress and their interactions in the Biologic yield (tons.ha^{-1}).

Stage / Humic acid	Control treatment S_0	Branches stage S_1	The grain filling stage S_2	Average
H_0	14.30	13.90	12.61	13.60
H_1	15.53	14.00	12.95	14.16
H_2	16.10	14.21	13.35	14.55
Average	15.31	14.04	12.97	
L.S.D 0.05	**H**	**S**	**H ×S**	
	0.15	0.15	0.65	

The grain yield (tons.ha^{-1})

The results of the statistical analysis show that the addition of the Humic acid and the water stress and their interaction between them have significantly affected the grain yield trait of the wheat crop, as the addition of the humic acid has increased the grain yield and the highest value was in the spraying treatment (H_2), which amounted to (6.10 tons.ha^{-1}) while the lowest value was for the control treatment, which amounted to (5.72 tons.ha^{-1}), with an increase of 6.64%. The water stress has a significant effect on grain yield, the control treatment (S_0) gave the highest value was in which amounted to (6.33 tons.ha^{-1}) compared to the two stress treatments in the branching stage and grain filling stage, which amounted to (5.89 and 5.50 tons.ha^{-1}), with an increase of (7.47 and 15.09%), respectively. The effect of the bi-interaction between the addition of the humic acid and the water stress significantly affected the increase in the grain yield, the treatment of H_2S_0 gave the highest value was (6.60 tons.ha^{-1}) while the interaction treatment H_0S_2 recorded the lowest value of (5.25 tons.ha^{-1}), with an increase of 25.71%.

Table 4: Effect of the addition of humic acid and water stress and their interactions in the grain yield (tons.ha^{-1})

Stage / Humic acid	Control treatment S_0	Branches stage S_1	The grain filling stage S_2	Average
H_0	6.15	5.75	5.25	5.72
H_1	6.25	5.93	5.56	5.91
H_2	6.60	6.00	5.71	6.10
Average	6.33	5.89	5.50	
L.S.D 0.05	**H**	**S**	**H ×S**	
	0.10	0.10	0.76	

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The harvest index

The percentage of grain weight to the weight of the rest of the plant parts on the surface of soil is called the harvest index (Attiya and Wahib, 1989). Table 5 shows that the addition of humic acid has significantly increased the harvest index. The spraying treatment with humic acid gave the highest harvest index of 42.14% while the lowest value of the harvest index was at the treatment of adding humic acid to soil which gave of (41.87%). The water stress had a significant effect on the harvest index, and gave water stress in the branching stage the highest harvest index was 42.52% compared to the water stress in the branching stage and the control treatment (without water stress), which reached (42.06, 41.43%), respectively, The effect of the interaction between the addition of the humic acid and the water stress had a significant effect on the harvesting index. The highest value of the harvesting index was at the H_0S_0 treatment, which gave 43.00% compared to the lowest value of the harvesting index at the H_1S_0 treatment, which recorded 40.31% %.

Table 4: Effect of the addition of humic acid and water stress and their interactions in the grain yield (tons.ha^{-1})

Stage / Humic acid	Control treatment S_0	Branches stage S_1	The grain filling stage S_2	Average
H_0	43.00	41.39	41.63	42.00
H_1	40.31	42.35	42.95	41.87
H_2	40.99	42.45	42.99	42.14
Average	41.43	42.06	42.52	
L.S.D 0.05	H	S	H ×S	
	0.03	0.03	1.75	

The weight of 1000 grain (g)

Table (6) indicates that all the indicators of the study represented by the spraying of humic acid, adding humic acid the soil, water stress and their interactions have significantly affected the weight of 1000 grain. It is noted that the addition of the humic acid led to increase the weight of 1000 grain, its highest value when the spraying treatment with the humic acid (H_2) of (37.15 g) compared to the treatments of H_1 and H_0, which amounted to (36.83, 36.49). The spraying treatment of with humic acid did not differ significantly from the H_1 treatment. However, the percentage increase in the treatment of H_2 from H_0 treatment was 1.81%. As for the effects of water stress treatments, the control treatment (S_0) gave the highest value of the weight of 1000 grain of 38.40 g compared to the two water stress treatments in the branching stage and the grain filling stage, which amounted to (36.72, 35.34 g), with an increase of (4.57, 8.66%), respectively, It is also noticed from the same table that the interaction treatments between the addition of humic acid and water stress had significantly affected the weight of 1000 grain. The highest value of this trait was at the interaction treatment of H_2S_0 of (38.61 g), while the lowest value at H_0S_2 treatment, which amounted to (34.95 g), with an increase of 10.47%.

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Table 4: Effect of the addition of humic acid and water stress and their interactions in the weight of 1000 grain (%).

Stage / Humic acid	Control treatment S_0	Branches stage S_1	The grain filling stage S_2	Average
H_0	38.10	36.41	34.95	36.49
H_1	38.50	36.75	35.25	36.83
H_2	38.61	37.00	35.83	37.15
Average	38.40	36.72	35.34	
L.S.D 0.05	H	S	H ×S	
	0.05	0.38	0.38	

The leaves content of proline

Table (7) shows that the effect of the addition of humic acid and water stress and their interactions in the leaves content of proline of the wheat plant (mg.g^{-1}) has significantly affected the increase in the wheat leaves content of proline, It is noted that the effect of the addition of humic acid was significant in the reduction of proline where the treatments H_1 and H_2 decreased from the control treatment by a reduction ratio of (10.8, 15.2%), respectively. As for the treatments of water stress, it is noticed from the same table that there is a significant effect of water stress. This trait has increased in the two water stresses at the branching stage and the grain filling stage for the treatments (S_1 and S_2), with an increase of (35.94, 11.07%), respectively, compared to the control treatment (S_0). The effect of the interaction between the addition of humic acid and water stress significantly affected the wheat leaves content of proline, the highest value was at H_0S_2 treatment of (3.60 mg.g^{-1}) while the lowest value at the treatment of H_2S_0 was (1.25 mg.g^{-1}), with an increase of 188%.

Table 4: Effect of the addition of humic acid and water stress and their interactions in the leaves content of proline (mg.g^{-1}).

Stage / Humic acid	Control treatment S_0	Branches stage S_1	The grain filling stage S_2	Average
H_0	1.55	2.34	3.60	2.50
H_1	1.41	2.18	3.10	2.23
H_2	1.25	2.00	2.15	1.8
Average	1.40	2.17	2.95	
L.S.D 0.05	H	S	H ×S	
	0.24	0.24	0.85	

Tables (3, 4, 5, 6, 7) shows that the addition of the humic acid to the spraying treatment (H_2) led to an increase in the biologic yield of the wheat plant, the grain yield, the harvest index, and the weight of 1000 grain except for the leaves content of proline as shown in Table (7)., which confirms the importance of this acid because it contains nutrient elements availability for absorption by the plant as well as improving the soil structure and increase its porosity, which led to the penetration of roots in it, for absorption of nutrient elements availability and reflected in the increase in the strength of vegetative growth and it activity, which reflected positively in the increase in the number of branches (Havlin et al., 2005). The spraying of humic acid increases vegetative growth and increases the efficiency of photosynthesis and leaf area

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of the plant. This increases the permeability of cellular membranes, stimulates enzymatic reactions, increases cell division, elongation etc, and increase the production of plant enzymes and stimulates intracellular vitamins. Its positive effect increases soil nutrient absorption (Brunetti et al., 2006), leading to an increase in grain yield and the weight of 1000 grains, which is positively reflected on the biologic yield and harvest index (Ghorbani et al., 2010). These results agree with (Sebastiano et al., 2005), which found significant increase in the biologic yield , the grain yield, the weight of 1000 grain, and the harvest index for the wheat plant when the spraying the humic acid on the plant compared to the control treatment. Humic acid has great importance in plant nutrition because it slowly increases the release of plant nutrients, increases the exchange capacity of ions and also increases the interaction of the micro elements. This is reflected positively on plant growth and its functional performance, which leads to increased straw and grain yield, thus increasing the biologic yield due to increased nitrogen content in the plant. (Gulser and Ayas, 2005). The addition of hemic acid increases the indicators of the study (Rahmat etal., 2010), which found a significant increase in the weight of 1000 grain, grain yield, the harvest index and the biologic yield when adding the humic acid, with two levels of (1.5, 3 $kg.ha^{-1}$) compared to the control treatment. This agrees with (Abbas et al., 2014), which showed a significant increase in the biologic yield, the grain yield and the harvest index when spraying the humic acid on the plant, with two levels of (3000, 6000 $ml.ha^{-1}$) compared to control treatment (Without adding). EL-Bassionny et al., (2014) showed significant differences in the weight of 1000 grains, grain yield, biologic yield, and harvest index when adding different levels of humic acid compared with the control treatment because of its great role in providing nutrient elements that support Plant growth which positively affects the increase of all the above study indicators (Awad, 2002). The effect of the proline content may be attributed to the role played by organic acids, including the humic acid in the ability of the soil to hold water for a longer period, which reduced the adverse impact of water stress, the content of these acids of nutrient elements, including nitrogen, phosphorus and potassium where the latter works in the limit the risks of the dehydration or dilution and regulate the plant's osmotic pressure and increase the processing of plants well with the element of potassium so increase its ability to retain water due to low rate of transpiration, which is due to the Potassium control on the mechanism of opening and closing the Stoma (Abu Dahi and Al-Younis, 1988). The tables also show that low water stress led to increase the biologic yield, the weight of 1000 grain and the grains yield excluding the harvesting index as shown in Table (6) and the leaves content proline as shown in Table (7), while those indicators decreased by increasing the water stress in the branching stage and the grain filling stage, In the low water stress, nutrient elements, especially nitrogen, phosphorus and potassium, are more availability to the plant, increasing their absorption, which in turn leads to increased cell growth, fragmentation and regularity of photosynthesis with increased accumulation The dry matter in the wheat plant at low water stress and the situation is reversed by increasing water stress, this agrees with (Al-Salmani et al., 1986), who indicated to that the biologic yield of wheat and grain increase increased soil moisture due to the effect of water in the growth (Gholami and Asdollahi, 2008), which indicated that the accumulation of dry matter decreased by (4.8, 26.5%) when the wheat crop was exposed to water stresses in the branching stage and the grain filled stage, respectively. As for the harvest index as shown in Table (5), it is noticed that low water stress led to a decrease in

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the harvest index (%) because the increase in straw is more than the increase in grain yield, this trait increased with increase water stress. This may be due to the fact that in the case of low water stress (control), the plant environment conditions are suitable, which leads to increased absorption of water and nutrients, and the biological processes are at their best and thus increase both the economic yield (grain) and the biologic yield, In the cases of high water stress in the branching stage and the grain filling stage, the harvesting index increases. This may be due to the lack of water in the soil being, which leads to a lack of the water and nutrients absorption, This is consistent with (Hochman, 1982; Ismail et al., 1999), who reported that the harvest index increases when different cultivars of wheat are exposed to water stress in all wheat growing stages Including the branching stage and the grain filling stage compared to low water stress, The results indicated in Table (7) that the leaves content of proline for wheat plant in the branching stage and the grain filling stage, from it notes that the plant leaves content of proline in the water stress of these two stages was high and decreased this index with decreasing water stress. The lowest content for it in low water stress (control), since this acid resulted from the destruction of protein and the formation of other amino acids, such as Aspartic and Glutamic, most of which have harmful effect of the effectiveness of enzymes, causing the dormancy of buds and seeds when collected in large quantities, especially in the grain filling stage, which is considering as a critical stage of the wheat growth stage, which is the opposite of the proline acid, which has little effect. Therefore, the conversion of these acids into the proline is one of the defensive means to reduce the effect of these acids (Stewart, 1983) as the proline gathers in all parts of the plant, all of which are subject to water stress It can also be attributed to the fact that the exposure of wheat plant to water stress, especially in the branching stage and the grain filling stage occur some of the biochemical reactions within the cells of the plant, which leads to the accumulation of some of the nitrogen compounds such as amino acids, especially amino acid (proline), this agrees with (Bartels, 2007; Farhd et al., 2011) who found an increase in the leaves content of proline with increasing water stress on the plant or may increase the accumulation with increased water stress due to the formation of new Proline under water stress conditions, with different stages of plant growth. Palfi et al., (1973), and (Rashmi and Agarwal, 1998) found that the reason for the formation of a new free prolin under water stress conditions is the result of the conversion of Glutamic acid to free proline or low prolulin oxidation under water stress conditions or the destruction of proteins rich in propylene, The accumulation of this acid in the leaves of wheat plant was low in the branching stage compared to its content in the grain filling stage as the increase in water stress lead to increased content of proline in the leaves, especially in the guard cells, which leads to lower water stress in these cells, increasing its ability to absorb water, because proline works as a storehouse of metabolic substances within the cell that regulates osmosis, This agree with (Hasegawa et al.,1984; Zhu, 2000), who indicated that increased water stress led to an increase leaves wheat content of proline in the branching stage and the high content in the grain filling stage. These results are consistent with (Castro and Alfredo, 2002), who noted that the intensity of water stress led to increase the leaves content of proline, The effect of bi-interaction between the spraying of humic acid on the plant and the addition of the humic acid to the soil with the water stress treatments represented by the control treatment S0 (without water stress), water stress in the branching stage S1 and water stress in the grain filling stage, The H_2S_0 treatment was excelled in the increasing the study indicators for the biologic yield,

ISSN 2072-3875

the grain yield and the weight of 1000 grain except for the harvesting index and the wheat leaves content of proline, this may be due to the organic acid content of the nutrient elements and the increase of N, P, K% in the grain. The importance of these nutrient elements comes from their role in many physiological processes, in particular the transfer and storage of substances and the relationships of water within the plant (Havlin et al., 2005), This may be due to the fact that in the case of low water stress, the root growth is dense, resulting in a larger soil volume and increased nutrient uptake in which they were dissolved or exchanged (Ebtisam, 2010). These results are consistent with (Shahryari, 2016) led to a significant increase in the biologic yield, weight of 1000 grains, grain yield except for the harvesting index under the water stress conditions of the different stages of plant growth and when adding 400 ml per 50 L compared to the control treatment.

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