# INFLUENCE OF GLYCINEBETAINE AND NITROGEN LEVELS ON GROWTH AND YIELD OF BARLEY (HORDEUM VULGARE L.) UNDER DROUGHT CONDITIONS

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#### ABSTRACT

Two field experiments were conducted in a sandy soil in the extension field in El–Salhia region, Sharkia Governorate during 2004/2005 and 2005/2006 seasons to study the effect of three levels of nitrogen (45, 65 and 85 kg N/fad) and three glycinebetaine rates (0, 8 and 12mM/fad) on the growth and yield of barley (Giza 124 cv.) under two irrigation intervals (weekly irrigated and di-weekly irrigated). The most important findings could be summarized as follows:

Irrigation every two weeks instead of one week reduced significantly all growth attributes. The relative decrease percentages were 25.41 and 22.93%, 22.63 and 19.98%, and 25.11 and 26.19% for flag leaf blade area, total chlorophyll and relative water content in both seasons, respectively.

Addition of 85 kg N/fad increased these traits by 12.80 and 13.63%, 1.88 and 3.50%, and 3.28 and 4.59% in the first and second seasons, respectively compared with the addition of 45 kg N/fad. Meanwhile, using 12 mM glycinebetaine (GB) increased these traits by 20.63 and 23.06%, 32.30 and 35.80%, and 22.57 and 30.40% in the first and second seasons, respectively compared with untreated plants. Under stress or non stress condition, increasing N level from 45 to 85 kg/fad without GB did not increase total chlorophyll. Meanwhile, this trait was increased significantly with increasing GB from zero to 12 mM.

Prolonging the period of irrigation from 7 to 14 day reduced significantly the yield and its attributes. The relative reduction percentages for grain and straw yields were 22.73 and 26.02% and 26.73 and 24.79% in both seasons, respectively.

Increasing N-level from 45 kg to 85 kg N/fad increased grain yield, straw yield and mean productivity by 12.84 and 12.78%, 16.43 and 14.7%, and 12.88 and 12.85% in both seasons, respectively. Meanwhile, the relative increase percentages due to application of 12mM GB/fad compared with zero GB were 21.27 and 24.77%, 24.82 and 26.83%, and 21.30 and 24.73% for these traits in both seasons, respectively.

The interaction between frequent irrigation, zero GB and 45 kg N/fad gave 11.38 ardab/fad compared with 15.34 ardab/fad which was obtained

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from frequent irrigation, 8mM GB/fad and 85 kg N/fad. Meanwhile, the interaction between drought condition, zero GB and 45 kg N/fad gave 8.66 ardab/fad compared with 11.69 ardab/fad which was obtained from drought condition, 8mM GB/fad and 85 kg N/fad treatments.

Key words: barley, glycinebetaine, N, drought, growth and yield.

# **INTRODUCTION**

Barley is one of the most important winter crops in the North West Coast and North Sinai. It is cultivated under rainfed conditions and under sprinkler irrigation system in the new reclaimed lands. It is used for feeding animals and also as a food by Arabian tribes who live in the desert and in dry regions. In addition, barley is the backbone of malting in brewing industry. Most of barley grown in sandy soil, is suffering from adverse conditions such as lake of water and poor soil fertility levels.

An increase in number of irrigations up to 4 enhanced barley growth as expressed in total number of tillers and dry matter accumulation at 40 and 80 days after sowing and at harvest. Significant improvement in effective tillers, grain number and weight per ear and yield was recorded by 4 irrigations (Tanoj- Chaudhary and Sharma, 2003). Meanwhile, drought during the vegetative growth stage indirectly affects yield potential by adversely affecting leaf area (Talukder, 1987) and photosynthetic capacity (Ni, 1992) that is available during grain set and filling. Moreover, drought during flowering reduces yield potential directly by decreasing the total number of grains (Ehaid *et al.*, 1988).

It was found that increasing N-application led to increase the water consumptive use, the water use efficiency of malting barley, and soil moisture extraction (Paramjit *et al.*, 2001). It is well known that N is largely used in synthesis of protein and structurally is a part of the chlorophyll molecule. Nitrogen fertilizer with marginal additional water use increased barley yield linearly by 45.15% (Sing and Kumar (1981). Basha and El-Bana (1994) and Abd Alla, Maha (2004) reported that splitting N rates into three equal doses significantly increased yield and its attributes of barley. Moreover, El-Moselhy and Zahran (2003) noticed that adding N fertilizer up to 60 kg N/fad to barley produced significant increase in each of plant height, spike length, number of spikes/m<sup>2</sup>, number and weight of grains/spike, straw yield and grain yield. In addition, El Bawab *et al.* (2003) showed that plant height, number of spikes/m<sup>2</sup>, and biological and grain yields were significantly increased due to increasing the amount of irrigation water up to 942 m3 and N fertilizer rate up to 60 Kg N/fad.

Accumulation of solutes, either actively or passively, is an important adaptation mechanism for plants in response to osmotic stress. The accumulation of stress metabolites like proline, sugars, amino acids and betaines to maintain structural and metabolic integrity, occurs in response to drought and other stresses. Glycinebetaine (N, N, and N- trimethylglycine) is accumulated by many species of Gramineae, Amaranthaceae, Malvaceae and Poaceae families. Glycinebetaine (hereafter referred to as betaine) is a common compatible solute in many different organisms, including higher plants (Grote *et al.*, 1994 and Rhodes and Hanson, 1993). For barley plants, an important response to stress conditions is the induction of synthesis of the osmoprotactant glycinebetaine (Rhodes and Hanson, 1993).

Using foliar application of glycinebetaine (GB) that protect the plant by acting as an osmolytic maintaining the water balance between the plant cell and the environment and by stabilizing macromolecules during cellular dehydration and at high salt concentration is a major goal for improving drought tolerance of barley in arid zones as in Egypt. It has been shown that GB, when applied to foliage, is translocated from leaves to other plant parts within several hours (Makela *et al.*, 1996), where it acts as a non-toxic cytoplamic osmolyte and plays a central role in adaptation to stress (Martin *et al*, 1997 and Jagendrof and Takab, 2001). Allard *et al.*, (1998) found that foliar applied GB prevented photoinhibition in wheat during drought stress. Exogenous glycinebetaine tended to increase photosynthetic activity, nitrogen fixation, leaf area and grain yield when it was applied to maize, sorghum and wheat (Agboma *et al.*, 1997 & Naryyar and Walia, 2004). Moreover, Abd Alla Kotb (2005) found that 8 and 12 mM GB/fad recorded the highest values of growth parameters, yield and its attributes of wheat.

Therefore, the present investigation aimed to investigate the effect of foliar application of glycinebetaine and N fertilizer level to improve drought tolerance of barley grown under desert conditions.

### MATERIALS AND METHODS

Two field experiments were conducted at the extension field in El –Salhia region, Sharkia Governorate during 2004/2005 and 2005/2006 seasons to study the effect of different levels of nitrogen (45, 65 and 85 kg N/fad) and glycinebetaine rates (0, 8 and 12mM/fad) on the growth and yield of barley (Giza 124 cv.) under two water intervals (weekly irrigated and di-weekly irrigated). The soil texture of experimental field was sandy with pH of 8.1. The available N, P and K contents were 18.2, 4.2 and 79 ppm, respectively.

A split-spilt plot design with 3 replicates was followed. Water intervals which began from 20 DAS using surface irrigation system were allotted to the main plots. The sub-plots were occupied by three levels of nitrogen, whereas, glycinebetaine rates were devoted to sub-sub plots. The sub plot area was  $10.5m^2$  (3 x 3.5) including 15 rows, 20 cm apart. The preceding crop was peanut in both seasons.

Barley grains (*Hordeum vulgare* L.) were sown on 16<sup>th</sup> and 19<sup>th</sup> of November in the first and second seasons, respectively at a seeding rate of 50kg/fad. Nitrogen levels as ammonium sulphate (20.6%N) were applied at three equal doses after 20, 48 and 62 days from sowing. Glycinebetaine (GB) rates (0 GB: spray with tap water, 8 mM equal to 0.84 kg/fad and 12 mM equal to 1.26 kg/fad were foliar applied in 80 liter water/fad after 35, 55, and 75 days from sowing. The recommend cultural practices for barley were applied as practiced in this region. Ten plants were randomly taken at 85 days from sowing for estimating the vegetative growth characters as follows:

- 1- Plant height (cm) was determined from the soil surface to the top of the main spike excluding awns.
- 2- Flag leaf blade area ( $cm^2$ ).
- 3- Total chlorophyll (mg/g):

Chlorophyll a and b were determined according to Fadeel (1962). The optical density of the extract was measured spectrophotometrically at 662 nm and 644 nm to determine chlorophyll a and b, respectively.

4- Relative water content (RWC) :

The relative water content was determined according to Schonfeld *et al.*, (1988), where the fresh weight of twenty discs, from the youngest fully expanded leaf, were determined within 2 hours after excision. Turgid weight was obtained after soaking the discs for 16 to 18 hours in distilled water. After soaking, discs were immediately and carefully blotted dried with tissue paper prior to the determination of turgid weight. Dry weight was obtained after drying the discs sample for 72 hours at 70C°. Relative water content was calculated using the following equation:

 $RWC = \underline{Fresh \ weight} - dry \ weight} \qquad x \ 100$ 

Turgid weight- dry weight

At harvest, an area of  $2m^2$  from each plot was harvested to determine:

- 5- Number of spikes/  $m^2$ .
- 6-1000 -grain weight (g).
- 7- Grain yield (ardab/fad).
- 8- Straw yield (ton/fad).
- 9- Mean productivity (ardab/fad):

Mean productivity is considered as the average yield in stress and non stress environment together. (Rosielle and Hamblin (1981).

In both seasons, the analysis of variance and least significant differences (LSD) were used separately to evaluate the response of each character within treatments according to Steel *et al.* (1997).

# **RESULTS AND DISCUSSION**

1- Plant growth characters

Prolonging irrigation interval from 7 to 14 days decreased significantly the values of all growth characters for barley. The relative reduction percentages due to water stress were 14.58 and 11.74%, 25.41 and 22.93%, 22.63 and 19.98%, and 25.11 and 26.19% for plant height, flag leaf blade area, total chlorophyll and relative water content in both seasons. It seems evident that subjecting barley plants to water stress, through prolonging irrigation interval reduced growth characters; probably due to impairing photosynthetic process and hence translocation of photosynthates from source to sink under water stress conditions. These results are in agreement with those obtained by Talukder (1987) and Abd Alla Kotb (2005).

It is evident from Table 1 that increasing N-level from 45 up to 85 kg N/fad produced gradual and significant increases in all growth characters. Moreover, these

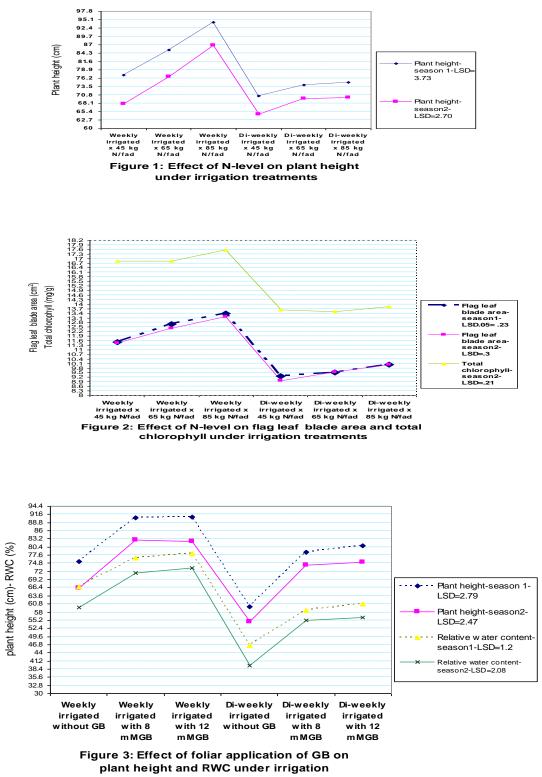
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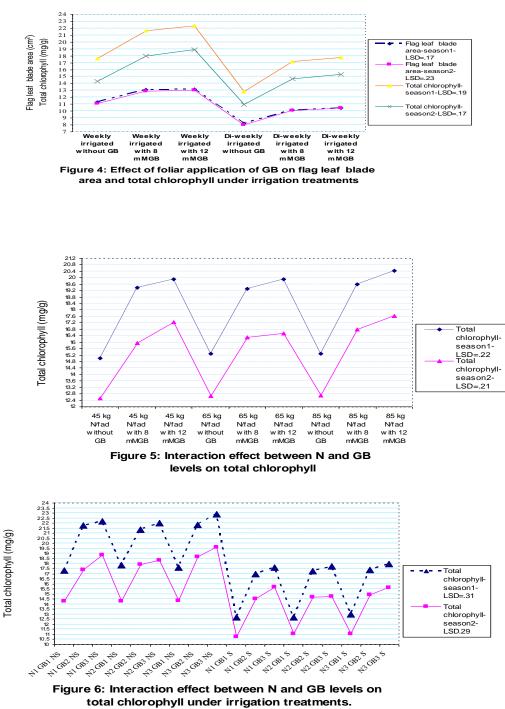
results showed that addition of 85 kg N/fad increased plant height, flag leaf blade area, total chlorophyll and relative water content by 14.52 and 12.07%, 12.80 and 13.63%, 1.88 and 3.50%, and 3.28 and 4.59% in the first and second seasons compared with the addition of 45 kg N/fad, respectively. Paramjit *et al.* (2001) reported that N-application led to increase the consumptive use of water, the water use efficiency of barley and soil moisture extraction. This might have played a role in improving plant drought tolerance, and consequently improved growth parameters of barley. These findings are in harmony with El Bawab *et al.* (2003) and El-Moselhy and Zahran (2003).

The effect of foliar application of GB on parley plant growth was significant in the both seasons (Table 1). The results indicated that the maximum values for growth characters were obtained from application of 12mM GB in both seasons. With increasing amount of GB up to 12 mM/fad, plant height recorded in the both seasons and relative water content in the second season did not respond to more than 8mM GB/fad. Moreover, the results showed that the relative increase percentages due to application of 12 mM GB were 21.49 and 30.35% for plant height, 20.63 and 23.06% for flag leaf blade area, 32.30 and 35.80% for total chlorophyll and 22.57 and 30.40% for relative water content in the first and second seasons, respectively compared with untreated plants. These results are in harmony with those obtained by Agboma *et al.* (1997), Naryyar and Walia (2004) and Abd Alla Kotb (2005).

Figures 1 to 6 explain the significant interactions effects among the three factors under study. The interaction between irrigation treatments and N fertilizer levels showed a significant effect on plant height, flag leaf blade area in both seasons and total chlorophyll in second season (Figures 1 and 2). The highest values of these traits were obtained from weekly irrigation with 85 kg N/fad. Meanwhile, the lowest values were obtained from di-weekly irrigation with 45 kg N/fad. Under water stress, increasing N-levels from 45 to 65 or to 85 kg N/fad increased significantly the values of these traits except the total chlorophyll which did not respond to N application under stress condition.

Concerning to the interaction between irrigation treatments and GB, results in Figures 3 and 4 showed that both of them interacted with each other significantly for all growth characters. Plant height, flag leaf blade area, total chlorophyll and relative water content were gradually increased by decreasing irrigation interval and increasing GB concentration up to 12mM/fad. Under drought condition and spray with 12 mM GB/fad, the response of plant height, flag leaf blade area, total chlorophyll and relative water content was 80.95 and 75.27 cm, 10.44 and 10.45 cm<sup>2</sup>, 17.78 and 15.35 mg/g, and 60.72 and 56.23% compared with 59.85 and 54.66 cm, 8.24 and 8.00 cm<sup>2</sup>, 12.78 and 10.94 mg/g, and 46.53 and 39.59% when the concentration of GB was decreased to zero in both seasons, respectively. Stressed plants received GB accumulated more chlorophyll and had higher relative water content than their untreated analogues. These results indicate, also, that barley plants responded to GB addition in both seasons under frequent irrigation as well as drought condition.





<sup>(</sup>N1, N2 and N3: 45, 65 and 85 kgN/fad. GB1, GB2 and GB3: 0, 8 and 12 mM GB/fad. NS and S: Weekly and Di-weekly irrigated, respectively)

Generally, Figure 5 shows that increasing N level from 45 to 85 kg N/fad with zero GB, total chlorophyll did not respond to N-levels in both seasons. Meanwhile, increasing GB concentration up to 12 mM / fad significantly ameliorate the values of this trait.

The same observation was obtained from the interaction among the three factors in both seasons (Figure 6). Under stress condition or non stress condition, increasing N level from 45 to 85 kg/fad with zero GB, the total chlorophyll did not respond to N-levels. Meanwhile, this trait was increased significantly with increasing GB from zero to 12 mM.

It could be concluded that stressed plants received GB accumulated more chlorophyll and had higher relative water content than their untreated analogues. This could be due to increasing the stability of chloroplast membranes (Mamedove *et al.* 1991), protection of photosystem 2 by GB (Papageorgiou *et al.* 1991), improved water status and reduced transpiration via effects on stomatal regulation. These beneficial effects of GB might have improved the growth of barley under adverse conditions. These results are in harmony with those obtained by Abd Alla Kotb (2005).

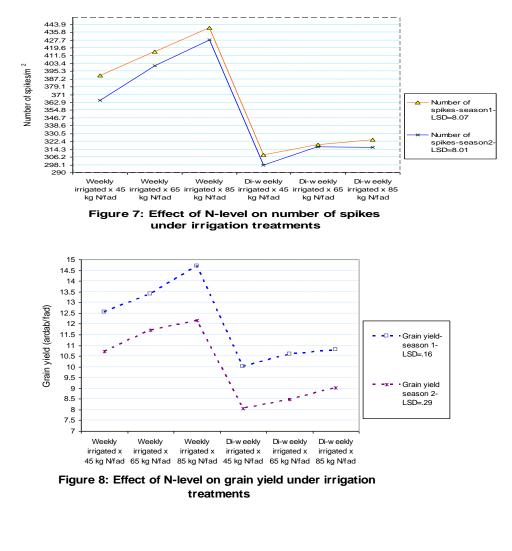
### 2- Barley yield and its attributes

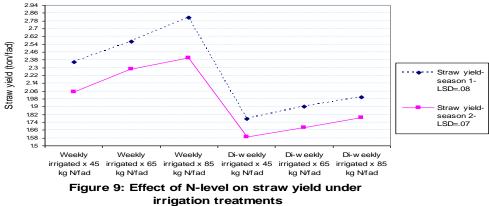
Prolonging the period of irrigation from 7 to 14 day reduced significantly the yield and its attributes in both seasons (Table 2). The relative reduction percentages were 23.68 and 22.03%, 14.82 and 14.48%, 22.73 and 26.02%, and 26.73 and 24.79% for number of spikes, 1000-grain weight, grain and straw yields/fad in both seasons, respectively. In the same trend, the results obtained by Talukder (1987) and Ni *et al.* (1992) refer that drought during the vegetative growth stage indirectly affected on yield potential by adversely affecting leaf area and photosynthetic capacity. These results are in agreement with Tanoj-Chaudhary and Sharma (2003) and Abd Alla Kotb (2005).

Table 2 showed also that number of spikes, 1000-grain weight, grain yield, straw yield and mean productivity were significantly increased by increasing N-level from 45 to 85 kg N/fad and GB concentration from zero to 12 mM GB/fad in both seasons. The relative increase percentages due to addition of 85 kg N/fad in comparison with 45 kg N/fad were 9.26 and 12.28%, 4.89 and 7.68%, 12.84 and 12.78%, 16.43 and 14.7%, and 12.88 and 12.85% in both seasons, respectively. These results are in harmony with those obtained by Basha and El-Bana (1994) and Abd Alla, Maha (2004). Meanwhile, the relative increase percentages due to application of 12mM GB/fad compared with zero GB were 22.18 and 25.50%, 12.49 and 15.35%, 21.27 and 24.77%, 24.82 and 26.83%, and 21.30 and 24.73% for these traits, respectively. These results mean that N had little effect compared with foliar application of GB on growth and yield of barley. In the same trend, Abd Alla Kotb (2005) reported that growth and yield of wheat were increased due to the application of 8 or 12 mM GB/fad.

Concerning to the interaction between water stress treatments and N-levels, the results showed significant effects on number of spikes/ $m^2$ , grain yield and straw yield (Figures 7, 8 and 9). Weekly irrigation with the addition of 85 kg N/fad gave the

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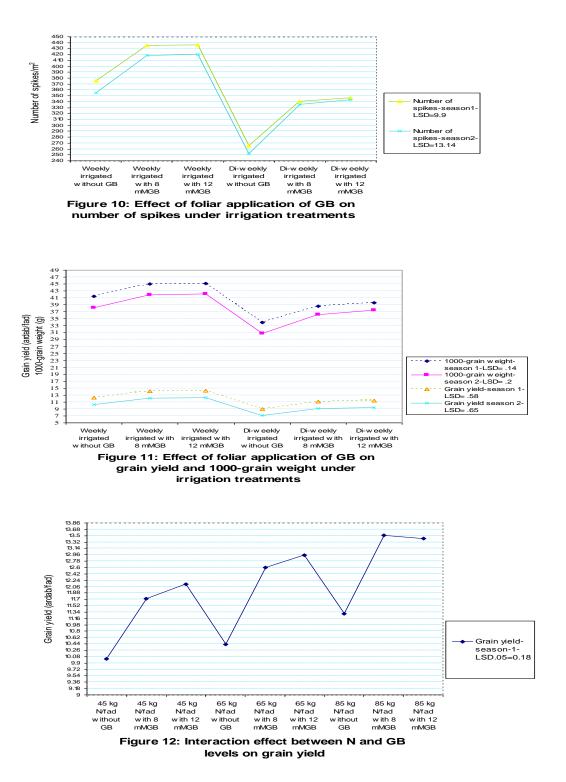
maximum averages of these traits. Under water stress, raising N level improved significantly these traits in both seasons. Sing and Kumar (1981) indicated that nitrogen fertilizer with marginal additional water use increased barley yield linearly by 45.15%. These results are in agreement with those obtained by Basha and El-Bana (1994) and Abd Alla, Maha (2004).

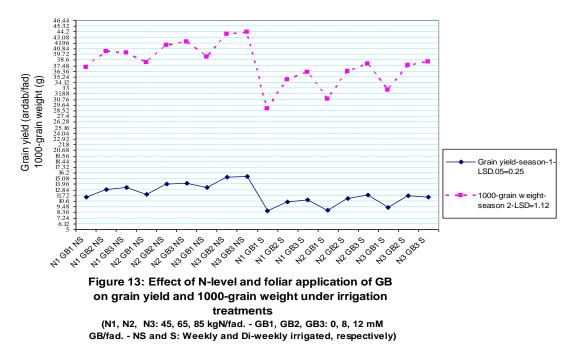
Figures 10 and 11 illustrate that under frequent irrigation or drought condition, increasing GB-levels from zero to 12 mM/fad produced gradual and significant increase in number of spikes, 1000-grain weight and grain yield in both seasons. Generally, the interaction between weekly irrigation and 8 or 12 mM GB/fad gave the highest values of these traits. Moreover, Figure (11) indicate that under drought condition and without GB addition, the response of grain yield was only 8.95 and 7.07 ardab/fad in comparison with 11.11 and 9.10 ardab/fad when the concentration of GB was increased to 8 mM/fad in both seasons, respectively.

Increasing both of N-level and GB concentration resulted in increasing of grain yield in the first season (Figure 12).

Figure 13 explain that under drought condition or frequent irrigation, increasing level of N and GB produced gradual and significant increases in 1000-grain weight in the second season and grain yield in the first season, respectively. Moreover, 11.38 ardab/fad was obtained from frequent irrigation, zero GB and 45 kg N/fad in comparison with 15.34 ardab / fad which was obtained from frequent irrigation, 8mM GB/fad and 85 kg N/fad. Meanwhile, the drought, zero GB and 45 kg N/fad treatment gave 8.66 ardab / fad compared with 11.69 ardab / fad which was obtained from drought, 8mM GB/fad and 85 kg N/fad treatment. It is clear that exogenous glycinebetaine might have had increased photosynthetic activity, nitrogen fixation, leaf area and grain yield as reported in maize, sorghum and wheat by Agboma *et al.*, (1997), Naryyar and Walia (2004) and Abd Alla Kotb (2005), in respective order.

From these results, it could be concluded that nitrogen had a higher positive effect on grain yield and its attributes under drought condition by irrigation every 14 days than under frequent condition. This may be due to nitrogen acted to ameliorate the consumptive use of water, the water use efficiency of barley, and soil moisture extraction (Paramjit *et al.*, 2001). In addition, foliar application of GB had also major effect on these traits under stress condition. This effect may be due to a possible decrease in the sensitivity of RWC in barley leaves and hence the photosynthetic pigments to photoinhibition caused by drought stress. These results indicate that GB acted as osmoregulation substance and enhanced the response of barley plants to water stress when was applied by a proper amount.





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# تأثير مستويات مختلفة من النيتروجين والجلايسين بيتايين على نمو ومحصول الشعير تحت ظروف الجفاف

أجريت تجربتان حقليتان بارض رملية بمنطقة الصالحية بمحافظة الشرقية خلال موسمي ٢٠٠٥/٢٠٠٤، ٢٠٠٥/٢٠٠٥ لدراسة تاثير معدلين من الاجهاد المائى ( الرى كل اسبوع، الرى كل اسبوعين)، ثلاث مستويات من النتروجين (٤٥، ٥٥، ٥٥ كجم/فدان) وثلاث معدلات من الجلايسين بيتاين (٠، ٨، ١٢ ملى مول/فدان) على نمو ومكونات المحصول فى صنف الشعير جيزة ١٢٤. يمكن تلخيص أهم النتائج المتحصل عليها كما يلى :

أدى الإجهاد المائي إلى نقص معنوي لكل صفات النمو المدروسة، حيث بلغ معدل النقص نحو الدي ٢٠ ، ٢٢ ، ٢٢ ، ٢٢ ، ١٩ ، ٩٩ %، ٢١ ، ٢٠ ، ٢١ ، ٢٢ % لكل من مساحة ورقة العلم، محتوى الكلوروفيل الكلى ومحتوى الماء النسبي للأوراق في الموسم الأول والثاني على الترتيب. مقارنة بالنباتات المعاملة ب ٤٤ حم ن/فدان فان إضافة ٨٥ كجم ن/فدان زاد من تلك المقاييس بمعدل ٢٠ ، ٢٠ مستويات المعاملة ب ٢٠ ، ٣٠ ، ٣٠ ، ٤٠ ، ٤ ، ٢٠ ما موسم الأول والثاني على الترتيب. مقارنة مستويات الجلاسين بيتاين من صفر إلى ٢٢ ملى مول/فدان إلى زيادة تلك المقاييس بمعدل ٢٠ ، ٢٠ مستويات الجلاسين بيتاين من صفر إلى ٢٢ ملى مول/فدان إلى زيادة تلك المقاييس بمعدل ٢٠ مروف الجفاف أو الري العادي مع عدم إضافة جلاسين بيتاين فان زيادة مستوى الثاني على الترتيب. تحت طروف الجفاف أو الري العادي مع عدم إضافة جلاسين بيناين فان زيادة مستوى النتروجين من ٤٥ إلى صفر إلى ٢٢ ملى مول/فدان الكلوروفيل، بينما أدي زيادة مستويات الجلاسين بيتاين من صفر إلى ٢٢ ملى مول/فدان النتج عنها زيادة محتوى الكلور وفيل الكلي.

أيضا أدى زيادة فترات الري من ٧ إلى ١٤ يوما إلى نقص معنوي للمحصول ومكوناته. وكان معدل هذا النقص هو ٢٢.٢٦، ٢٠.٢٢%، ٣٢.٢٦، ٣٠.٢٤% لكل من محصول الحبوب والقش في الموسم الأول والثاني على الترتيب. مقارنة بالنباتات المعاملة ب ٤٥كجم ن/فدان فان إضافة ٨٥ كجم ن/فدان نتج عنها زيادة بمعدل ١٢.٨٤، ١٢.٧٨، ٣٦.٢١، ٢٠.٤٤%، ٨٨.٢١، ٥٥.٢٤% لكل من محصول الحبوب والقش ومتوسط الإنتاجية لكلا الموسمين على الترتيب. بينما أدى زيادة مستويات الجلاسين بيتاين من صفر إلى ١٢ ملى مول/فدان إلى زيادة تلك المقاييس بمعدل ٢٢.٢٧، ٢٠.٢٧ الجلاسين بيتاين من صفر الى ٢٢ ملى مول/فدان إلى زيادة تلك المقاييس بمعدل ٢٢.٢٠ و الأسبوعي، صفر جلاسين بيتاين مع ٤٥ كجم ن/فدان الى الردب/فدان مقارنة مع ٢٠.٢٠ و فعلى التفاعل ( الري الأسبوعي، صفر جلاسين بيتاين مع ٤٥ كجم ن/فدان) ١٠.٣٠ إر دب/فدان مقارنة مع ٢٠.٤٠ إر دب/ فدان المتحصل عليها من التفاعل ( الري الأسبوعي، ٨ ملي مول جلاسين بيتاين مع ٨٥ كجم ن/فدان). بينما أعطى التفاعل ( الري الأسبوعي، ٨ ملي مول جلاسين بيتاين مع ٢٠ ٨.١٢، الردب/فدان مقارنة مع ٢٠.١٢ إر دب/فدان المائي، صفر جلاسين بيتاين مع ٢٠ ١٢، ١٠ يودان). مقارنة مع ٢٠.١٠ مائي مول جلاسين بيتاين مع ٢٥ كجم ن/فدان). و معان المتحصل عليها من التفاعل ( الري الأسبوعي، ٨ ملي مول جلاسين بيتاين مع ٢٠ ٨. إردب/فدان و مقارنة مع ٢٠.١٠ إردب/فدان المائي، صفر جلاسين بيتاين مع ٢٠ ٨.١٠ إردب/فدان و مقارنة مع ٢٠.١٠ إردب/فدان).