

WHEAT SEED PRIMING (*Triticum aestivum* L.) FOR TOLERANCE DROUGHT

B. S. Alobaidy*

K. A. Jaddoa**

ABSTRACT

A field experiment was carried out in field crops Department field-Faculty of Agriculture-University of Baghdad during the season 2013-2014. The objective was to investigate the effect of seed priming wheat before sowing on the wheat plants tolerance to the drought compared with the non-priming seeds and its relationship with the Improving physiological traits and grain yield. Use the R.C.B.D design in the arrangement of split-plot with three replicates. The drought treatment (50,70, and 90%) depletion of available water of occupied the main plots. While seed priming treatments (Gibberellins, Kinetin, Cycocel, Salicylic acid, KCL, Ascorbic acid, Distilled water and Dry seeds). The results of the experiment showed no significant differences between moisture stress levels 50% and 70% in content chlorophyll and grain content, indicating 25% water availability. The average chlorophyll content increased from 78.4% for the treatment of the dry seeds to 87.4% when use Gibberellin increased by 11.47%. The interdiction between the levels of moisture stress and seed priming treatment indicates a difference in the behavior of seed priming coefficients towards moisture stress levels. This means the ability of growth regulators to induce broad antioxidant systems and provide protection for vegetable plastids and other cellular structures by reducing the toxic effects of stress free radicals. Water and increase drought tolerance efficiency in wheat plant.

INTRODUCTION

To be successful in a region of deficit water condition and severe dry climates, plants have to be programmed to recognise and respond ideal water content for growth and environmental cues which indicates a threat of impending damage. The water has become a limiting factor for almost all of summer and winter field crops equally due to the lack of water in Tigris and Euphrates in Iraq. In addition to the scarcity of rain considered another limiting factor especially in the north of the country in areas that depend on rain. When the water is not readily available in required quantities for each plant, therefore this will adversely affect growth characteristics and plant development in terms of anatomy, morphology, physiology and biochemistry which lead to reduction in plant size, leaves area and yield (8,10,22,35). The seriousness of drought encourages researchers in order to investigate methods and approaches that would support crops to tolerate drought and give acceptable yield. Seed priming which means treat seeds with different materials such as water, vitamins, phytohormones and minerals before sowing has been proved to be effective and useful in technique synchronous germination and vigour seedlings (15,25). However, the regulation of germination might be a trigger for pre-adaptation responses, when abiotic stresses (e.g. water deficit) are expected to happen, eventually

* College Of Agric., Al-Anbar Univ., Baghdad , Iraq.

** College Of Agric., Baghdad Univ., Baghdad , Iraq.

leads to alleviation stress effects on growth and yield of plants (1,8,10,11,14). In order to improve our understanding of the physiological and chemical effects of drought stress enhanced by seed priming with of vitamins and hormones under different levels of field capacity, the present study investigated drought effect on wheat in relation to antioxidant enzyme (peroxidase) and some physiological characteristics under drought treatments in plants their seeds were treated with different seed primers.

MATERIALS AND METHODS

Seed treatments

Grains of wheat cv. Bohoth 22 were soaked in solutions of plant hormones (Table 1). The concentrations of the seed priming treatments were chosen based on lab experiment (not included). The seeds were soaked in each treatment for 24 hours and then seeds were dried to their original moisture before planting.

Table 1: seed priming treatments' names and concentrations

Test materials	Concentration (ppm)	symbol
Ascorbic acid	40	AS
Salicylic acid	50	SA
Gibberellin	600	GA3
Cycocel	1000	CCC
Kinetin	40	KIN
KCl	30	KCl
Hydro priming	Distilled water	DW
Check 1	without priming (Dry)	D

Plant materials

Seeds were sown in experimental field of Field crops department, Agriculture College / Baghdad University during the winter season of 2013-2014. The field was well prepared and the phosphorus fertilizer was added and well mixed with the soil in amount of 100 Kg P₂O₅.ha⁻¹, the nitrogen fertilizer was partitioned into three parts in amount of 200 Kg CO(NH₂)₂. The first part of nitrogen was added at the sowing date and the second at tillering stage while the third was added at flowering stage (23). Plants were harvested at maturity when seed moisture reached 14%.

Soil moisture content estimation

Plants were subjected to three water moisture levels. The 50% depletion of soil field capacity (control), 70% and 90% as mild and severe water deficit respectively. The moisture of the study soil was estimated according to the predict soil water retention curve. The relation between volumetric water content (Θ) and water potential (ψ) was estimated for soil samples and water content at potentials 33 and 1500 KPa which was 0.38 and 0.20 respectively in order to estimate the soil capacity to retention water which lead us to determine the best date for irrigation.

The irrigation dates differed according to the soil available water retention and depth of irrigation which was 0-30 cm at the first growth stages and 0-40 cm at the late growth stages. The soil capacity to retain the available water after germination based on the difference between the filed capacity and permanent wilting point. The monitoring of soil water content was done by taking frequent soil samples randomly from experiment filed and then dried in

electric oven at 105°C for 24 hours and then the moisture can be gotten according to the equation of Hillel (20).

$$P_w = \left(\frac{M_{sw} - M_s}{M_s} \right) * 100$$

Where:

P_w: percentage of weighing moisture

M_{sw}: mass of wet soil

M_s: mass of dry soil

However the required amount of the irrigation water for each plot was calculated according to Kovda et al (26)

$$d = D * (\theta_{fc} - \theta_d)$$

Where:

d: added water depth (mm)

D: soil depth (mm)

θ_{fc}: volumetric water content at field capacity

θ_d: volumetric water content at which added water should be applied according to the depilation of available water capacity for plant.

The volumetric water content was calculated according to the following equation (20,21)

$$Q_v = Q_w * \partial b$$

Where:

Q_v: water content based on volume

Q_w: water content based on weight

∂b: soil bulk density

$$\theta = \frac{P_w}{100} * \frac{\ell b}{\ell w}$$

Where:

θ: volumetric water content (cm³.cm⁻³)

ℓb: soil bulk density (Mg.m⁻³)

ℓw: water density (Mg.m⁻³)

All plots were irrigated equally at planting in order to grantee complete field establishment. Then plots were irrigated according to 90, 70 and 50 depilation from available water at two depths 0-30 cm and 0-40 cm according to growth stages. A pan (2*3*3 m) covered with transparent polyethylene (2mm) was used for irrigation purpose. The irrigation was done by plastic tube connected to electric pump. The water gauge was fixed on the tube in order to measure the amount of water according to the relevant treatments.

Physiological indicators

Chlorophyll content in leaves

Chlorophyll content was estimated at elongation and flowering stages as an average of five leaves in each experimental unit by using SPAD 50 2Minolta Apparatus (33).

Relative water content (RWC) in leaves

According to Taiz and Zeiger (36) fresh leaves were randomly chosen and then 4four discs (diameter 2 mm) were taken from their middle. Discs were then put in plastic bags in order to preventing the loss of their moisture then were put in distilled water for 12-14 hours under light and temperature of room. Then discs were dried with filter paper and turgid weight was taken (TW). Discs then were put in oven at 85 °C for three hours and then dried weight was taken (DW). Finally, relative water content was estimated according to the following equation (6).

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

Where:

RWC: relative water content

FW: Fresh weight

TW: Turgid weight

DW: dried weight

Proline content in leaves

Free proline was estimated in leaves according to Palfi et al (31). The method was used to estimate proline in fresh plant tissues (mmol.gm⁻¹).

Peroxidase enzyme activity

The activity of POD was estimated according to Müftügil (29) equation.

Protein content in seeds

Protein was estimated in mature seeds by using semi-micro Kjeldal according to methods that mentioned in Horwitz et al.(22) and the protein percentage was gotten by multiplying nitrogen percentage by (5.7).

Plant Yield (tonne.ha⁻¹)

Plants were harvested when moisture of seeds got to 14%. Then yield of plants were calculated according to the yield in each experimental unit in gm.m⁻² then it was converted to tonne.ha⁻¹ (22).

Experimental design and statistical analysis

The experiment was layout in a split-plot arrangement in randomized complete block design (RCBD). The main plots included irrigation levels (50, 70 and 90%), while the sub-plot which included seed priming treatments (table 1). The data then was subjected to ANOVA analysis using Genstat software and the means and standard errors were calculated using Excel (MS 2010). The significant differences between means were examined according to LSD at P<0.05.

RESULTS DISCUSSION

The effect of moisture depletion

Statistical analysis shows that chlorophyll content is similar at all moisture levels (Figure 1). However the relative water content in leaves increased as moisture decreases then declined when plants exposed to sever stress. Relative water content in leaves at 90% depletion of soil moisture is significantly lower in comparison with 70% moisture depletion but it is not significantly different comparing with 50%.

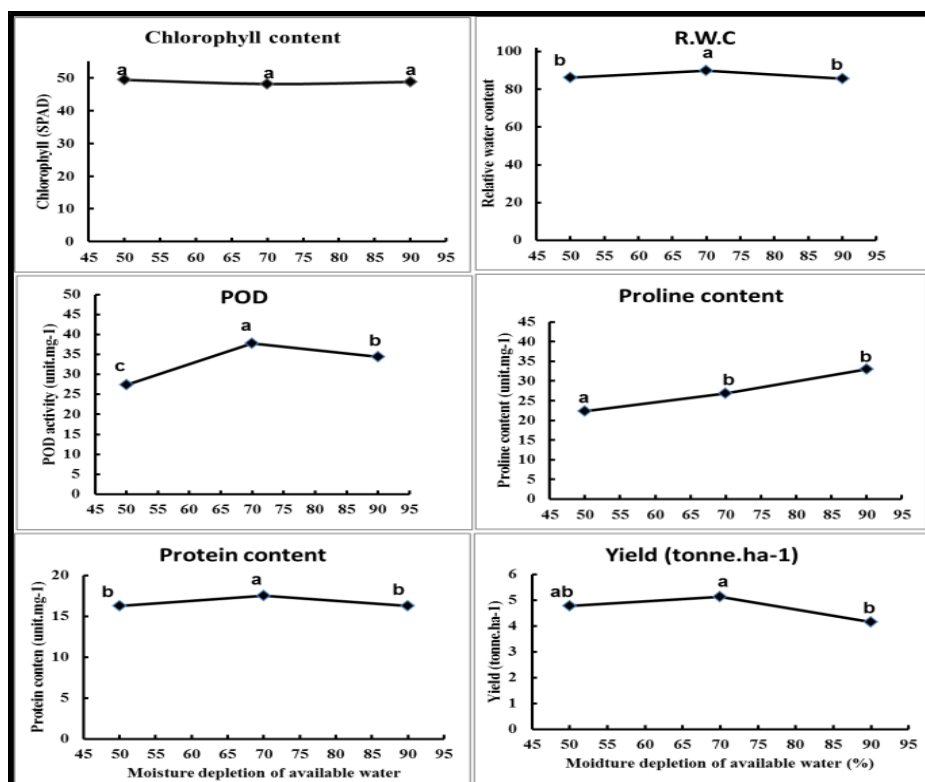


Fig.1: effect of moisture depletion on wheat physiological characteristics and yield (means with same litter are statistically similar while different letters refers to significant difference between means)

The enzyme activity is at its normal when plants are not stressed, but as plants undergo a stress the activity of enzymes are in increase to certain level of stress. In figure (1), statistical analysis shows that Peroxidase enzymes increased when plants are subjected to 70% depletion of available water compared to 50% depletion while decreased again when plants are subjected to 90% depletion of available water. It is obvious that proline is significantly increased in leaves as plants under moisture depletion levels; therefore the increase of proline was progressive along with moisture depletion from study soil (Figure 1).

As for protein content, it has been found that protein content increased when plant subjected to mild stress and then declined at 90% depletion of moisture. Data presented in figure (1) showed that protein content increased when plants were growing at 70 in comparison with plants is under normal conditions as well as under severe stress. It has been proved that plant can tolerate stress to certain levels, therefore the yield was higher in plants growing at 70% depletion of moisture and it was not significantly different from yield in plants growing under normal conditions (Figure 1).

The effect of seed priming treatments

Figure (2) is showing the statistical analysis of all characteristics under effect of different treatments of seed priming. The cycocel treatment achieved significant increase in chlorophyll content in the leaves and values similar to the values achieved by the Ascorbic acid, Salicylic acid and Distilled water stimulation with a significant increase of 13.42%, 12.75% and 12.08% respectively in the compared to the dry seed treatment (Figure 2) . The result showed the treatment of Ascorbic acid in a significant increase in relative water

content of the leaves gave 89.31 mg. 1 - wet weight superior to the average of the dry seed treatment.

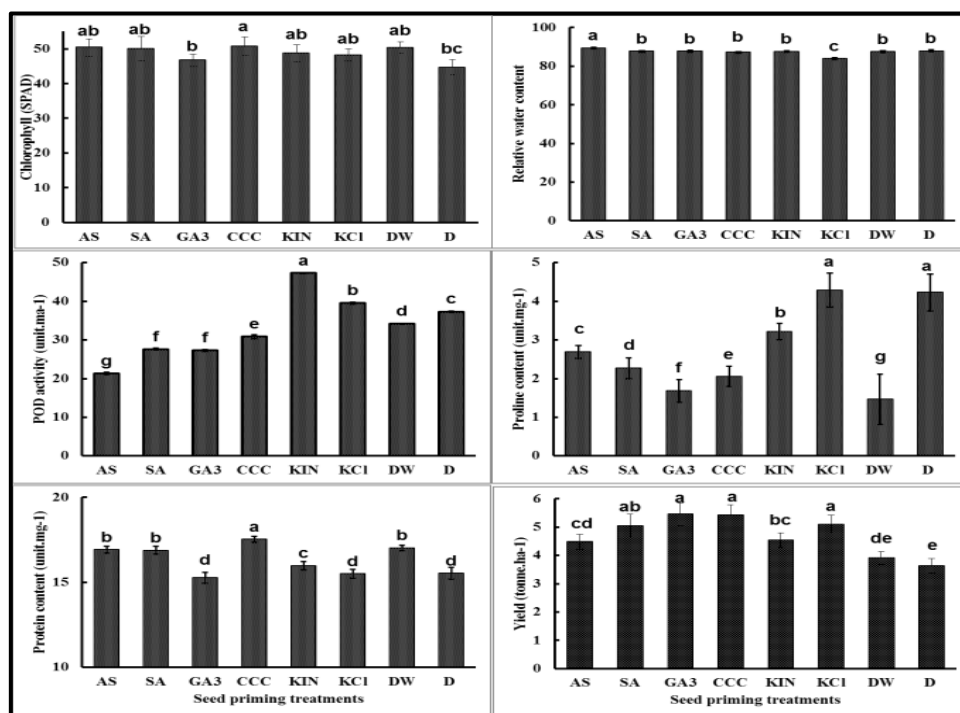


Fig.2: effects of seed priming (AS: Ascorbic acid, SA: Salicylic acid, GA3: Gibberellin, CCC: Cycocel, KIN: Kinetin, KCl: Potassium chloride, DW: distilled water, D: Dry seed) on wheat physiological characteristics and yield (means with same litter are statistically similar while different letters refers to significant difference between means; error bars are presented as standard errors (means±SE))

The stress caused by water stress caused a significant increase in the proline content in the leaves by 47.34% at the level of wet stress 90% and 20.17% at the level of moisture stress 70% compared to 50%. Pre-planting seed stimulation improved the functional status of wheat plant, causing a significant decrease in proline content for most treatments except KCL compared to dry seeds treatment. Peroxidase as part of the enzymatic protection against water stress is strongly affected by high wet stress. The effectiveness of this enzyme increased to 34.39 mg / kg⁻¹ at 90% with a significant increase of 25.60% than the mean enzyme activity at 50% which reached 27.38 mg unit protein⁻¹. The effect of the seed priming treatments on the effectiveness of the peroxidase enzyme varied with the different materials used (Fig. 2). Gibberellin stimulated a significant increase in the average grain yield to 5,453 tonnes. h- 1 - higher than the average dry seed ratio of 50.42%. Seed stimulation before planting can play an important role in increasing the effect of stress by increasing nutrient uptake. The increase is due to increased dry matter, number of seeds and seed weight.

The interaction between seed priming effects and the moisture levels

Results presented in table (2) show that there is a great interaction between seed priming and moisture levels. It also seems that not all seed primers showed same response to moisture levels and also not all traits behaved in similar way under different combination of treatments. Chlorophyll content was higher when Cycocel treatment was applied and plant were grown under normal

conditions (50% depletion), while lowest content was also at 50% depletion of moisture when plants were produced from seeds that treated with Kinetin.

Table 2: the interaction between the moisture levels and seed primers on the traits under study.

Hormones	Moisture depletion (%)	Chl. Content	R.W.C (%)	POD (unit.mg -1)	Proline (unit.mg -1)	Protein (unit.mg -1)	Yield (tonne.ha -1)
Ascorbic acid	50	51.73 ^{abc}	84.29 ^{hi}	18.7 ^{mn}	19.99 ^o	17.51 ^d	4.48 ^{cdefg}
	70	50.77 ^{abc}	88.27 ^e	15.46 ⁿ	22.21 ^m	17.81 ^{cd}	3.71 ^{gh}
	90	48.67 ^{abc}	95.38 ^{ab}	29.79 ^{ghij}	38.39 ^e	15.43 ^f	5.05 ^{bcd}
Salicylic acid	50	52.2 ^{ab}	93.84 ^c	27.52 ^{ijk}	20.23 ⁿ	17.58 ^d	4.63 ^{cdef}
	70	48.4 ^{abc}	88.1 ^e	31.29 ^{ghij}	24.12 ^j	17.5 ^d	4.6 ^{cdef}
	90	49.6 ^{abc}	80.97 ^k	23.98 ^{kl}	23.64 ^l	15.55 ^f	5.91 ^b
Gibberellin	50	46.13 ^{bcd}	88.11 ^e	21.58 ^{lm}	13.45 ^u	12.97 ^g	6.97 ^a
	70	45.67 ^{bcd}	88.43 ^e	31.52 ^{gh}	18.31 ^q	15.37 ^f	4.64 ^{cdef}
	90	48.27 ^{abc}	86.41 ^g	29.51 ^{ghij}	18.54 ^p	17.48 ^d	4.7 ^{cdef}
Cycocel	50	53.1 ^a	78.09 ^l	27.7 ^{hijk}	14.29 ^t	17.6 ^d	4.94 ^{cde}
	70	50.77 ^{abc}	94.87 ^b	30.44 ^{ghij}	18.52 ^p	21.79 ^a	7.46 ^a
	90	48.33 ^{abc}	88.45 ^e	34.3 ^{ef}	28.96 ^h	13.18 ^g	3.87 ^{fgh}
Kinetin	50	45.17 ^{cd}	91.31 ^d	38.86 ^d	26.27 ⁱ	15.44 ^f	4.1 ^{efgh}
	70	50.07 ^{abc}	90.64 ^d	51.18 ^b	23.98 ^k	16.51 ^e	5.91 ^b
	90	51.07 ^{abc}	80.5 ^k	51.81 ^b	46.61 ^b	16 ^{ef}	3.6 ^{gh}
KCl	50	47.13 ^{abc}	80.33 ^k	27.96 ^{hij}	37.75 ^f	13.5 ^g	4.36 ^{defg}
	70	47.8 ^{abc}	85.6 ^h	58.6 ^a	46.4 ^c	15.4 ^f	5.88 ^b
	90	49.73 ^{abc}	85.57 ^{gh}	32.05 ^{fg}	44.76 ^d	17.56 ^d	5.06 ^{bcd}
Distilled water	50	51.43 ^{abc}	82.69 ^j	26.83 ^{jk}	15.05 ^r	16 ^{ef}	5.29 ^{bc}
	70	51.07 ^{abc}	96.67 ^a	39.29 ^d	14.64 ^s	17.5 ^d	4.16 ^{efgh}
	90	51.73 ^{abc}	82.97 ^{ij}	36.39 ^{de}	14.55 ^s	17.57 ^d	2.27 ^j
Dry seed	50	50.77 ^{abc}	90.74 ^d	29.85 ^{ghij}	31.92 ^g	19.82 ^b	3.45 ^{hi}
	70	48.67 ^{abc}	87.67 ^{ef}	44.82 ^c	46.42 ^c	18.33 ^c	4.75 ^{cdef}
	90	52.2 ^{ab}	85.16 ^{gh}	37.25 ^{de}	48.64 ^a	17.45 ^d	2.68 ^{ij}
L.S.D (0.05)		6.788	1.391	3.942	0.132	0.668	0.884

The results of combination treatments were not consistent with the effect of individual factors (Figure 1 and 2). Relative water content also responded to the interaction between seed priming treatments and levels of moisture. The highest RWC was achieved when seeds were hydro primed (Distilled water) at 70% depletion of the moisture (96.67%), while the lowest RWC was achieved under normal conditions (50% depletion) in seeds that treated with cycocel 78.09% (Table 2). As for the activity of POD enzyme, the highest and lowest activity were achieved in plants that subjected to the mild water stress (70% depletion), but with KCl for the higher (58.69 unit.mg⁻¹) and Ascorbic acid (15.46 unit.mg⁻¹) for lower enzyme activity. These results were to some extent consistent with the results of individual factors (Table 2, and Figure 1, 2). Proline is being produced in plant under stress conditions in order to enable to decrease the osmotic potential in cells eventually assure the continuity of water absorption under stress condition. Therefore, results in table (2) show that the highest proline content was found in plants growing under 90% depletion of the moisture, when plant were produced from seeds that are not primed followed by Gibberellin and those results were consistent with results of each factor individually, while the lowest content of proline was found in plants that subjected to the treatment of 90% depletion with Gibberellin seed primer (Table 2 and Figure 1, 2). The highest protein content was found when plants were growing under mild stress and seeds were primed with Cycocel, however the

lower content of protein were obtained from Gibberellin treatment but in plant grew under normal condition (Table 2). As sum of all effects of all treatments (individuals and their combinations), the great yield was achieved under mild conditions when seeds were primed with Cycocel (7.46 tonne.ha⁻¹), while the lowest yield was obtained from plants growing under severe stress when seed were not primed (Table 2).

The results of yield were consistent with results under effect of individual factors (Figure 1 and 2).

Effects of water deficit on plant growth and production have been extensively studied (7). Water stress imposed to wheat plants by depletion of available water from soil leads to biological reactions, eventually reduction in leaf size, plant growth and total yield. The current study showed a significant overlap in the grain yield between the seed priming factors and the water stress factors (Table 2). This indicates a difference in the behavior of the seed priming coefficients towards the water stress levels. For example, the grain yield increases with the increase of the water stress using Salicylic acid and Ascorbic On the contrary the rest of the stimuli were followed. Although there are some indications of the direct effect of hormones on the rate of transition of representative substances, studies have shown that their effect is indirect through their effect on downstream demand. Therefore, plant hormones contribute to regulating the storage capacity of single grains, regulating their nutrient uptake Period of fullness. ALDESUQUY et al. (2) has found same results, when he found that Salicylic acid could increase grain yield, The increase in the yield of cereals when used the Salicylic acid can be attributed to several factors, which have a stimulating effect on flowers and increased flowers age, which may be a prerequisite for the synthesis of oxytin or cytokinein or by the effect of Salicylic acid on the hormonal balance of growth-promoting hormones, The reduction of ABA stress hormone coincided with an increase in relative water content and chlorophyll content (Fig. 2) and its role in providing protection for vegetable plastids and other cellular structures by reducing the effects of water stress(25).The overlap of the 90% humidity level and the Ascorbic acid treatment showed an increase in the relative water content of the leaves reached 95.38% with a significant increase of 4.62% compared to the comparison treatment (90% water stress level with Dry seeds). Seed priming by Ascorbic acid has led to the harmful effects of water stress, increased chlorophyll content and relative water content of leaves, reduced the effectiveness of antioxidant enzymes and maintained the stability of membranes in response to stress and improved nutrient uptake (14).This demonstrates the positive role of Ascorbic acid by enhancing these indices . The reason for the increase in grain yield is that the factors of seed priming before planting have increased the capacity of holding papers for water, and gained the plant drought resistance, which reflected the increase and quality. The treatment of wheat seeds with cycocel increased the chlorophyll content in the tissue. One of the reasons for the superiority of the treatment of the seeds of wheat by Cycocel, is that Cycocel helped to collect chlorophyll and then the formation of green plastids and delay the aging of leaves(9). The positive effect of catalysts on the increased concentration of chlorophyll content can be attributed to several factors, including that seed priming has provided protection for vegetable plastids and other cellular structures by reducing the toxic effects of free radicals caused by water stress.

Bohoth 22 has been chosen as tolerant genotype based on lab experiment (not included) therefore RWC was higher when plants were subjected to 90% depletion of the available water. But it is normally that RWC decrease with the reduction in soil moisture (1,7). When wheat plants are exposed to water stress during reproductive stage reduce its RWC (34). Researchers explained that RWC was not effected during vegetative stage is due to plant ability to recover the growth normally when it is irrigated again . It is evident that ABA is being produced in plant under stress as it closes stomata and reduces water loss in plant (27,38). On another hand, plants from seeds that were soaked in KCl have less variance in RWC (11). Plants have a defence system in order to protect cellular and subcellular system against the harmful components (e.g. ROS) under environment stress (19,30). Mechanisms of ROS scavenging exists in plants through antioxidant enzymes (e.g. POD) when they are exposed to environmental stress. The changes in the level of antioxidant enzymes are responses to environmental stresses on plant metabolism and modulation of their activities is important in the resistance of plant to the environmental stresses(30). Therefore POD activity increased under mild water stress and then declined when plants exposed to sever water stress. The results of this study were consistent with findings of Alipoor and Moradi (3) and Raza et al. (33) which confirmed the increase of POD activity in wheat plants under water stress. This increase depends on level of stress and the duration of the stress. POD is scavenging the harmful effect of ROS by giving compound that binds to H₂O₂ therefore it can inhibit its effect while it is converting it into H₂O and O₂. In addition to its role in the stability of membranes and chlorophyll therefore its activity is increasing as response to protect plant from effect of water stress (2,3,24). Antioxidants normally increase in plant tissues to protect cells and their components against harmful of stresses by hunting ROS that are produced under stress (37). However, the results also were consistent with findings of Azadi et al. (5) who found that seed priming has increased the activity of antioxidant enzymes such as CAT, POD and Ascorbase in comparison with control treatment. Plants have ability to increase proline as response to abiotic stresses such as salinity or water stress (28). The abundance of ROS in plant tissues under stress (e.g. H²O², O², OH etc.) is leading to fat oxidation in membranes causing reduction in protein production and increase in analysis of protein (4,13). ROS can also affect electron transport chain in photosynthesis and stroma enzymes in chloroplast (16); therefore proline presence is useful in scavenging those ROS (18). The exposure to water stress during wheat reproductive stage led to reduction in protein content in seeds. ROS that are produced in wheat plants during stress affected cellular membrane as well as metabolic processes which then reduced protein formation in plant in general (12).

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تحفيز بذور الحنطة لتحمل الجفاف بواسطة

منظمات النمو

بشرى شاكر العبيدي* خضير عباس جدوع**

الملخص

نُفذت تجربة حقلية في حقل تجارب قسم المحاصيل الحقلية - كلية الزراعة - جامعة بغداد خلال الموسم 2013-2014 لدراسة تأثير تحفيز حبوب *seed priming* الحنطة قبل الزراعة على تحمل النباتات للجفاف أكثر من مثيلاتها الناتجة عن حبوب غير محفزة *Unprimed* وعلاقة ذلك بتحسين الصفات الفسيولوجية والحاصل. استخدم تصميم القوالب الكاملة المعشاة (R.C.B.D) بترتيب الألواح المنشقة بثلاثة مكررات في التجربة الحقلية، احتلت الألواح الرئيسة مستويات الاستنفاد الرطوبي، وهي 50% و70% و90% من الماء الجاهز للسعة الحقلية، فيما مثلت معاملات تحفيز الحبوب بمنظمات النمو (*Salicylic acid* و *Cycocel*، *Kinetin*، *Gibberellin*) والأملح (KCl) والفيتامينات (*Ascorbic acid*) إضافة إلى معاملي السيطرة (*Dry* و *Distilled water*) في الألواح الثانوية.

أظهرت نتائج التجربة، عدم وجود فروق معنوية بين مستوى الاجهاد الرطوبي 50% و70% في محتوى الكلوروفيل وحاصل الحبوب مما يدل على امكان توفير 25% من المياه، وان متوسط محتوى الكلوروفيل زاد من 78.4% لمعاملة المقارنة عدم التحفيز *Dry seeds* إلى 87.4% باستعمال التحفيز بـ *Gibberellin* بزيادة معنوية بلغت 11.47%. بدل التداخل المعنوي بين مستويات الاجهاد الرطوبي ومعاملات تحفيز البذور يدل على اختلاف في سلوكية معاملات تحفيز البذور باتجاه مستويات الاجهاد الرطوبي، وهذا يعني مقدرة منظمات النمو على استحداث أنظمة واسعة من مضادات الاكسدة وتوفير الحماية للبلاستيدات الخضراء والتراكيب الخلوية الاخرى بتقليل التأثيرات السمية في الجذور الحرة الناتجة عن الاجهاد المائي وزيادة كفاءة تحمل الجفاف في نبات الحنطة.

جزء من اطروحة الباحث الاول.

كلية الزراعة، جامعة الانبار، الانبار، العراق.

كلية الزراعة، جامعة بغداد، بغداد، العراق.

